Evidence Report:

Risk of Acute and Late Central Nervous System Effects from Radiation Exposure

Human Research Program
Space Radiation Program Element

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I. PRD Risk Title: Risk of Acute (In-flight) and Late Central Nervous System Effects from Radiation Exposure

Possible acute and late risks to the central nervous system (CNS) from galactic cosmic rays (GCR) and solar particle events (SPE) are concerns for human exploration of space. Acute CNS risks may include: altered cognitive function, reduced motor function, and behavioral changes, all of which may affect performance and human health. Late CNS risks may include neurological disorders such as Alzheimer's disease (AD), dementia and premature aging. Although detrimental CNS changes are observed in humans treated with high-dose radiation (e.g., gamma rays and protons) for cancer and are supported by experimental evidence showing neurocognitive and behavioral effects in animal models, the significance of these results on the morbidity to astronauts has not been elucidated. There is a lack of human epidemiology data on which to base CNS risk estimates; therefore, risk projection based on scaling to human data, as done for cancer risk, is not possible for CNS risks. Research specific to the spaceflight environment using animal and cell models must be compiled to quantify the magnitude of CNS changes in order to estimate this risk and to establish validity of the current permissible exposure limits (PELs). In addition, the impact of radiation exposure in combination with individual sensitivity or other space flight factors, as well as assessment of the need for biological/pharmaceutical countermeasures, will be considered after further definition of CNS risk occurs.

II. Executive Summary

The possible acute and late risks to the CNS from GCR and SPEs are a documented concern for human exploration of our solar system (NAS 1973; NAS-NRC 1996; NCRP 2006; NAS 2008; NRC 2008; NCRP 2014). In the past, the risks to the CNS in adults exposed to low to moderate doses of ionizing radiation (0 to 2 gray (Gy), where 1 Gy = 100 rad or 1 Joule absorbed per kg) have not been a major consideration, as this is the typical dose fraction used in radiotherapy and does not produce widespread cell killing or frank tissue degradation. However, the heavy ion component of space radiation presents distinct biophysical challenges to cells and tissues compared to artificial terrestrial forms of radiation. Soon after the discovery of cosmic rays, the concern for CNS risks originated with the prediction of the light flash phenomenon from single high charge and energy (HZE) nuclei traversals of the retina (Tobias 1952), which was confirmed by the Apollo astronauts. HZE nuclei are capable of producing a column of heavily irradiated and potentially damaged cells, or a microlesion, along their path through tissues, raising the concern over serious impacts on the CNS (Todd 1989, 1986). In recent years, other concerns have arisen with the discovery of neurogenesis, new regulatory pathways, improved mapping of neuronal pathways, new functional molecular assemblies in the CNS, and their vulnerability to HZE nuclei in experimental models of the CNS.

Human epidemiology is used as a basis for risk estimation for cancer, acute radiation risks, and cataracts. However, this approach is not viable for estimating CNS risks from space radiation because there are no human data for low-linear energy transfer (LET) radiation with which to develop a quantitative scaling approach for space radiation, and HZE particles likely produce qualitatively different CNS effects compared to X rays or gamma-rays. At doses above a few Gy, detrimental CNS changes occur in humans treated with radiation (such as gamma rays and protons) for cancer. Here, local treatment doses of 50 Gy are typical, which is well above the exposures in space even if a large SPE were to occur. Thus, of the four categories of space radiation risks

(cancer, CNS, degenerative, and acute radiation syndromes), the CNS risk relies most extensively on experimental data in animals for its evidence base. Understanding and mitigating CNS risks requires a vigorous research program that draws on basic understanding gained from cellular and animal models and includes the organization of radiation-induced pathophysiological effects according to adverse outcome pathways linked to known diseases and the development of approaches to extrapolate risks and the potential benefits of countermeasures for astronauts.

Many experimental studies using heavy ion beams simulating space radiation provide constructive evidence of the CNS risks from space radiation, although the studies are limited by the small number of GCR particles considered and the restriction of past studies to rodent models, which are only partially reflective of the human brain. First, exposure to HZE nuclei at low doses (10-50 cGy) has been demonstrated to induce neurocognitive deficits in several mouse and rat behavioral paradigms. Exposures to equitoxic doses in excess of 2 Gy of low-LET radiation (e.g., gamma rays and X-rays) do not necessarily show similar effects. Performance deficits therefore have been demonstrated at doses similar to those expected for design reference Mars missions (<1 Gy). The threshold for performance deficits following exposure to HZE nuclei depends on both the physical characteristics of the particles, strain, sex, age at exposure, and post-irradiation evaluation time. Second, exposure to HZE disrupts neurogenesis in the hippocampal dentate gyrus (DG) of rodents at low doses (<1 Gy), exhibiting a significant dose-related reduction of new neurons and oligodendrocytes in the subgranular zone (SGZ) correlated with increases in numbers of activated microglia. Neurogenesis contributes to hippocampal memory-related functions. Third, reactive oxygen and nitrogen species (ROS/RNS) in neuronal precursor cells arise following exposure to charged particles at low doses (<10 cGy). Their levels rise more rapidly after high-LET radiation exposure *in vitro* and *in vivo* and remain elevated for several months. In mutants with elevated or reduced antioxidant enzyme levels, multiple neurological endpoints show corresponding improvements and impairments after irradiation. Antioxidants and anti-inflammatory agents could potentially be used to mitigate adverse changes. Fourth, neuroinflammatory markers are elevated following exposure to HZE nuclei and protons but generally require doses > 1 Gy. Fifth, a variety of new studies show that persistent reductions in neuron arborization and synapse number (dendritic spines) may result from doses of HZE below 50 cGy. Sixth, electrophysiological properties of individual neurons and functionally integrated populations of neurons and support cells show impairments below 1 Gy of protons and HZE. Finally, studies using transgenic mice predisposed to developing pathologies reflective of AD show that low doses of HZE may accelerate the onset of such pathologies and related molecular biomarkers.

Research with animal models irradiated with HZE nuclei has shown that important changes to the CNS occur with the dose levels of concern to NASA. However the operational significance of these results on the performance or morbidity of astronauts has not been established. One classic model of late tissue effects (Rubin and Casarett 1968) suggests that significant effects will occur at lower doses, but with increased latency. It should be noted that the majority of studies to date have been carried out with relatively small numbers of animals ($N \leq 10$ per treatment group) and short post-irradiation times ($\leq$ 90 days); therefore, dose threshold effects (if any) at the lowest doses may not yet have been detected. Extrapolation of space radiation effects in animals to humans will be a challenge for space radiation research and may be limited by the population sizes and time course of animal studies. Another important limitation of studies using charged particles is the lack of dose protraction to more closely match the steady low dose rate of the space environment; however, some recent studies have begun to address this issue with dose

fractionation over times on the order of 1 week. The NASA Space Radiation Laboratory (NSRL) is limited to particles of energy 1 GeV/n, which is the median energy of GCR particles, but ongoing equipment upgrades will soon extend this to 1.5 GeV/n (Slaba et al. 2015). So, currently, the effects of 50% of the GCR HZE particles of highest energy have not been directly measured. Similarly, the space environment is characterized by a complex mixture of particles and energies unlike the single-ion, single-energy experiments typically carried out at NSRL. Technical upgrades are now being implemented to enable a limited simulation of the GCR environment using multiple ions in rapid succession to replicate the fluence and LET characteristics of the natural radiation field. An approach to extrapolate existing observations to possible GCR environment-induced cognitive and performance degradation or late CNS effects in astronauts has not been discovered. However, organizing radiation-induced changes into adverse outcome pathways reflective of pathologies occurring in humans and guided by new approaches in systems biology may offer exciting tools to address this challenge. Recently, 8 knowledge gaps reflecting human CNS morbidities have been identified to guide projection of CNS risks. New approaches to risk assessment tuned to CNS properties and responses, rather than carcinogenesis and mortality criteria, may be needed to develop space radiation risk projection models of the CNS.

III. Introduction

Both GCR and SPEs are of concern for CNS risks. The major GCR are composed of protons, helium nuclei, and HZE nuclei with a broad energy spectra of interest ranging from a few 10s of MeV/n to above 10,000 MeV/n, with a median energy of about 1 GeV/n. Secondary particles produced through nuclear reaction in shielding and tissue, including neutrons, protons, helium nuclei, mesons, and gamma-rays, are also a concern. In interplanetary space, a GCR organ dose and dose equivalent of more than 0.2 Gy and 0.6 Sv per year, respectively, are expected (Cucinotta et al. 2003, 2006, 2014). The high energies of GCR allow them to penetrate to 100s of cm through any material, thus precluding radiation shielding as a comprehensive mitigation measure for GCR risks on the CNS. For SPEs, the possibility exists for absorbed organ doses over 0.5 Gy from a SPE if the crew is performing extra-vehicular activity (EVA) or remains in a thinly shielded spacecraft throughout the duration of the event (Parsons and Townsend 2000; Kim et al. 2007). The energies of SPEs, although substantial (10s to 100s of MeV), do not preclude radiation shielding as a potential countermeasure in reducing risks to the CNS. However, the costs of shielding may be high to protect against the largest events.

GCR exposures occur at low fluences, with each cell in an astronaut's body being traversed by a proton about every three days, helium nuclei once every few weeks, and HZE nuclei about once every few months. For groups of interacting cells, GCR traversals are much more frequent. The fluence of charged particles hitting the brain of an astronaut has been estimated several times in the past (Craven and Rycrof 1994; Curtis et al. 2000, 1998, 1989). One estimate is that during a 3-year mission to Mars at solar minimum (assuming the 1972 GCR spectrum), 20 million out of 43 million hippocampus cells and 230 thousand out of 1.3 million thalamus cell nuclei will be directly hit by one or more particles with charge Z>15 (Curtis et al. 2000, 1998; Cucinotta et al. 2011 - *see Table below in Section VI).* Parihar et al. (2015) provide an additional calculation of traversal probability for several neuron structures where geometric cross sections are >1000 μm^2 for the dendritic tree, ~100 μm^2 for the cell soma, and ~5 μm^2 for filopodial spines. Their calculations yield ratios of traversal probabilities of 200:20:1 for the individual structures at a given fluence and suggest that most dendritic tress will be traversed while individual filopodial

spines will not be. These numbers do not include the additional cell hits by energetic electrons (delta-rays) produced along the track of HZE nuclei (Cucinotta et al. 1998) or correlated cellular damage (Cucinotta et al. 1999; Ponomarev and Cucinotta 2006) as well as the much more frequent interactions with protons and alphas particles. Norbury et al. (2014) and Slaba et al. (2015) estimated that within a spacecraft with 10 or more g/cm^2 shielding, the dominant contributions to dose at all locations in the human body will come from protons and helium nuclei. Calculations indicate that the average hits per cell nucleus per year will approximate 126 and 7 hits per cell nucleus for H and He, respectively vs 0.5 for all HZE. In terms of dose, the values will be about 86, 22, and 8.9 mGy/yr. The contributions of delta-rays from GCR and correlated cellular damage increase the number of damaged cells two- to three-fold from estimates of the primary track alone and present the possibility of heterogeneously damaged regions, respectively. The importance of such additional damage is poorly understood, but Parihar et al. (2015) point out that with maximum delta-ray ranges of ~ 1 cm, essentially all neuronal structures would receive multiple ionizations.

At this time, the significance of potential detrimental effects to an astronaut's CNS from the HZE component of GCR has yet to be identified. This is largely due to the lack of a human epidemiological basis to estimate risks and the relatively small number of experimental studies with animals that have been published. More recent studies by NASA Space Radiation investigators have broadened to a large extent the types of early and late CNS effects that may occur. However, studies are limited by the number of GCR particles considered and the use of only a few doses or dose-rates. To accurately characterize radiation quality and dose response relationships, a large number of particles must be considered (>6) at sufficiently low to moderate doses (at least 5 doses below 0.5 Gy). Intensive effort is now going into creating a simulated GCR environment with multiple charged particles at the NSRL facility by late 2017 (Slaba et al. 2015). There is also a limitation in the animals that have been considered, with only mice and rats studied in the past. The use of a primate animal model is more representative of humans and has been considered for the NSRL due to the large differences between the brains of primates and rodents (Weatherall 2006; Herculano-Houzel 2012). The Russian Space Agency is currently conducting non-human primate studies with 170 MeV protons and 500 MeV/n ^{12}C ions (Krasavin 2015). For estimating cancer risks, relative biological effectiveness (RBE) factors are combined with human data for low-LET radiation exposure to estimate risk. Since this approach is not possible for the CNS risks, new approaches to risk estimation will be needed. Thus biological research is required to establish risk levels in space, to establish risk projection models, and, if risks are found to be significant, to design countermeasures.

Determining radiation exposure risk to the CNS is qualitatively different than that for cancer, where the risk measure is mortality. NASA has identified two main classes of risks to the CNS, namely, 1) cognitive/performance impairments that could compromise missions, and 2) enhanced morbidity or decreased latency to late degenerative diseases. There are currently no common standards for defining "significant" cognitive/performance impairments, and late degenerative conditions are usually detectable only when they reach clinical thresholds (Anger, 2003).

NCRP report # 153 (NCRP 2006) and four reviews (Obenaus et al. 2012; Wong et al. 2004; Tofilon et al. 2000; Schultheiss et al.1995) have summarized known high-dose responses of the CNS that may not sufficiently predict the consequences of space-like low-dose, low-dose-rate exposures to mixed fields of charged particles. Recent reviews of data for space-like radiation fields and low-dose photon studies through 2014 (NCRP 2014; NCRP 2006; Nelson 2009; Cucinotta et al. 2014) conclude that there is evidence for significant alterations in behavioral, neurogenic, neurochemical, inflammatory, and electrophysiological changes to the CNS elicited

by space-like radiation fields generated by accelerators. New observations described below extend these largely phenomenological observations to more mechanistic levels and lower doses.

A. Description of CNS Risks of Concern to NASA

Acute (during missions) and late CNS risks from space radiation are of concern for exploration missions beyond low-Earth orbit (LEO), including missions to the moon, asteroids, or Mars. Acute CNS risks include changes in cognition, motor function, behavior, and mood, which may affect performance and human health. Specific examples of human behaviors and cognitive function of interest that may be affected by space flight include short-term memory, learning, spatial orientation, motor function, emotion recognition, risk decision making, vigilance, reaction time, processing speed, circadian regulation, fatigue, and neuropsychological changes (NASA SP-2009-3405, 2009; Strangman et al. 2014). The late CNS risks are possible degenerative neurological disorders such as AD, dementia, and premature aging. The effects of protracted exposure to low-dose-rate (< 20 mGy/h) exposures to protons, HZE particles, and neutrons of the relevant energies for doses up to ≈ 0.5 to 1 Gy (corresponding to exposures estimated for design reference missions in deep space) on the CNS are of concern. Current Mars design reference mission exposure estimates vary between 0.25 Gy and 0.5 Gy from galactic cosmic radiation with shielded SPE exposures on the order of 0.15 to 0.5 Gy to internal body organs within a typically shielded spacecraft. Approximate relative dose (Gy) contributions to total organ exposure from GCR include protons delivering ~50-60% of the dose, alphas delivering approximately 10-20%, high LET particles of $3 \leq Z \leq 9$ contributing ~5-10%, high LET particles of $Z \geq 10$ contributing ~5-10%, and secondary radiation, including neutrons, pions, and muons, contributing on the order of 10% of the total dose.

The NCRP has recommended that all clinically significant acute risks must be avoided, but there may be subclinical performance decrements that could compromise mission success and crew safety. CNS experimental studies with charged particles have established that statistically significant structural, functional, and behavioral changes can be quantified after exposure of rodents to space-relevant doses. However, the definition of acute CNS risks based on functional (or mission operational) significance to humans must be established in order to set dose limits, and this is under-developed at this time. For late effects, such as increased risk of neurodegenerative diseases such as AD, the occurrence of the disease is fatal, with a mean time from early-stage AD to death of about 8 years. Therefore, if AD risk or decreased latency derived from space radiation exposure is established, it could be included in the overall Risk of Exposure Induced Death (REID) risk formalism for space missions (Cucinotta 2015).

B. Current NASA Permissible Exposure Limits (PELs)

PELs for short-term and career astronaut exposure to space radiation have been approved by the NASA Chief Health and Medical Officer. The PELs set requirements and standards for mission design and crew selection as recommended in NASA-STD-3001, Volume 1, Rev A (NASA 2014). NASA has used dose limits for cancer risks and the non-cancer risks to the blood forming organs (BFOs), skin, and lens since 1970. For exploration mission planning, preliminary dose limits for the CNS risks are based largely on experimental results from animal models. However, further research is needed to validate and quantify these risks and to refine values for dose limits. The CNS PELs correspond to the doses at the deep limbic system region of the brain called the

hippocampus and are set for time periods of 30 days, 1 year, or a career with values of 500, 1,000, and 1,500 mGy, respectively. The unit mGy-Eq will be considered in the future, but the RBEs for CNS effects are largely unknown; therefore, a physical dose limit (mGy) is used, with an additional PEL requirement for particles with charge Z>10. For particles with charge Z≥10, PEL requirements limit the physical 1-year and career doses (mGy) to 100 mGy and 250 mGy, respectively. NASA uses computerized anatomical geometry models to estimate the body self-shielding at the hippocampus.

IV. Evidence

A. Review of Human Data

Evidence for the deleterious effects of terrestrial forms of ionizing radiation on the CNS has been derived from radiotherapy patients, although the associated doses are much higher and inhomogeneous than would be experienced in the space environment (Greene-Schlosser 2012a,b). Behavioral changes, such as chronic fatigue and depression, occur in many patients undergoing irradiation for cancer therapy. Neurocognitive effects are observed at lower doses, especially in children (Schultheiss et al. 1995; BEIR-V 1990).

Reviews of intelligence and academic achievement of children after treatment for brain tumors indicate that radiation is related to a decline in intelligence and academic achievement, including low score of intelligence quotients (IQ), verbal, and performance IQ, as well as in academic achievement in reading, spelling, mathematics, and attention functioning (Butler and Haser 2006; Zeltzer 2009). Similarly, in lower dose whole-body exposures for treatment of pediatric acute lymphocytic leukemia (ALL), adult survivors of the treatment exhibit decrements in intelligence scores (Armstrong et al. 2013; Brouwers and Poplack 1990). Recent studies have found evidence for deficits in specific cognitive processes, including information-processing speed, memory, attention, and learning. Such cognitive impairments generally are not observed in the first year of radiation therapy but are seen during long-term follow-up.

Radiotherapy for the treatment of several tumors with protons and other charged particle beams provides opportunistic observations for considering radiation effects on the CNS. NCRP Report No. 153 notes charged particle usage "for treatment of pituitary tumors (Kjellberg and Kliman 1979; Linfoot 1979), hormone-responsive metastatic mammary carcinoma (Tobias 1979), brain tumors (Castro et al. 1985; Suit et al. 1982), and intracranial arteriovenous malformations and other cerebrovascular diseases (Fabrikant et al. 1989, 1985, 1984; Kjellberg et al. 1983; Levy et al. 1989; Steinberg et al. 1990). In these studies, associations with neurological complications are found, such as impairments in cognitive functioning, language acquisition, visual spatial ability, memory, and executive functioning, as well as changes in social behaviors. Similar effects did not appear in patients treated with chemotherapy. In all of these examples, the patients were treated with extremely high doses that were below the threshold for necrosis (Goldberg et al. 1982; Keime-Guibert et al. 1998).

Atomic bomb and Chernobyl accident victims receiving low to moderate doses of radiation (≤ 2 Gy) show evidence of memory and cognitive impairments. They are more frequently seen medically for psychiatric disorders and exhibit altered electroencephalographic (EEG) patterns (Bromet et al. 2011; Ron et al. 1982; Hall et al. 2004; Ishikawa et al. 1981, Mickley et al. 1989; Yamada et al. 2002, 2009; Loganovsky, et al. 2001, 2000). These studies are limited by individual

dosimetry uncertainties and cultural inhibitions regarding reporting of mental disorders. A study of A-bomb survivors by Yamada et al. (2009) found no increased risk of dementia, but this study was limited by the small sample set (2000), short observation period, and difficulties in dementia classification. Mental retardation was observed in the children of the atomic bomb survivors in Japan exposed prenatally at moderate doses (<2 Gy) during the 8-15 week period post-conception, but not at earlier or later times since conception (BEIR-V 1990; Otake 1998).

B. Review of Space Flight Issues

The first proposal of the effect of space radiation on the CNS was by Cornelius Tobias in his 1952 description of the light flash phenomenon caused by single HZE nuclei traversals of the retina (Tobias et al. 1952). Light flashes were observed by the astronauts during the early Apollo missions and dedicated experiments subsequently performed on later Apollo and Skylab missions (Pinsky et al. 1974). More recently, studies of light flashes have been made on the Russian Mir space station and the International Space Station (ISS) (Narici 2008; Sannita et al. 2004). A 1973 report by the National Academy of Science considered these effects in detail. This phenomenon, known as a phosphene, is the visual perception of flickering light. It is considered a subjective sensation of light since it can be caused by simply applying pressure on the eyeball (NCRP 2006). The traversal of a single highly charged particle through the occipital cortex or the retina was estimated to be able to cause a light flash. Possible mechanisms for HZE-induced light flashes include direction ionization and Cerenkov radiation within the retina.

The observation of light flashes by the astronauts brought attention to the possible effects of HZE nuclei on brain function. The microlesion concept also originated at this time, which considered the effects of the column of damaged cells surrounding the path of a HZE nucleus traversing critical regions of the brain (NAS 1973; Todd 1989, 1986). A more modern view might also consider functional modification rather than damage in the genotoxic sense. An important task still remains to determine whether and to what extent such particle traversals contribute to functional degradation within the CNS.

The possible observation of CNS effects in astronauts participating in past NASA missions is highly unlikely because the lengths of past missions were relatively short and the population sizes of astronauts are small, as well as because astronauts are partially protected by the Earth's magnetic field and the solid body of the Earth in LEO, which together reduce the GCR dose-rate by about 2/3 from its free space values. Furthermore, the GCR in LEO has lower LET components compared to the GCR to be encountered in transit to Mars or on the lunar surface because the Earth's magnetic field repels nuclei with energies below about 1,000 MeV/n, which are of higher LET. For these reasons, the CNS risks are of a higher concern for long-duration lunar missions or for a Mars mission than for missions on the ISS. *Furthermore, radiation safety standards would not allow for missions where clinically significant CNS risks would occur during the mission and would limit late CNS effects to an acceptable risk level.* Therefore, it is highly critical to understand for which space radiation exposure levels violation of safety standards would occur, well before long-term space missions occur.

C. Radiobiology Studies of CNS Risks for Protons, Neutrons and HZE Nuclei

This section presents a review of selected studies on the effects of space radiation in cell, tissue, and animal models of the CNS using charged particles from accelerators and photons from

X-ray or gamma sources at doses < 2 Gy. Selected data from higher dose studies are also included when they provide information regarding dose response trends or potential biological responses not yet measured at low doses with charged particles. The section emphasizes the most recent findings from study designs using space-relevant doses of charged particles.

1. Overall observations

Over the last few years, a large amount of new information regarding the CNS has emerged from investigations of cell and animal models irradiated with space-like radiation fields. The proliferating population of neurons in the hippocampus is inhibited from reproducing, and patterns of differentiation are altered. This prevents new neurons from integrating into circuits associated with learning and memory. Persistent oxidative stress develops along with inflammatory responses to generate an altered microenvironment for the neuronal network. The blood capillary network undergoes a reversible decrease in its connectivity with likely reductions in tissue oxygenation. Low doses of many different particles can result in the remodeling of neurons such that the complexity of their dendritic branches and the number of their dendritic spines (and associated synapses) are reduced, which would interfere with information processing. Electrical properties of individual neurons and their cell membranes are altered, and the ability of neurons to transfer information from one to another across synapses or to strengthen their connections after stimulation is impaired. Levels of numerous molecules associated with synapse structure, ion movements across membranes, inflammatory signaling, cell survival, and DNA repair are altered. There is an impairment of the ability of the tissue to recycle damaged proteins. Additionally, most importantly, these changes are associated with alterations in behaviors reflecting cognitive abilities and memory. The dose responses can be complex and non-linear. There are regional differences in tissues, and effects are sex-, age-, species-, and genetic background-dependent. Overall, the evidence points to persistent measureable changes in the functional status of the CNS similar to those seen during aging and in some neurological diseases, but we do not yet know if these changes rise to the level of operational or clinical significance in humans.

2. Effects in Neuronal Cells and the CNS

a. CNS Structure

The CNS consists of the brain, spinal cord, and retina and is composed of neurons, glial cells, and vasculature. NCRP Report No. 153 (NCRP 2006) and NCRP Commentary #23 (NCRP 2014) provide short introductions on the composition and cell types of interest for radiation studies as well as excellent reviews of many issues addressed in this Evidence Report. The cerebral cortex is the largest subdivision of the human brain. It is involved in processing and analyzing sensory and motor information as well as processes underlying cognition. There are between 1 and $2x10^{10}$ neurons in the cerebral cortex portion of an adult human and 5 to 10 times as many glial cells (Blinkov and Glezer 1968). Brain tissue is often described as consisting of gray matter and white matter. Structures comprised of neuronal cell bodies and their processes are the gray matter, which is organized into layers. Structures comprised of axon fiber bundles are the white matter, so-named because of the appearance of the white insulating myelin sheaths. White matter structures are much more prominent in humans and primates than in rodents and reflect the need to connect structures over larger distances.

The main anatomical units of the CNS are neurons, which exhibit a variety of sizes, shapes, connectivity, and neurotransmitter and receptor specializations. Information processing is carried out by neurons organized into circuits and pathways of varying complexity. Certain nuclei or centers consist of closely packed neuron cell bodies (e.g., the granular layer of the hippocampus' DG), while in other cases, cell bodies may be separated by considerable distances. Each neuron is organized into three main parts: the soma or cell body, a dendritic tree, and an axon. The dendritic tree and soma receive and integrate signals from other neurons, while the axon is the transmitting structure. The dendritic tree consists of one or more branches covered with small protrusions called spines, the location of most synapses. Spines and the shafts of the dendrites receive input from axons of other neurons. Axons are thin (1 to 20 μm) processes that extend from the soma for long distances and usually branch at their termini where they exhibit swellings or boutons, the location of synapses. Many axons are covered with a lipid-rich myelin sheath comprised of concentric layers of glial cell membrane and serve to increase conduction velocity of the axon.

Synapses are the structures that mediate transmission of signals from one neuron to another, and each neuron may possess thousands of synapses on its surface. In mammals, the majority of synapses are considered "chemical synapses", while a minority are termed "electrical synapses" that function essentially as gap junctions. Chemical synapses are 1-μm scale complexes that have a presynaptic component (usually from an axon) and a postsynaptic component (usually from a dendrite or dendritic spine) separated by a thin space or synaptic cleft. Neurotransmission is the process by which an electrochemical signal (action potential) is transferred across the synaptic cleft by a chemical messenger that initiates electrochemical signals in the recipient cell. The process involves a highly regulated sequence of trans-membrane voltage changes, ion movements, neurotransmitter release, and neurotransmitter binding to specific receptors on the postsynaptic membrane. Numerous small molecules act as neurotransmitters, including acetylcholine, norepinephrine, serotonin, dopamine, glycine, glutamic acid, γ-aminobutyric acid, and several peptides. Their binding may result in depolarization (excitatory) or hyperpolarization (inhibitory) of the recipient neuron.

Of additional importance are the glia, which are supporting cells and consist of astrocytes, oligodendroglia, and microglia. These cells permeate and support the nervous tissue of the CNS, providing a scaffold. The most numerous of the neuroglia are Type I astrocytes, which constitute about half the brain in primates (a smaller fraction in rodents) and greatly outnumber the neurons. They cooperate with the vasculature to maintain the blood brain barrier, regulate extracellular concentrations of neurotransmitters, and mediate inflammatory responses. Oligodendrocytes are responsible for the production of myelin sheaths. Microglia are the resident monocytes or macrophages of the CNS and serve innate immunity functions, but they also participate in the maintenance of synapses. Glia retain the capability of cell division in contrast to neurons, and, therefore, the responses to radiation differ between the cell types.

In recent years, studies with stem cells uncovered that cell proliferation and differentiation (neurogenesis) occur in the adult subventricular zone and hippocampus of mammals, which is linked to cognitive activities such as memory and learning (Squire 1992; Eisch 2002). Neuronal progenitor cells (NPCs) proliferate throughout life in mammals and differentiate into glia and neurons that are incorporated into neuronal circuits. Damage to this population is associated with the neurocognitive impairments that appear following cranial radiation (Monje 2012).

A final important tissue component of the brain is the vasculature, which exhibits a comparable vulnerability to radiation damage to that found elsewhere in the body (Reinhold and

Hopewell 1980). Radiation-induced damage to oligodendrocytes and endothelial cells of the vasculature accounts for major features of the pathogenesis of high-dose brain damage. Based on studies with low-LET radiation and cultured cell killing plus white matter necrosis observation, the CNS is considered a radioresistant tissue. For example, during radiotherapy, early brain complications in adults usually do not develop if daily fractions of 2 Gy or less are administered with a total dose of up to about 50 Gy (NCRP 153). The "tolerance dose" in the CNS (a therapy concept based primarily on overt tissue destruction), as with other tissues, depends on the volume and on the specific anatomical location in the human brain that is irradiated (Schultheiss et al. 1995).

b. Neurogenesis

Pluripotent neural precursor cells are the most radiosensitive cells of the mammalian brain (Mizamatsu et al. 2003; Monje et al. 2002; Tofilon and Fike 2000; Limoli et al. 2007; Fike et al. 2009). Studies with low-LET radiation showed that radiation impairs not only proliferation of NPCs, but also persistently impairs their differentiation into neurons and other neural cells (Casadesus et al. 2004, 2005; Rola et al. 2004a,b, 2005, 2008). NCRP Report 153 (NCRP 2006) notes that cells in the dentate subgranular zone (SGZ) undergo dose-dependent apoptosis and that the production of new neurons in young adult male mice is significantly reduced by relatively low ($\leq$ 2 Gy) doses of X-rays. Survival and proliferation of NPCs is inhibited above 0.5 Gy of charged particles, but patterns of differentiation for descendent cells are altered at doses below 0.5 Gy. These responses also hold true for neutrons (Yang et al. 2010). Rivera et al. (2013) found that dose fractionation had little effect on the inhibition of neurogenesis by iron particles. Increases in the numbers of newly-born activated microglia (possibly infiltrating monocytes) accompany decreases in neurons (Rola et al. 2005; Monje et al. 2002) and may persist for up to 2 months.

Supporting observations on widespread NPC death from brains of developing rodents and fish have been used to estimate RBEs with values from 1.4 to 9.8 for ^{12}C and ^{56}Fe ions and neutrons (Ishida et al. 2006; Yoshida et al. 2012; Yasuda et al. 2011), which agrees with the value of 3.4 estimated by Guida et al. (2005) for cultured human neuroblasts (hNT2 cells). While neurogenesis is impaired, the magnitude of its contribution to overall cognition is not yet well established.

c. Oxidative Stress

In vitro studies using rodent neural precursor cells from the hippocampus grown in the form of neurospheres show an increase in ROS following X-ray exposure (Limoli et al. 2007, 2004). Similar results are observed with 250 MeV proton exposures (1 to 10 Gy) at post-irradiation times (6 to 24 hours) compared to unirradiated controls (Giedzinski et al. 2005). The increase in ROS after proton irradiation is more rapid than that observed with X-rays and shows a well-defined dose response at 6 and 24 hours, increasing up to 10-fold, but by 48 hours post-irradiation, ROS levels fell below those of controls and coincided with minor reductions in mitochondrial content. Use of the antioxidant α-lipoic acid (before or after irradiation) was shown to reduce the radiation-induced rise in ROS levels. High-LET radiation led to significantly higher levels of oxidative stress in neurosphere NPCs compared to lower LET radiations (γ-rays, protons). Tseng et al. (2013) and Limoli et al. (2007) and Acharya et al. (2011) demonstrated persistent oxidative stress in ^{1}H-, ^{16}O-, ^{48}Ti-, and ^{56}Fe-irradiated mouse and human neurospheres at < 1 cGy, against which α-lipoic acid was again radioprotective (Manda et al. 2008). Baulch et al. (2015) extended these observations using cultured human neural stem cells irradiated with 5–100 cGy doses of ^{16}O, ^{28}Si, ^{48}Ti, or ^{56}Fe particles (600 MeV/n; 10–50 cGy/min) and ^{28}Si and ^{56}Fe particles at energies of 300 and 1000

MeV/n. Radiation-induced oxidative and nitrosative stress was found to be dose-dependent but largely independent of the LET of the incident particles. All particles resulted in ROS/RNS elevations at ≥ 25 cGy, and in some cases at doses as low as 5 cGy; ^{28}Si and ^{56}Fe were equally effective at all three energies tested. Figure 1 below illustrates results of iron ions, protons, and x-rays on cultured human neural precursor cells grown in 3D neurospheres (Limoli et al. 2007).

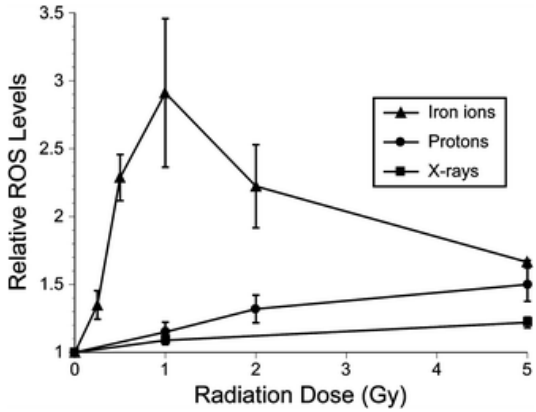

Figure 1. Dose response for oxidative stress after ^{56}Fe ion irradiation. Human hippocampal precursor cells subjected to ^{56}Fe ion irradiation were analyzed for oxidative stress 6 hours after exposure. At doses ≤1 Gy a linear dose response for the induction of oxidative stress was observed. At higher ^{56}Fe doses, oxidative stress fell to those values found before using lower LET irradiations (X-rays, protons). Experiments represent a minimum of three independent measurements (±SE) and were normalized against unirradiated controls set to unity. ROS levels induced after ^{56}Fe-irradiation were significantly ($P < 0.05$) higher than those in controls (Limoli et al. 2007).

In vivo radiation exposure is associated with acute and chronic elevation of oxidative stress. Baluchamy et al. (2012, 2010) and Suman et al. (2013) demonstrated induction of lipid peroxidation and ROS in mouse brains accompanied by reduced levels of glutathione and superoxide dismutase activity following γ-ray, ^{1}H, and ^{56}Fe ion exposures. In mice, persistent oxidative changes are induced by <1 Gy of charged particles. At early times (<1 week) after irradiation, ROS and RNS increases were generally dose responsive but were less dose-dependent weeks to months post-irradiation (Tseng et al. 2014). Exposure to ion fluences at less than one ion traversal per cell nucleus was sufficient to elicit radiation-induced oxidative stress. When antioxidant enzyme levels were assessed in brain tissue, whole-body irradiation triggered a compensatory response in the rodent brain with increased antioxidant enzyme activities 2 weeks after exposure that returned to baseline levels by 4 weeks.

The Raber and Fike laboratories addressed the impact of superoxide dismutase isoform deficiencies on neurogenesis, activation of microglia, and cognitive impairment and found that x-ray-induced effects were reduced in knockout mutant mice for all isoforms of superoxide dismutase (Fishman et al. 2009; Raber et al. 2010; Rola et al. 2007) even though baseline neurogenesis was impaired. In a pharmacological approach, the cell-permeable superoxide dismutase mimetic, metalloporphyrin compound (MnTE-2-PyP), was observed to reduce apoptosis induced by 1 and 4 Gy of protons in the rat retina (Mao et al. 2012) when given before irradiation.

Enhancing H_2O_2 detoxification capacity using a catalase-overexpressing transgenic mouse (MCATtg) suppresses proton-induced impairment of neurogenesis (Liao et al. 2013) and cognition (Olsen et al. 2013; Parihar et al. 2015). Manda et al. (2008) and Villasana et al. (2013) showed that α-lipoic acid administration ameliorated lipid peroxidation and impaired memory elicited by ^{56}Fe exposure in mice. Together, these observations support a functional role for ROS in mediating the pathogenesis of radiation effects in the brain.

ROS play normal roles in signaling when their concentrations and cellular locations are controlled. It is when dysregulation occurs that adverse consequences arise (Joseph and Cutler, 1994). Critical regulatory sites for neuronal activity are receptor-gated ion channels, and recent evidence suggests that multiple channels are regulated by their redox status. It was shown that oxidation of K+ channels (which control neuronal excitability and survival) by ROS is a major mechanism underlying the loss of neuronal function in a eukaryotic model and survival (Sesti et al. 2009). Similarly, γ-aminobutyric acid type A receptors, important in inhibitory neuron function, were found to be susceptible to oxidation, which resulted in changes in ion conductance and channel opening probability. Altered reduced glutathione levels were effective in modulating these responses (Amato et al. 1999). NMDA and acetylcholine receptors also have shown redox modulation of activity (Derkach et al. 1991; Janaky et al. 1993). Thus, there is a potential for perturbation of the regulation of major ion channels directly by radiation or the persistent radiation-induced metabolic shift toward pro-oxidant tissue status.

Another potential redox regulatory site is the cytoskeleton of neuronal processes. The protein cofilin, which regulates the actin filament system in dendrites and spines, has been shown to be redox-sensitive (Samstag et al. 2013), and cofilin and its regulatory network have recently been shown to be highly responsive to irradiation (*cf.* below, Kempf et al. 2014a).

Rabin and Shukitt-Hale (2014) and Raber et al. (2005, 2009, 2015) reviewed the similarities between the effects of aging and radiation exposure on neuronal and behavioral function and drew attention to the efficacy of natural product anti-oxidants in ameliorating deficits from both causes. In particular, components of berry-rich diets are shown to participate in signaling pathways involved in neurotransmission and plasticity, inflammation, and cell survival such that treatments reducing oxidative stress and inflammation also improve performance in older animals and irradiated subjects.

d. Neuroinflammation

Neuroinflammation is a fundamental reaction to brain injury and is associated with the progression of numerous disease processes. Neuroinflammation and microvascular changes are well-known pathological sequelae of cranial irradiation (Greene-Schloesser et al. 2012b), and microvasculopathy, blood brain barrier dysfunction, and neuroinflammation are now clinically recognized as interrelated processes contributing to a wide range of acute and delayed neurological disorders that affect CNS function (Obermeier et al. 2013; Zlokovic 2011). The brain and immune system are are linked by bi-directional communication activities of neurons (e.g. vagus) and the various cytokines and chemokines that coordinate inflammation, cell trafficking and immune cell differentiation (Maier 2003). Thus, many topics addressed below should also be viewed in the context of immunity in the spaceflight environment. This is described in the Immune Discipline Evidence Report "Risk of Crew Adverse Health Event Due to Altered Immune Response" describing changes in immune system associated with spaceflight has been added to the SRPE ERs. https:// humanresearchroadmap. nasa.gov/evidence/reports/Immune_2015-05.pdf?rnd= 0.566203442665843

Neuroinflammation disturbs CNS function and is mediated by altered activation states of microglia and astrocytes, interruption of the blood brain barrier, and local expression of a wide range of inflammatory mediators, including pro-inflammatory cytokines, chemokine receptors, and adhesion molecules (Tofilon and Fike 2000). Microglial activation and inflammatory cytokine production have been implicated in cognitive deficits (Jenrow et al. 2013). Myeloid cell recruitment appears by 6 months following exposure. Acute and chronic neuroinflammation has been studied in the mouse brain following exposure to HZE. The acute effect of HZE is easily detectable at 6 and 9 Gy; however, fewer studies have investigated lower doses. Rola et al. (2005) estimated the RBE value for induction of an acute neuroinflammatory response by HZE irradiation compared to gamma irradiation at ≈ 3. COX-2 pathways are implicated in neuroinflammatory processes caused by low-LET radiation. COX-2 up-regulation in irradiated microglia cells leads to prostaglandin E2 production, which appears to be responsible for radiation-induced gliosis (over proliferation/activation of astrocytes in damaged areas of the CNS) (Kyrkanides et al. 2002; Moore et al. 2005; Hwang et al. 2006). Robbins and colleagues demonstrated the importance of MAP kinase pathways in radiation-induced microglial activation and neuroinflammation (Schnegg et al. 2012).

Moravan et al. (2011), York et al. (2012), and Morganti et al. (2014) found that mouse cranial exposure to γ-rays and protons at doses above 1 Gy elicits persistent elevation of TNF-α, CCL2, T cell infiltration, GFAP, MHC II+, and CD11c+, accompanied by T lymphocyte infiltration and increased numbers of activated microglia. Sweet et al. (2014) found persistent (1 - 12 months) decreases in ICAM-1 after ≥ 10 cGy whole-body irradiation of mice with 1 GeV/n protons with a pronounced sex difference; specifically, females were sensitive while males were not. Rosi et al. (2008) and York et al. (2012) found significant increases in activated microglia numbers (x-rays and protons) that were correlated with reductions in behaviorally-induced gene expression. Poulose et al. (2011) observed astrocyte activation (rat hippocampus) after ^{16}O exposure accompanied by altered neurotrophic factor signaling, and Sanchez et al. (2010) reported reductions in cultured human astrocyte glutamate transport following ^{1}H, ^{12}C, and ^{56}Fe irradiation. Kempf et al. (2014b) quantified the number of CD11b+ cells in the hippocampus and found increases of 49 - 62% depending on the field 6-7 months after 1 Gy of ^{60}Co γ-rays. These changes were accompanied by increased hippocampal transcription and translation of TNFα after 1.0 Gy and a 69-82% elevation of GFAP+ astrocytes in the hilus at doses ≥ 10 cGy. CCR2-/- knockout mice (involved in monocyte activation and trafficking) were resistant to 10 Gy head-only ^{137}Cs γ-ray-induced decrements in cognitive and memory behaviors (hippocampus-dependent Morris water maze but not hippocampus-independent short delay novel object recognition, see below), behaviorally-induced Arc gene expression, and neurogenesis (Belarbi et al. 2012). These results highlight a role of CCR2 signaling in radiation-induced cognitive impairment and inflammation.

The neuroinflammatory response to radiation is not brain-autonomous. Body-only irradiation can elicit the production of pro-inflammatory cytokines in the brain in a process mediated by the vagus nerve. Thus, Marquette et al. (2003) showed that 15 Gy ^{60}Co-γ to rats elicited IL-1β, TNF-α, and IL-6 production 6 hrs post-irradiation in the thalamus, hypothalamus, and hippocampus; vagotomy abrogated this response. Inflammation generated by peripheral lipopolysaccharide administration modified ^{56}Fe-induced electrophysiological responses with a complex time course (Vlkolinský et al. 2008, 2007). Taken together, these findings suggest that radiation-induced inflammatory processes may have a causal role in CNS dysfunction, affecting many component cell types and processes and developing with a complex time course.

e. Microvascular Changes

Late necrotic brain tissue damage after high radiation doses is well known to be associated with damage to the vascular system (Lyubimova et al. 2004), but limited evidence now suggests that low doses of charged particles also disrupt vascular structure and function. Yang and Tobias (1984) observed petechial hemorrhages on the cortical surfaces of 1.5-day-old rat neonates 24 hrs post-irradiation using 670 MeV/n Ne, 600 MeV/n Fe, and 225 kVp X-rays at doses from 0.5 to 8 Gy and calculated an RBE of 1.4 - 2.1. Recently, Mao et al. (2010) demonstrated substantial (34%) microvessel loss at 9-12 months (with later recovery) in the mouse hippocampus after 0.5 - 2 Gy of ^{1}H or ^{56}Fe exposure. The CA1 region was markedly more sensitive than the DG, possibly reflecting the greater vessel connectivity in the DG. In rat retinas, vessel loss was linear with time, with rates that increased with proton dose above 8 Gy (Archambeau et al. 2000). When male C57BL mice were brain-only irradiated with 20 Gy of 6 MV photons and scored at 3 - 120 days post-irradiation, reductions in the number of anatomical vessels and perfused vessels were observed from 3 to 7 days with later recovery; measures of hypoxia accompanied the reduction of perfused vessels, and effects were reduced by blocking of TNF-α (Ansari et al. 2007).

Monolayers and 3D cultures of human umbilical cord endothelial cells (HUVECs) and cultured human brain microvascular endothelial cells were irradiated with 10 to 75 cGy of 1 GeV/n ^{56}Fe and evaluated for maintenance of cell layer integrity for up to 72 hrs post-irradiation (Grabham et al. 2013; Sharma et al. 2014). Transendothelial electrical resistance was progressively compromised after ≥ 50 cGy, accompanied by permeability to fluorescent dextrans (3 and 10 kDa). Tight junction (ZO-1 immunofluorescence) breakdown occurred in both 2D and 3D cultures of HUVEC cells. These observations suggest that impaired perfusion, hypoxic conditions, and loss of the blood brain barrier may ensue from space-like radiation exposures.

f. Neuronal and Brain Tissue Structural Changes

The topology of neuronal networks and structural plasticity are important regulators of cognitive performance, as they control synapse number, strength, and organization. Recent neuronal morphometry investigations using Golgi silver stain in mice and rats and fluorescence microscopy of transgenic mice expressing enhanced green fluorescent protein (EGFP) in neurons have demonstrated that γ-rays, protons, and ^{56}Fe radiation cause reductions in hippocampal neuron arborization (>50% at 30 days) as well as loss of dendritic spines, each of which would limit the complexity of signal processing (Chakraborti et al. 2012; Parihar et al. 2013; Quasem et al. 2007). Parihar et al. (2014, 2015b) further showed reduction of dendritic complexity 10 and 30 days after 1 Gy of 250 MeV protons and spine reductions at ≥ 10 cGy. Immature filopodial spines were more sensitive than stubby or mushroom-shaped spines. The presynaptic marker synaptophysin was reduced in these tissue samples, while the post-synaptic marker PSD-95 was elevated. Most recently, investigations have shown that 600 MeV/n ^{16}O and ^{48}Ti ions at doses of 30 cGy can cause ~30% reductions in dendritic length and branching parameters 8 weeks post-irradiation in the median prefrontal cortex (mPFC), an area associated with executive functions (Parihar et al. 2015a). The number of dendritic spines was also significantly reduced at 5 and 30 cGy (see Figure 2, below), but neurons exhibited ~60% increased postsynaptic PSD-95 levels in the mPFC, perhaps a compensatory change. Notably, spine density correlated with cognitive performance using Novel Object and Object in Place paradigms. High-dose experiments with primary rat hippocampal neurons exposed to 30+ Gy of 140 kVp X-rays and immediately fixed indicated that structural modifications can be quite rapid (< 30 minutes). Reductions of

filopodial spines were the most salient feature, accompanied by F-actin and drebrin puncta reductions and increases in PSD-95 (Shirai et al. 2013).

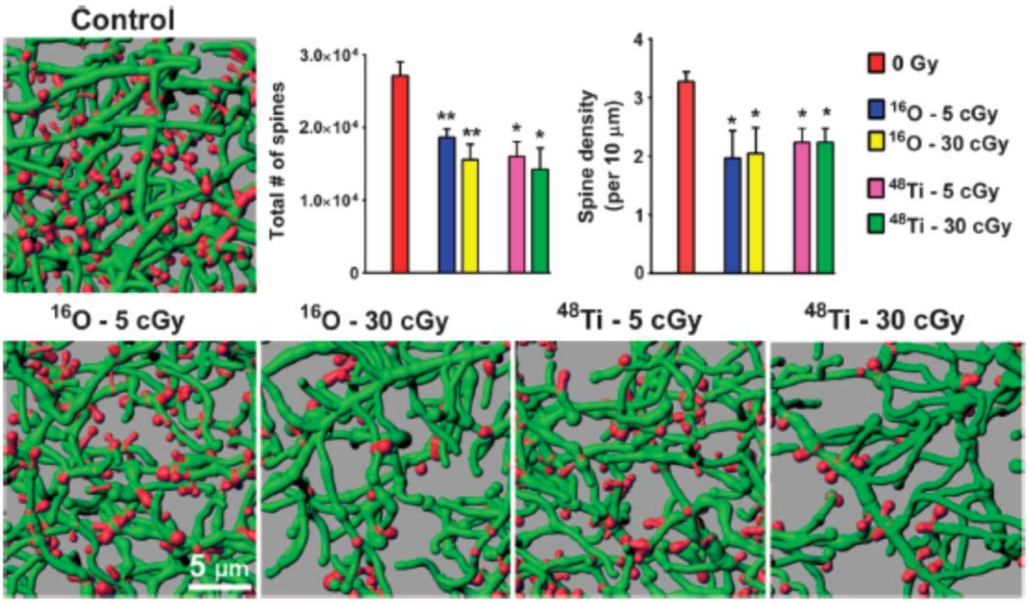

Fig. 3. Reductions in dendritic spine density in the mPFC after HZE particle exposure. Representative digital images of 3D reconstructed dendritic segments (green) containing spines (red) in unirradiated (top left panel) and irradiated (bottom panels) brains. Dendritic spine number (left bar chart) and density (right bar chart) are quantified in charged particle–exposed animals 8 weeks after exposure. *P = 0.05, **P = 0.01, ANOVA.

Figure 2. The figure above, reproduced from Parihar et al. (2015a) illustrates dendritic spine reduction after low doses of ^{16}O and ^{48}Ti ions.

Axonal processes are also damaged by radiation. In chick embryo dorsal root ganglion explants (peripheral neurons), up to 70% growth cone collapse was elicited 48 hrs post-irradiation by 10-Gy exposures to 200 kVp X-rays (Al-Jahdari et al. 2008). Collapse first became significant after 12 hrs with 5 Gy exposures. In chick embryo retinal explants (CNS neurons), Vazquez and Kirk (2000) demonstrated that neuritogenesis was inhibited in a dose-dependent manner after exposure to 1000 MeV/n ^{56}Fe ions.

In mouse hippocampal neuronal HT22 cells irradiated with 0.5 - 4 Gy of ^{137}Cs γ-rays, proteomic analysis at 4 and 24 hrs post-irradiation indicated that signaling pathways related to synaptic actin-remodeling were significantly affected at 1.0 and 4.0 Gy but not at 0.5 Gy (Kempf et al. 2014a). The decreased expression of miR-132 and Rac1 was associated with an increase in hippocampal cofilin and phospho-cofilin, which control synaptic actin filament formation in spines and synapses. Similar findings were observed *in vivo* at 24 hrs after 1 Gy ^{137}Cs γ-ray irradiation of 10-day-old NMRI mice. Pathways associated with Rho family GTPases (key regulators of spine and synapse morphology) were all perturbed by irradiation and, overall, the pathways shared several proteins, such as Rac1, PAK, LIMK, and cofilin, which all are constituents of the Rac1-Cofilin pathway. The results suggest that a Rho/Rac1/Cofilin-based mechanism may underlie spine and dendrite remodeling observed post-irradiation. Notably, cofilin organizes surface receptor complexes in the "immunological synapse". Its activity may polymerize or depolymerize

actin depending on the availability of G-actin, and cofilin activity is under direct redox control, which implicates it in oxidative disturbances of actin dynamics (Samstag et al. 2013). This may render it acutely sensitive to radiation or to persistent oxidative stress.

Subtle widespread remodeling of brain structures after low-dose irradiation may also occur. Thus, MRI imaging of ^{60}Co γ-ray and ^{56}Fe-irradiated rat and mouse brains shows dynamic changes in apparent diffusion constants and T1/T2 relaxation times in multiple regions, suggesting microscopic tissue structure changes. Complementary MR spectroscopy showed alterations in levels of several neuronal damage markers (Huang et al. 2010, 2009; Kumar et al. 2013; Obenaus et al. 2008). In a study by de Guzman et al. (2015), 16 - 36-day-old C57Bl/6 mice were irradiated head only with 3 - 7 Gy of ^{137}Cs γ-rays. Brains were imaged by high-resolution MRI, and regional volume decrements were mapped as biomarkers of radiosensitivity. Results showed that age, dose, and region-dependent anatomical alterations in brain development occurred and were consistent with human pediatric patient neurocognitive outcomes. Notably, the hippocampus and olfactory bulb were the most sensitive at all ages. Newly initiated studies by C. Lemere and colleagues at Brigham and Women's Hospital in Boston are applying PET imaging to understanding late neurodegeneration following charged particles. Such approaches might be used to monitor astronauts pre- and post-flight.

g. Electrophysiology

Early studies through the 1960s using X-rays and gamma rays showed that the conduction velocities and total conduction block of compound action potentials in peripheral nerves of frogs and rats were very resistant to radiation, while implanted electrodes in rabbits and rats detected altered frequencies and amplitudes of spontaneous spike trains in many brain regions after < 5 Gy (Ordy et al. 1968). Later, *in vitro* experiments suggested that spontaneous discharges of hippocampal neurons could be induced by x- and γ-rays at as little as 8 cGy (Peimer et al. 1986, Mickley et al. 1989). Pellmar demonstrated that synaptic efficacy (dendritic response) and population spikes (somatic response) were modified acutely in guinea pig brain slices after photon doses above 30 Gy (Pellmar et al. 1990, 1993). Finally, Clatworthy et al. (1999) demonstrated that 5 - 15 Gy of ^{137}Cs γ-rays induced changes in excitability of *Aplysia* sensory neurons after 48 hrs. Together, these observations suggested that intrinsic nerve properties were resistant but that synapses might be sensitive targets. Recent electrophysiological experiments with low doses of charged particles have now explored neuronal functional responses over periods from weeks to 1.5 years post-irradiation and revealed that both intrinsic properties and synaptic parameters change. The principal model being used is the rodent acute brain slice (usually containing the hippocampal field). In this preparation, freshly isolated 300 - 400-micron-thick slices of tissue from irradiated animals are kept in oxygenated, glucose-supplemented, artificial cerebrospinal fluid, and pairs of stimulating and recording electrodes (or microelectrode arrays) record from ensembles of several hundred neurons, (field recordings) or, alternatively, single neurons are targeted with microelectrodes (patch clamp recordings).

In the intact neuronal networks of mouse hippocampal slices, stimulation of fields of axons from CA3-area neurons (Schaeffer collaterals) results in transmission of signals to CA1 field pyramidal cells in which recordings from either dendritic regions or cell soma regions are conducted. In such extracellular field recordings, synaptic transmission is found to be altered by ^{1}H, ^{28}Si, and ^{56}Fe exposure with a complex dose and ion species pattern. Input-output curves (excitability), pre-pulse facilitation (presynaptic glutamate release), and paired pulse inhibition (recurrent inhibitory transmission) measurements have assessed synaptic coupling of axons to

dendrites and short-term synapse strengthening following stimulation. In both the CA1 and DG fields, synaptic excitability is modified by accelerated ion exposure at doses as low as 0.1 Gy in a brain region and ion-specific way. Long-term potentiation (LTP), a tissue-level model of memory formation, was used to assess stimulation-induced synaptic strengthening and also exhibited hippocampus field-, dose-, and ion-specific modulation consistent with dysregulation of the balance between excitatory and inhibitory activity post-irradiation (Vlkolinský et al. 2008, 2007). Figure 3 illustrates reduction of LTP after ^{56}Fe-particle irradiation.

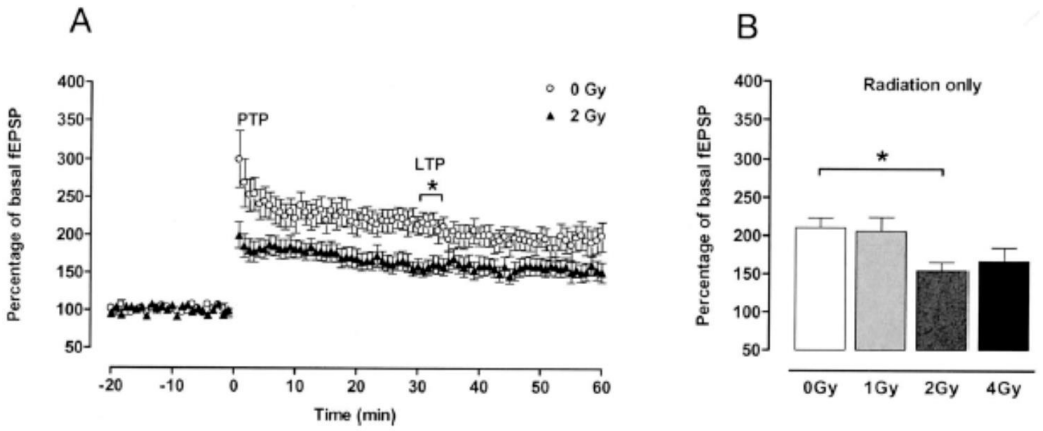

Figure 3. Reproduced from Figure 3 of Vlkolinsky et al. (2007). Effect of ^{56}Fe-particle radiation on synaptic plasticity. Panel A: In slices from control mice, high-frequency stimulation induced prominent LTP of the dendritic fEPSP slope. The early phase of the fEPSP enhancement is post-tetanic potentiation (PTP); the later phase is LTP. Compared to nonirradiated controls, the dose of 2 Gy had a significant inhibitory effect on the magnitude of LTP (one-way ANOVA, $P < 0.05$). Panel B: While LTP in the 2-Gy group was significantly reduced, significant changes were not observed in the 1- and 4-Gy groups.

Experiments with 25 and 100 cGy of 600 MeV/n ^{28}Si ions in C57Bl/6J mice demonstrated an interaction between cognitive testing (contextual freezing) and radiation (Raber et al. 2014). ^{28}Si radiation enhanced LTP at 25 and 100 cGy in the dorsal hippocampus. Behavioral training also enhanced LTP and further potentiated the radiation response at 25 cGy but not 100 cGy, which matched the inverted U-shaped dose response for the behavior. Rudobeck et al. (2014) examined the effects of 25 and 100 cGy of ^{28}Si radiation on the ventral hippocampus of C57Bl/6J mice (previous work was performed on the dorsal hippocampus). Extracellular recordings of excitatory postsynaptic potentials (EPSPs) and population spikes showed prominent decrements in population spike amplitudes and reduced maximal neuronal output without changes in dendritic field EPSPs. Such reduced EPSP-spike coupling is a novel finding suggesting impaired information transfer.

Reduced presynaptic glutamate release and decreased abundance of glutamate receptors in purified rat synaptosomes after ^{56}Fe exposure supports both the pre-pulse facilitation and LTP observations and implicates post-synaptic remodeling (Machida et al. 2010). Thus, both intrinsic properties as well as the dynamic remodeling and strengthening of synapses are sensitive to charged particles in a brain region-, cell type-, and radiation species-specific pattern, which predicts inappropriate signal processing and behavior.

In a different model, motivated by cosmic ray-induced light flash observations in astronauts, Sannita et al. (2007) showed that pulses of ^{12}C ions were able to generate prompt electroretinogram and visual cortex signals in irradiated mice, suggesting direct depolarization of neurons from particle traversals.

Patch clamp studies were conducted on single neurons in acute C57Bl/6J mouse hippocampal slices following irradiation with protons at 0.1 to 1 Gy. The data revealed that at 90 days post-irradiation, 1-Gy exposures significantly hyperpolarized cell resting membrane potentials (V_{RMP}) by ~4 mV, decreased input resistance (R_{in}) by ~22 MΩ (megaOhm), upregulated persistent sodium current (I_{NaP}), and increased the rate of miniature excitatory post-synaptic currents (mEPSC), indicating a general reduction in pyramidal neuron excitability in the CA1 (Sokolova, et al. 2015). These small alterations in passive membrane properties had a dramatic impact on network function in a computational model of the CA1 microcircuit, leading to a 50% decrease in rhythmic theta oscillation power at the 4-Hz peak frequency (see below under modeling).

In the DG, characterized by enhanced inhibitory tone compared to CA1, patch clamp studies focused on inhibitory neurotransmission in DG granule cells in mice 90 days after exposure to 150 MeV ^{1}H, 600 MeV/n ^{28}Si, and 600 MeV/n ^{56}Fe ions at 0.1 to 1 Gy (Marty et al. 2014). Proton exposure (10, 50, 100 cGy) increased synaptic excitability with a dose-dependent decrease in amplitude and charge transfer of miniature inhibitory post-synaptic currents (mIPSCs), but no changes were detected in the expression of $GABA_A$ receptor subunits a2, b3, or c2. Field recordings using a microelectrode array also indicated a dose-dependent increase in granule cell excitability. Exposure to Si ions (25 and 100 cGy) had no significant effects on synaptic excitability, mEPSCs, or mIPSCs. Fe ion exposure (25 and 100 cGy) had no effect on synaptic excitability and mIPSCs but significantly increased mEPSC frequency at 1 Gy, without changes in mEPSC kinetics, suggesting a presynaptic mechanism. Together, these findings illustrate the ion and tissue field specificity of the radiation responses and suggest that preferential radiation-induced impairment of inhibitory activity leads to increased overall excitability in the DG.

h. Molecular Marker Changes

Altered gene expression in brain tissue has been shown to be dose-, dose rate-, and radiation species-dependent. In mice, ^{1}H exposures altered neurotrophin and receptor-signaling pathway-related gene expression changes in the hippocampus (Chang et al. 2010). Brains from mice exposed to protons also showed dysregulation of miRNAs (Khan et al. 2013), highlighting a role for epigenetic regulation. With gamma rays, Lowe et al. (2009, 2012) found that low doses primarily altered expression of genes regulating ion channels, synaptic plasticity, and vascular damage, while high-dose responses affected oxidative stress and amyloid processing genes. They also have shown alterations in choroid plexus and cerebrospinal fluid components such as transthyretin which serves a chaperone function in amyloid protein removal and suggest the use of calcium regulator troponin T1 (Tnnt1) as a useful biomarker for radiation exposure. Unbiased proteomic analysis of γ-ray-irradiated mouse brains showed changes in 6% of 997 peptides (Lim et al. 2011), and proteomic data from ^{56}Fe-irradiated rats have been analyzed in serum and brain (Britten 2010, 2014) and proteomic signatures associated with high and low performance scores have been identified. These data and others are being scrutinized for applicability to biomarkers that might be obtained from astronauts to monitor potential pathologies (Straume et al. 2008).

Brain acetylcholine metabolism changes have been detected after ≤ 0.24 Gy of β irradiation (Egana 1962), while in rat brains, tyrosine hydroxylase levels (dopamine pathway) are unaffected

by ^{56}Fe irradiation (Rice et al. 2009) and neural cell adhesion molecule (NCAM; a synaptic plasticity regulator) is down-regulated (Casadesus et al. 2005). Casadesus et al. (2005) also demonstrated changes in the microenvironment associated with HZE-induced neurodegeneration as shown in Figure 4. It was noted that the observed changes are similar to those found in aged animals, suggesting that irradiation responses may share pathways with those of aging.

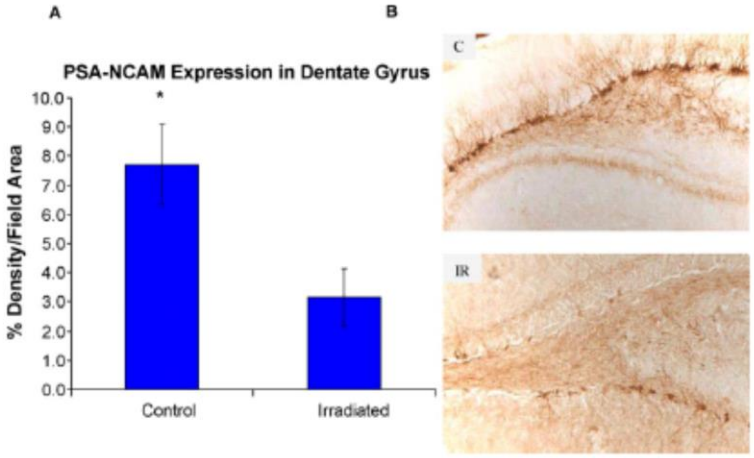

Figure 4. Reproduced from Casadesus et al. (2005). (Panel A) Expression of polysialylated isoforms of the neural cell adhesion molecule (PSA-NCAM) in the hippocampus of rats irradiated (IR) with 2.5 Gy of ^{56}Fe high-energy radiation and control (C) subjects as measured by % density/field area measured. (Panel B) PSA-NCAM staining in the dentate gyrus of representative irradiated (IR) and control rats.

In a study by Kempf et al. (2014b), 10-day-old NMRI albino mice were whole-body irradiated with 2 - 100 cGy of ^{60}Co γ-rays and analyzed 6 - 7 months post-irradiation with respect to the proteome, transcriptome, and several miRNAs in the cortex and hippocampus. Signaling pathways related to synaptic actin remodeling, such as the Rac1-Cofilin pathway, were altered after ≥ 50 cGy in the cortex and hippocampus, while MAP-2 and PSD-95 were elevated at 100 cGy. Synaptic plasticity genes Arc, c-Fos, and CREB were reduced at 1.0 Gy, coupled with increased levels of the associated microRNAs miR-132/miR-212 and miR-134. NMDA, AMPA, and metabotropic glutamate receptor levels were also decreased after 1 Gy. These changes at 6-7 months were preceded at 2 months post-irradiation by impairments in open field behavior at doses ≥ 50 cGy. Below, figure 5 illustrates an excerpt from figure 2 of Kempf et al. (2014b) shows changes in signaling pathways related to synaptic structure and plasticity 6 - 7 months post-irradiation.

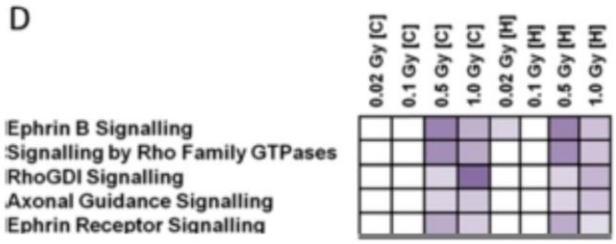

Figure 5. (Excerpt from Figure 2 in Kempf et al. 2014b). Analysis of signaling pathways from proteomic experiments. Associated signaling pathways of all dose-dependent significantly deregulated proteins using the Ingenuity Pathway Analysis (IPA) software are shown in panel D. Higher color intensity represents higher significance (p-value), whereas all colored boxes have a p-value of ≤ 0.05; white boxes have a p-value of > 0.05 and are not significant. Hippocampal and cortical data result from four and five biological replicates, respectively. H: Hippocampus, C: Cortex.

Glutamate levels in the brain are controlled by astrocytes whose specific uptake mechanisms prevent excessive buildup in the intercellular space which can lead to excitotoxicity. Sanchez et al. (2009, 2010) found that radiation alters the levels of several glutamate transporters in cultured astrocytes, neurons and mixed cultures of human hNT2 cells differentiated with retinoic acid.

The behaviorally-induced immediate early gene Arc was investigated by Rosi et al. (2008, 2010) and Raber et al. (2013) for its expression in the dentate gyrus of mice. Both messenger RNA and protein levels in neurons showed behaviorally-induced upregulation which was inhibited by exposure to X-rays and low doses of ^{56}Fe ions.

When adult rats were exposed to fractionated 40 Gy whole-brain ^{137}Cs-γ irradiation, the protein Homer1a was temporarily (at 48 hrs) up-regulated in the hippocampus but down-regulated in the cortex. Homer1a is a protein under the control of the radiation-inducible ERK signaling pathway and binds to postsynaptic, G-protein coupled, metabotropic glutamate family 1 receptors (mGluR1), which modulate NMDA receptors and are linked to cognition. Two months later, the early changes correlated with decreases in hippocampal mGluR1 and increases in cortical mGluR1, suggesting that the ERK signaling pathway may function through Homer 1a to influence cognitive processes through glutamate receptors (Moore et al. 2014).

Genotoxic changes are also seen in the brain. Chang et al. (2007) found a persistently elevated lacZ transgene mutation frequency in the brains of mice irradiated with ^{1}H and ^{56}Fe, and there was a suggestion of clonal expansion, which may implicate the neurogenic cell population as preferential targets. Zhang et al. (2015) measured mRNA levels of Rad9, Rad1, and Hus1 DNA repair genes in 129 strain mouse tissues 2 - 48 hrs after 10 Gy ^{60}Co γ irradiation. They found that Rad-1 was unresponsive but Rad-9 and Hus-1 were transiently 8- and 145-fold greater, respectively, at 2 hrs and 12- and 4-fold greater at 12 hrs, illustrating a highly dynamic DNA 9-1-1 repair pathway response. Finally, head-only irradiation of mice with x-rays led to out-of-field genotoxic effects and altered methylation in the spleen (Koturbash et al. 2008), while a combined cranial γ-ray and ^{12}C ion protocol showed both adaptive and out-of-field responses in mice for reductions in reproductive pituitary hormones, testis weight, and sperm count (Zhang et al. 2006).

i. Loss of Autophagy

In a series of studies conducted over many years, Rabin and co-workers showed that even though exposure to HZE particles occurs at low fluence rates, the cumulative effects of long-term exposure result in molecular changes similar to those seen in aged animals. Recently, they assessed (Poulose et al. 2011) markers of autophagy, a dynamic process for intracellular degradation and recycling of toxic proteins and organelles (associated with neurodegenerative processes), stress, and inflammatory responses, in the brains of Sprague-Dawley rats irradiated at 2 months of age with 5, 50, and 100 cGy of 1000 MeV/n ^{16}O particles. Exposure to ^{16}O particles significantly inhibited autophagy function in the hippocampus as measured by ubiquitin inclusion bodies (P62/SQSTM1) and autophagosome markers (MAP1B-LC3, beclin1, and mTOR). The changes also

correlated with protein kinase Cα, nuclear factor kappa B (NF-κB), and GFAP, indicating glial cell activation 75 days after exposure, indicative of oxidative stress and inflammation.

3. Behavioral Effects

a. Overall Observations

While many molecular, structural, and functional alterations in CNS activity can be quantified after low doses of radiation, the complexity of the brain, its redundancy, its distributed processing, and its capacity for adaptation may work together to compensate for damage to structures or disruption of processes. Therefore, it is important to assess CNS responses at the system level by behavioral testing to determine whether function has been altered by the interplay of contributing responses. Behavioral effects are difficult to quantify, and it is well established that behavioral outcomes are dependent on the animal species, strain, age, sex, and assessment method used (Buckner and Wheeler 2001). For example, spatial learning and memory tests, such as the Barnes maze and Morris water maze, may be more or less reliable in mice versus rats (Raber et al. 2004; Shukitt-Hale et al. 2003, 2000). The age at evaluation and irradiation affects the responses to charged particles (Rabin et al. 2012) and X-rays (Forbes et al. 2014). Sex and genotype (e.g., ApoE allele and ATM) are important variables (Acevedo et al. 2008; Benice and Raber 2009; Haley et al. 2012; Higuchi et al. 2002; Villasana et al. 2006, 2010, 2011; Yamamoto et al. 2011; Yeiser et al. 2013; Johnson et al. 2014; Parihar et al. 2014). Additionally, observations comparing head only-, body only-, or whole body-irradiated animals demonstrate a significant role for the periphery in determining behavioral responses (Rabin et al. 2014). Finally, extrapolation of animal behaviors to humans is a challenging task due to the lack of human data, differences in functions of different brain regions, and vast differences in abilities, but some behavioral test analogs exist, such as the Novel Image Novel Location test (Raber 2015) and the Psychomotor Vigilance Test (Davis et al. 2014). Methods to estimate risk must take these considerations into account. Despite these cautions, published studies now provide convincing evidence that space radiation does affect the behavior of animals in a complex manner dependent on dose and radiation quality.

Overall, whole-body or head-only irradiation reliably elicits quantifiable behavioral impairments in rodents at doses ≥ 50 cGy, which may appear acutely or develop over many months. With the caveat that brain functions are not strictly localized to specific anatomical regions, most observations to date have interrogated hippocampus-dependent memory, cortex-dependent executive function and cognition, and amygdala-dependent anxiety and fear. Recent experiments have detected behavioral changes at doses < 50 cGy and, in some cases, below 5 cGy. Most tests have been performed on irradiated young adult inbred animals tested after 1 - 3 months. The most commonly employed tests include the Morris water maze and Barnes maze (Britten et al. 2012; Villasana et al. 2010), novel object recognition, object in place recognition, (Casadesus et al. 2004; Kumar et al. 2013; Shukitt-Hale et al. 2000; Tseng et al. 2013), and contextual fear conditioning (Raber 2013, 2011) for hippocampus-dependent memory (especially spatial memory) but with strong associations with the cortex as well. Cognitive behaviors more closely associated with the frontal cortex include operant conditioning (Rabin et al. 2007; Rice et al. 2009), attentional set shifting (Britten et al. 2014; Lonart et al. 2012), and psychomotor vigilance tests (Heinz et al. 2008; Davis et al. 2014). Anxiety and fear are commonly assessed with open field tests and elevated plus or zero mazes (Kumar et al. 2013). Many other tests have been employed as well, such as acoustic startle (Haerich et al. 2005).

Radiation types investigated to date are X-rays, gamma rays, electrons, and charged particles, including $^{1}H^{+}$, $^{12}C^{6+}$, $^{16}O^{8+}$, $^{28}Si^{14+}$, $^{48}Ti^{20+}$, and $^{56}Fe^{26+}$, with energies from 150 MeV/n to 5 GeV/n. Dose responses have been described as linear or non-linear (e.g., U-shaped), and responses elicited by different ions may be opposing, which presents problems for estimating the effects of multiple ion exposures and interpreting RBE values. Clear patterns for the dependence on LET remain elusive. Dose responses utilizing mixed fields, such as those planned for the GCR simulator at NSRL, will be important in evaluating behavioral responses relevant to space radiation exposures. Selected observations from a variety of experiments utilizing behavioral testing are presented below.

b. Sensorimotor Tests

Sensorimotor deficits and neurochemical changes were observed in rats exposed to low doses of 1 GeV/n ^{56}Fe (Joseph et al. 1993, 1992). Doses below 1 Gy were able to reduce performance on the wire suspension test. Changes occurred as early as 3 days after radiation exposure and lasted up to 8 months. A negative result was reported by Pecaut et al. (2004), where no behavioral effects were seen in female C57BL/6 mice 2 to 8 weeks after exposure to 0 - 200 cGy of 1 GeV/n ^{56}Fe as measured by open-field, rotorod, or acoustic startle habituation.

c. Conditioned Taste Aversion

There is evidence that deficits in conditioned taste aversion (CTA) are induced by very low doses of heavy ions (Hunt et al. 1989; Rabin et al. 1989, 1991, 1994, 2000). The CTA test is a classical conditioning analysis that assesses avoidance behavior that occurs when ingestion of a normally acceptable food item is associated with illness (Riley and Tuck 1985). CTA involves the amygdala and insular cortex, dopaminergic, cholinergic, and glutamatergic neurotransmitters, as well as the expression of MAP kinase and CREB signaling pathways. It was established that the effects of radiation on CTA in Sprague-Dawley rats is somewhat LET-dependent and that ^{56}Fe ions are the most effective of the various low- and high-LET radiation types that have been tested (Rabin et al. 1989, 1991). Doses as low as 20 cGy of ^{56}Fe ions can impair CTA. Attempts to establish an RBE (detection threshold dose) vs. LET relationship by comparing ^{56}Fe, ^{48}Ti, and ^{28}Si particles of different energies suggest that the RBE of different particles for behavioral dysfunction cannot be predicted from LET alone (Rabin et al. 2007).

d. Operant Conditioning

Operant conditioning tests measure the effect of motivation and responsiveness to environmental stimuli in modifying voluntary behaviors. Studies by Rabin et al. (1994, 2003, 2005, 2011a, 2011b) examined the ability of rats to perform "an operant order" to obtain food reinforcement using an ascending fixed-ratio schedule (FR); i.e., rats were trained to press a lever an ever-increasing number of times to obtain a food pellet. The behavior is associated with the striatum and dopaminergic system of the brain. Detection limits for ^{56}Fe, ^{48}Ti, and ^{28}Si particles of energies from 600 MeV/n to 1000 MeV varied from 25 to 200 cGy, with lower energy particles tending to be more effective. When male rats were exposed to 25–200 cGy of 1 GeV/n ^{56}Fe particles at 25 - 200 cGy at ages of 2 - 16 months and evaluated 2 - 4 months later, the results showed that older rats exhibited a performance decrement compared to younger rats (Rabin et al.

2012). When 8-week-old rats were whole-body- or partial-body-irradiated with 1 - 25 cGy of 1 GeV/n ^{16}O ions and tested 8 weeks later, provocative and controversial differences were reported (Rabin et al. 2011b, 2014). While head-only irradiation significantly impaired behavior at 1 cGy only, whole-body exposed animals were impaired at all doses, as seen below in Figure 6, and body-only exposures exhibited intermediate effects. This is the lowest effective dose reported to date for behavioral effects and draws attention to the interactions between the CNS and soma.

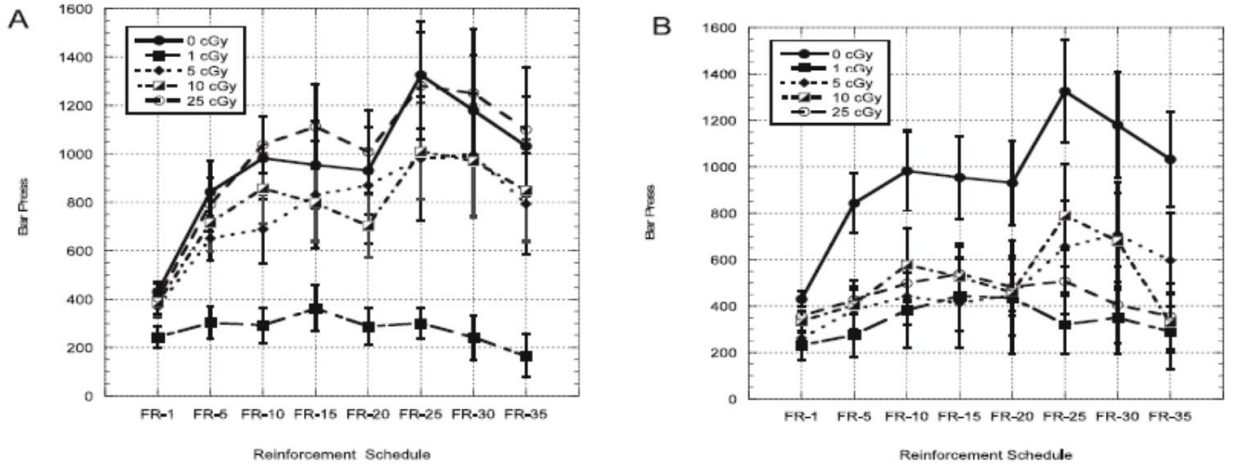

Figure 6. Effects of partial-/whole-body exposure to ^{16}O particles on operant responding on an ascending fixed-ratio schedule. Mean ± standard error of the mean (SEM). Panel A: Head-only exposure; panel B: whole-body exposure. [Panels C & D not shown]. Excerpt of Figure 3 reproduced from Rabin et al. (2014).

e. Learning and Memory

Spatial learning and memory behaviors have been the most widely used tests to probe the effects of charged particle exposure on behavior and have sometimes proven to be the most sensitive. Mazes are often used to assess hippocampus-dependent spatial memory, as they require animals to learn to find an escape location (which may remain in one location or be moved) by referencing distant visual cues. Water mazes and Barnes mazes both have an element of fear motivation from being in water or in a bright and sometimes noisy location.

Studies with young Sprague-Dawley rats using the Morris water maze were among the first and examined effects of whole-body irradiation with 1.5 Gy of 1 GeV/n ^{56}Fe ions 1 month post-irradiation. In this test, animals must locate and remember the position of a submerged platform. Irradiated rats demonstrated cognitive impairment analogous to decrements observed in aged Fischer rats, leading to the suggestion that increased oxidative stress may be responsible for the induction of both radiation- and age-related cognitive deficits (Shukitt-Hale et al. 2000). Denisova et al. (2002) also exposed rats to 1.5 Gy of 1 GeV/n ^{56}Fe ions and tested their spatial memory in an eight-arm radial maze. Radiation exposure impaired the rats' cognition, leading to more errors than those made by control rats, and the animals were unable to adopt a spatial strategy to solve the maze. These findings were reproduced by Raber and others as well at somewhat lower doses (Raber et al. 2004; Villasana et al. 2011).

An alternative maze design is the Barnes maze, in which animals on a brightly-lit circular platform must learn the position of a single dark escape hole located at the periphery of the field

containing 40 or more false holes (Britten et al. 2012; Villasana et al. 2010). Britten et al. (2012) used young male Wistar rats exposed head-only to 20 – 60 cGy of 1 GeV/n ^{56}Fe ions or 8 – 13 Gy 125 kVp X rays and tested 3 months later for spatial memory performance in the Barnes maze. Results showed that escape latency time in the Barnes maze was increased (impaired performance) after ≥ 20 cGy of high-LET iron particles but only after > 10 Gy but ≤ 13 Gy of low-LET X-rays (see Figures 7 and -8). The authors suggest that an RBE of ~50 may apply to the threshold for observing impairments and is unlikely to involve significant cell killing.

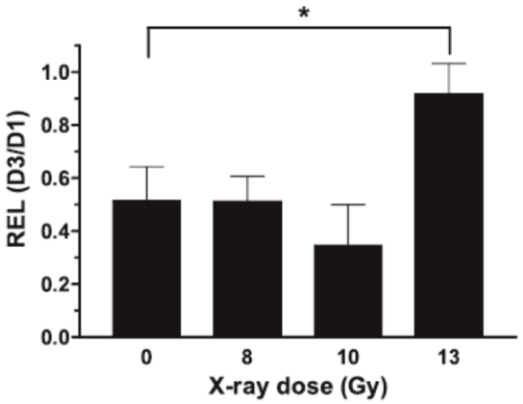

Fig. 7 X-ray response.

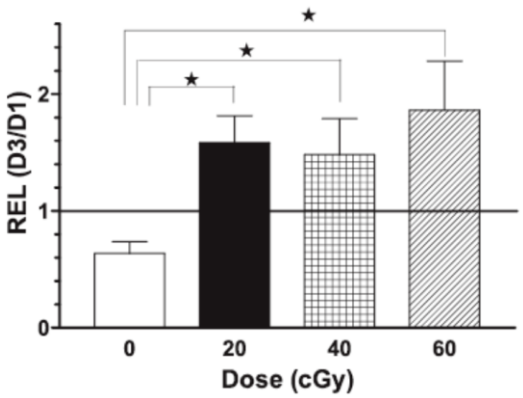

Fig. 8 Fe ion response.

Figure 7. [Left] Effect of X radiation on the relative escape latency. Figure shows the relative escape latency time (day 3/day 1 escape latency times), REL(D3/D1), of rats exposed to 0, 8, 10, and 13 Gy of X rays. Values are means ± SEM. *P < 0.05 compared to the unirradiated population, analyzed by two-tailed Mann-Whitney test. Reproduced from Figure 1 of Britten et al. (2012).

Figure 8. [Right] Effect of 1 GeV/u ^{56}Fe-particle radiation on the relative escape latency. Figure shows the relative escape latency time (day 3/day 1 escape latency times), REL(D3/D1), of rats exposed to 0 (open bar), 20 (solid bar), 40 (cross-hatched bar), and 60 (diagonally hatched bar) cGy of 1 GeV/u ^{56}Fe particles. Values are means ± SEM. *P < 0.05 compared to the unirradiated population, analyzed by the two-tailed Mann-Whitney test. Reproduced from Figure 2 of Britten et al. (2012).

Another design that uses both fear motivation and elements of spatial memory is contextual fear conditioning, in which animals are trained to anticipate a foot shock in one spatial environment coupled to a sound cue. They are then placed in either the same or a different spatial environment ± the sound cue to test their association of the cue and environmental references. Whole-body irradiation of C57Bl/6 mice with 50 or 100 cGy 600 MeV/n ^{56}Fe irradiation resulted in impaired contextual but not cued fear freezing, which correlated (cued fear freezing) with expression of the behaviorally-induced immediate early gene, Arc, in the dentate gyrus (Raber et al. 2013). Similar tests run with 600 MeV/n ^{28}Si ions elicited an enhancement of contextual fear freezing at 25 cGy but not 100 cGy - evidence of an inverted U-shaped dose response (Raber et al. 2014). When 6-7-month-old B6D2F1 female and male mice were irradiated with 20 - 160 cGy of 1 GeV/n protons, 263 MeV/n ^{28}Si ions, or 1 GeV/n ^{48}Ti ions and tested for contextual and cued freezing after 3 months, no effects were observed for protons or ^{48}Ti, but ^{28}Si-irradiated mice were impaired in

contextual freezing with 160 cGy of ^{28}Si (Raber et al. 2015). This contrasts with the enhancement of freezing observed with 25 cGy Si in C57Bl/6 mice and illustrates contributions of strain and particle type on cognitive outcome measures. Sweet et al. (2014) irradiated C57Bl/6 mice with 0 - 200 cGy of 1 GeV protons and did not observe contextual fear changes out to 12 months.

f. Novel Object Recognition

In the novel object recognition (NOR) task, an animal is placed in an open field with 2 (or more) objects whose position and features it learns. The animal is removed and placed back in the "arena" in which one object has been replaced with another (previously shown to elicit equal interest as the first). Rodents normally spend more time exploring the novel object, and the proportion of time spent exploring the new object divided by the total object exploration time is used as the discrimination index. Haley et al. (2013) studied the effects of ^{56}Fe particles on hippocampal function in male and female C57Bl/6J mice irradiated with 10 – 50 cGy of 600 MeV/n ^{56}Fe ions and tested those 2 weeks later. Compared to sham irradiation, radiation impaired novel object recognition and spatial memory retention in female and male C57Bl/6J wild-type mice at an early time point at doses as low as 0.1 Gy. There were no effects of irradiation on contextual fear conditioning or spatial memory retention in the water maze for the same animals. Figure 9 illustrates the disruption of preferential attention to the novel object (both sexes pooled). The results also illustrate how different behavioral tests may differ in sensitivity in the same animals.

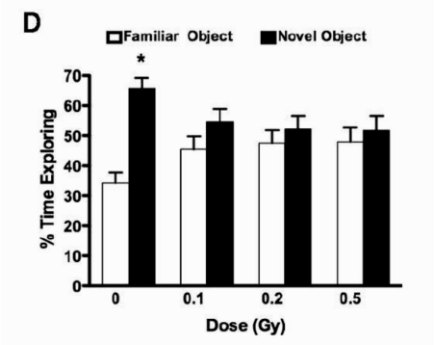

Figure 9. Novel object recognition of sham-irradiated and irradiated male and female mice analyzed (panel D) [as time] spent exploring the familiar and novel objects. n = 8 mice/sex/treatment. *P < 0.05 versus the familiar object. Excerpt from Figure 1 of Haley et al. (2013).

Parihar et al. (2015) have extended the results of the NOR task and complementary novel location or object in place (OiP) test to very small doses of 600 MeV/n ^{16}O and ^{48}Ti particles using 6-month-old male transgenic mice [strain Tg(Thy1-EGFP) expressing the Thy1-EGFP transgene]. These animals were significantly older than those examined by Haley et al. (2013) above. The data showed substantial impairment in NOR and OiP performance 6 weeks post-irradiation after 5 - 30-cGy exposures depending on ion type, as shown in Figure 10.

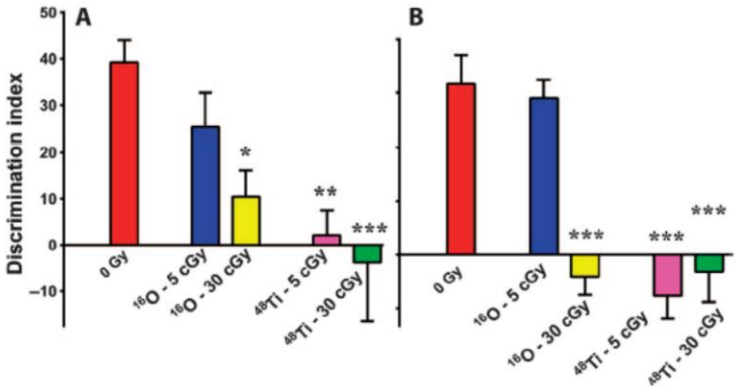

Figure 10. Behavioral deficits measured 6 weeks after charged particle exposure. (A) Performance on a NOR task reveals significant decrements in recognition memory indicated by the reduced discrimination of novelty. (B) Performance on an OiP task shows significant decrements in spatial memory retention, again indicated by a markedly reduced preference to explore novelty. *P = 0.05, **P = 0.01, ***P = 0.001, analysis of variance (ANOVA). Reproduced from Figure 1 of Parihar et al. (2015).

Unlike the mouse studies, when 8-week-old Sprague-Dawley rats were exposed to whole-body or partial-body irradiation at 1 - 25 cGy low doses of 1 GeV/n ^{16}O ions, no effects on novel object or place recognition were observed at 3 weeks (Rabin et al. 2014) in animals that later showed operant conditioning decrements, nor were anxiety measures altered in elevated plus maze tests.

g. Tests of Executive Function

The laboratory of Britten (Lonart et al. 2012) has considered the possibility that neurocognitive tasks regulated by the prefrontal cortex could also be impaired after exposure to low doses of HZE particles. They used juvenile male Wistar rats receiving either sham treatment or head-only irradiation with 20 cGy of 1 GeV/n ^{56}Fe and tested those 3 months later for their ability to perform attentional set shifting (ATSET). This test employs changes in associations between olfactory cues for food rewards that must be located by the natural behavior of digging in clean sand. Irradiated rats showed significant impairments in completion of the ATSET test battery. Specifically, 17% completed all stages compared to 78% of control rats. Most failures (60%) occurred at the first "reversal stage", and half of the remaining animals failed at the "extradimensional shift" phase of the complex test sequence. These observations suggest that exposure to mission-relevant doses of 1 GeV/u ^{56}Fe particles results in the loss of executive function in several regions of the cortex: medial prefrontal cortex, cingulate cortex, and basal forebrain.

Britten et al. (2014) next compared both juvenile (6 week old) and socially mature (6 – 11 months old) Wistar rats that were whole-body-irradiated with 10 – 30 cGy of 1 GeV/n ^{56}Fe and tested 3 months post-exposure. Importantly, animals segregated into high- and low-performing groups prior to irradiation such that ~25% of juveniles and ~40% of older animals could not maintain attention in the task and were removed from the study. Results of irradiation were analyzed for the high-performing groups and indicated that 15 and 20 cGy doses (but not 10 cGy) impaired performance in several parameters of attentional set shifting (Figure 11). Also of interest

in these animals were observations on purified synaptosomes in which hyperosmotic sucrose-stimulated release of acetylcholine (but not GABA) was inhibited at 20 cGy. This is a measure of presynaptic neurotransmitter vesicle secretion form the "readily releasable pool".

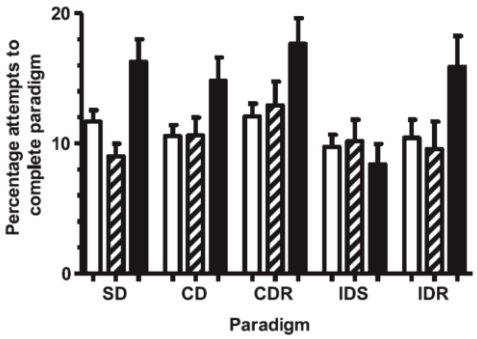

Figure 11. Effect of whole-body exposure to 1 GeV/nucleon ^{56}Fe particles on the paradigm-specific performance of retired breeder rats: number of attempts required to reach the criterion following sham-irradiation (open bar) and whole-body exposure to 15 cGy (hatched bar) or 20 cGy (solid bar) 1 GeV/nucleon ^{56}Fe. Graphs show means ± SEM. HAB: habituation; SD: simple discrimination; CD: compound discrimination; CDR: compound discrimination reversal; IDS: intradimensional shifting; IDR: intra-dimensional shifting reversal; EDS: extra-dimensional shifting; EDR: extra-dimensional shifting reversal. Reproduced from Figure 5 of Britten et al. (2014).

Davis et al. (2014) exposed young Long-Evans rats to 25 - 200 cGy head-only 150 MeV protons and tested them from 25 to 251 days post-irradiation using the rodent Psychomotor Vigilance Test (rPVT), which was adapted from a human test battery. The rPVT test uses light cues, nose-poke responses, and food rewards to measure reaction times, performance accuracy, persistence of attention, and impulsivity (premature responding) to randomized cues. Consistent differences were not initially observed when averaged across all animals in each treatment group. However, when animals' early post-irradiation performance scores were subjected to hierarchical clustering analysis, they fell into two distinct groups, radiation sensitive and insensitive. There was a progressive radiation impairment of performance in sensitive animals at all doses tested over 251 days (Figure 12), which reached stable values after 2 months. Sensitive animals also showed greater radiation-induced changes in dopamine transporter protein and dopamine D_2 receptor levels than insensitive animals. Earlier experiments by these investigators showed impaired reaction times in Long Evans rats after 5 Gy head-only ^{137}Cs γ-ray exposure (Heinz et al. 2008).

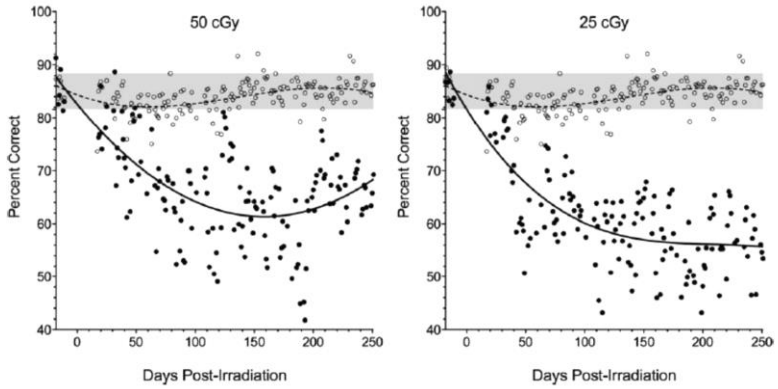

Figure 12. Examples of performance accuracy for animals showing pronounced deficits when exposed to 150 MeV/n protons at 25 - 200 cGy. The percent correct scores are shown as a function of days post-exposure, with each dot representing a separate session. Data points in the far left on each graph indicate baseline performances prior to exposure. Shaded areas indicate the range of a 95% confidence interval around the pre-exposure baseline performances of all non-exposed control animals. Closed circles: Animals identified by cluster analysis as being radiation-sensitive; open circles: Average performances of all non-exposed control animals. Solid and dashed lines: Visual fits of data trends to the data, based on centered third-order polynomial transforms. Reproduced from an excerpt of Figure 1 from Davis et al. (2014).

The results of these complementary sets of investigations highlight the importance of individual differences in executive functioning, which is sensitive to charged particles at ≥ 15 - 25 cGy. The authors also cite studies showing high/low performance groups for rats based on Barnes maze performance and even in astronauts with high/low sensitivity to sleep deprivation, further emphasizing the importance of inter-individual variation and cautioning against global averaging.

h. Emesis

Within 24 hours following exposure to low-LET radiation, the immediate CNS effects are anorexia and nausea (Fajardo et al. 2001). These prodromal risks are dose-dependent and provide indicators of the exposure dose. Thus, ED_{50} estimates are 1.08 Gy for anorexia, 1.58 Gy for nausea, and 2.40 Gy for emesis. These doses are at the high end of those estimated for the largest SPEs for an astronaut in a minimally shielded environment and prompted investigation of emesis in a non-rodent animal model, as mice and rats do not vomit. In a study by Sanzari et al. (2013), 12-16-week-old female Fitch ferrets were whole-body-irradiated with 0.25 to 2 Gy of ^{60}Co γ-rays or spread out Bragg peak protons from a 155 MeV beam (to simulate the SPE spectrum) at 0.5 Gy/min or 0.5 Gy/hr and followed for up to 7 hours for retching- and vomiting-related endpoints. The high-dose-rate cohort exhibited ED_{50} (95% CI) values of 0.48 (0.16–0.81), 1.01 (0.91–1.12), and 0.89 (0.69–1.08) Gy for retching after protons and vomiting after gamma rays or protons, respectively. Low dose rates were less effective. Rabin et al. (1992) found similar values in adult male ferrets for 600 MeV/n ^{56}Fe particles, fission spectrum neutrons, and 18.5 MeV electrons and reported ED_{50} values of 0.35, 0.40, and 1.38 Gy, respectively. Thus, there is a dependence of ED_{50} on radiation type, with higher LET species being more effective.

4. Neurodegenerative Changes

Investigators funded by the Space Radiation Program Element have begun to study the effects of space radiation on increasing or accelerating the time of appearance of pathologies and neuronal markers of AD using transgenic mouse models. Vlkolinsky examined whether HZE particle radiation accelerated age-related neuronal dysfunction using transgenic mice overexpressing human amyloid precursor protein (APP). APP23 transgenic mice exhibit age-related behavioral abnormalities and deficits in synaptic transmission. Vlkolinsky (2010) exposed 7-week-old APP23 transgenic males to brain-only ^{56}Fe-particle radiation (600 MeV/n; 1 - 4 Gy) and recorded synaptic transmission in hippocampal slices at 2 - 24 months. The results showed that radiation accelerated the onset of age-related EPSP decrements recorded at the population spike threshold from 14 months of age to 9 months and reduced synaptic efficacy. At 9 months, radiation also reduced population spike amplitudes.

Using a different mouse transgenic model, the laboratory of O'Banion (Cherry et al. 2012) examined the effects of ^{56}Fe particle irradiation in the APPswe/PSEN1dE9 (APP/PS1) mouse model of AD. APP/PS1 mice show Alzheimer's pathologies at an old age, and the goal of the study was to determine whether low doses of space radiation accelerated the age of appearance of AD pathologies. At 6 months after exposure to 0.1 and 1.0 Gy ^{56}Fe radiation, APP/PS1 mice show decreased cognitive abilities measured by contextual fear conditioning and novel object recognition tests. Male mice also showed acceleration of Aβ plaque pathology (Figure 13). Increases were not due to higher levels of amyloid precursor protein (APP) or increased cleavage as measured by levels of the beta C-terminal fragment of APP.

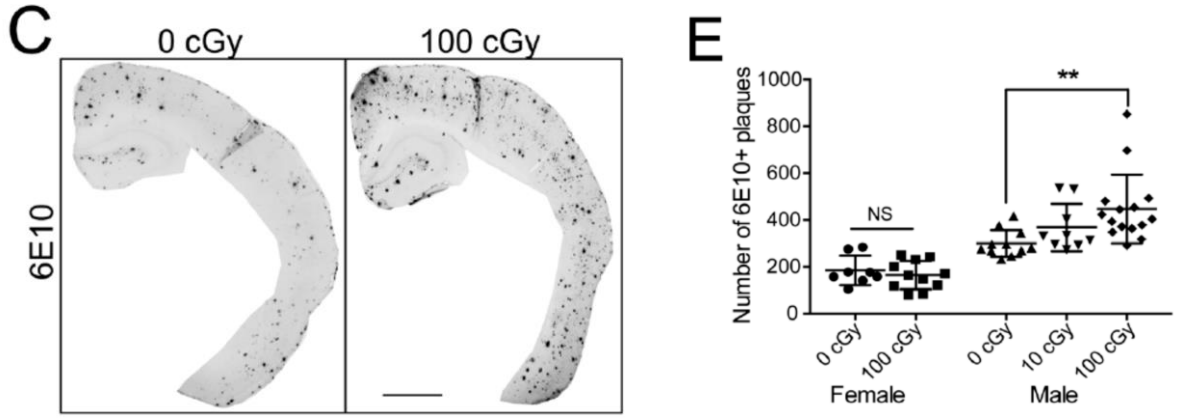

Figure 13. [Excerpt of Figure 2 reproduced from Cherry et al. (2012) panels C & E] Immunohistochemical staining for Congo red and 6E10 increases after ^{56}Fe particle irradiation. (A, C) Representative images of half male brains stained for 6E10 (C) 6 months after 0 cGy or 100 cGy ^{56}Fe particle radiation. Scale bar is 1 mm. In addition, the total number of individual 6E10 positive plaques (E) was determined. Each dot represents a single animal measured as the percent area of the cortex and hippocampus combined. Data were analyzed with Student's t-test for the females and one-way ANOVA with a Bonferroni post-test for the males. Data are displayed as the mean ± SD, n = 8–14 animals per dose. *P < 0.05, **P < 0.01.

Unlike the findings with charged particles in transgenic animals, Wang et al. (2013) found no acceleration of amyloid-β or tau protein pathology for up to two years in 10 cGy X-irradiated

wild-type C57BL/6J Jms mice, nor was Morris water maze performance impaired. While two of the 84 AD-related genes (Apbb1 and Lrp1) were down-regulated acutely (4 hr) in the hippocampus, only Il1-α was down-regulated after 1 yr. In a follow-up study using 5 and 10 cGy of 290 MeV/n ^{12}C ions (Wang et al. 2014), there again was no evidence of accelerated amyloid-β or tau protein pathology; however, a different suite of 6 genes showed acute expression level changes, and Il1-α was again down-regulated after 1 yr. Thus, in mouse models predisposed to pathogenic changes, there may be an acceleration of neurodegenerative pathology by charged particles. However, this may not extend to wild-type animals.

D. Non-Human Primate Research

Essentially all animal research with charged particles has been conducted using convenient rodent models, which can only approximate the human condition. To better understand the implications of the rodent-based research, it will eventually be necessary to conduct well-informed, targeted experiments with higher species and, in particular, non-human primates.

1. Rationale

Cucinotta et al. (2014) offered a thoughtful rationale for the use of non-human primates in the evaluation of CNS radiation risks. The authors pointed out that:

> Non-human primates (NHP) and humans are quite similar in their genetic, physiological, pharmacokinetic, and neurobiological characteristics while there are a large number of important differences between rodents and humans (Weatherall 2006; Dorus et al. 2004; Heekren et al. 2008). Non-human primates are used widely for specific areas of research including HIV/AIDS and infectious diseases, and neuroscience research (reviewed by the Weatherall Report 2006). Research on drug addiction, Parkinson's disease, Alzheimer's disease and stroke includes the use of NHP is being pursued in the U.S. and many other countries. Because of cross-species differences between humans and rodents, the determination of clinical significance for CNS health risks remains an important problem, especially if based on studies in rodents alone. This important issue is compounded for CNS cognitive risks which are known to originate in the frontal cortex, which is highly under developed in rodents compared to humans although rodents do provide some indication of cognitive risks related in the frontal cortex (Davis et al. 2014; Lonart et al. 2012).
> ...
> In broad-terms, mice and rats are used to investigate biological mechanisms and possible dose levels of concern, however are limited in representing human risks due to biological differences as summarized in Table2 [*not shown*]. However, NHP research requires much higher costs, and extra-levels of review and expanded ethical considerations before being considered. It is important that such studies be preceded with extensive research in cell and rodent models in order to first indicate if potential CNS risks are possible. Considerations of the feasibility of deep space missions and time-lines for missions planning relative to research maturity are also needed.

With these considerations in mind, after a solid body of knowledge of behavioral consequences of radiation is established for rodent models, focused NHP-based studies should be considered to establish the existence of and dose responses for corresponding adverse behavioral outcomes in a species with structural and functional characteristics much closer to those of humans. Such studies would ideally incorporate doses and compositions of radiation fields comparable to those in space, delivered at the lowest practical dose rates or fractionated over time scales corresponding to significant mission segments.

Some guidance on what may be expected in non-human primates is provided by earlier studies, but none of these studies employed charged particles comparable to those found in space, which can now be simulated at particle accelerator facilities such as the NASA Space Radiation Laboratory at Brookhaven National Laboratory.

2. Previous Behavioral Studies with Irradiated Non-Human Primates

In the years immediately following the development of the atomic bomb, numerous experimental programs were initiated to understand the health effects of radiation exposure, with particular interest in establishing dose responses for morbidity (acute radiation syndrome) and mortality (LD_{50}). Similarly, at the beginning of human space exploration, additional work focused on morbidity and mortality that might arise from space radiation exposure was conducted. Gamma rays, x-rays, neutrons, and protons were used in these studies with doses ranging beyond 10 Gy. For perspective, the $LD_{50/60}$ for the rhesus monkey was determined to be about 7.5 Gy (Hankey et al. 2015). Thus, an 11-yr program at the Armed Forces Radiobiology Research Institute (AFRRI) along with a 24-year program shared between the Air Force School of Aerospace Medicine (AFSAM) and NASA conducted many studies using non-human primates. Some tests also involved exposures at the Nevada Test Site for nuclear weapons. These studies began around 1954, with high levels of activity extending through the 1960s, and ended around 1990. They primarily used rhesus macaques but also used cynomolgus macaques and chimpanzees.

Studies in the very high dose range have limited value in assessing behavioral impairments due to the complications of acute radiation sickness and small sample sizes, but some test series included subjects exposed to doses < 2-3 Gy, and these results may be useful for identifying trends in dose dependence and to help define the current state of knowledge. Published results were sometimes classified for many years or appeared in agency technical reports not usually identified during modern computer-based literature searches. Some provocative reports are presented below and suggest that behavioral decrements can be measured in non-human primates at doses on the order of 1 Gy, are progressive and persistent for many years, involve cognitive and motor performance, and exhibit dose responses, but questions of thresholds or RBEs remain highly uncertain. The results should be interpreted with caution.

The joint NASA/AFSAM bioeffects program was reviewed by Dalrymple et al. (1991) and other authors in a focused issue of *Radiation Research* (Vol 126, 1991). These studies employed whole-body irradiations of rhesus monkeys with several energies of protons ranging from 55 - 2300 MeV to simulate SPE spectra. While cataract incidence (Lett et al. 1991) and cancer incidence (Wood 1991) were well-documented along with general pathology, effects on the brain did not include behavior, and the only salient finding was an elevated incidence of astrocytoma and glioblastoma primarily due to 55 MeV protons.

Davis et al. (1962) reported results from male rhesus monkeys administered 60 Gy of 250 kVp X-rays during partial head exposure in two 30-Gy monthly fractions to either the anterior frontal lobes, inferior parietal lobe, or both. Animals were pre-trained for 6 months on the Wisconsin General Test Apparatus (WGTA), requiring object food reward pairings, bent wire problems, patterned string tests, and the “elevator detour problem”, and then continuously for 60 days covering the second radiation exposure and a further 30 days post-irradiation. General disturbances in all tests and overall hyperactivity were noted early in all test subjects, but only the "elevator detour problem" requiring fine motor tasks clearly differentiated the irradiated groups.

Nine 5 - 17-year-old chimpanzees (3 females and 6 males) received whole-body doses of 3.75 or 4 Gy ^{60}Co-γ over 12 hours (~ 0.5 cGy/min) and were tested post-irradiation on a set of 25 different tasks involving rapid movement, repetitive responses, detection of minor differences in stimulus cues, and spatial memory (Riopelle 1962). Early deterioration of performance (3 weeks) was associated with acute radiation syndrome, but three of the 14 tests that could discriminate irradiated versus unirradiated groups indicated loss of performance (4-choice oddity, visual acuity, and size discrimination), which was persistent for 3-5 years. For example, in the 4-choice oddity test, animals had to select which of a set of 4 wooden plaques covered with a complex wallpaper pattern was unique, which would result in a food reward. Shown below in figure 14 are data from 3-5 years post-irradiation.

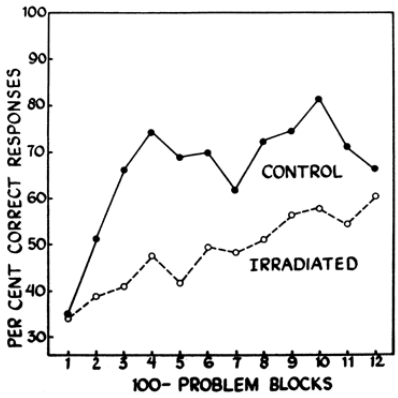

Figure 14. Performance of normal and irradiated chimpanzees on a task in which they had to select the unique stimulus from three identical stimuli. [Reproduced from Figure 3 of Riopelle, 1962]

Brown et al. (1962) and Melville et al. (1966) reported on a group of male rhesus monkeys that in 1964 were subjected to a series of 16-hr exposures to fast neutrons and gamma rays from a Polonium-Beryllium source repeated in 20 (4-day interval) to 40 (12-day interval) fractions to achieve cumulative doses ranging from 76.5 to 609 cGy [using 1 rep (Roentgen equivalent physical) = 0.93 cGy conversion]. These animals were followed for more than 7 years post-irradiation. The principal early effect noted was a transient decrease in peripheral blood cell counts noted in the higher dose group. The principal late effects involved a reduction in visual acuity and a series of persistent behavioral changes along with testicular damage. Testing on an object-quality discrimination learning set, bent-wire detour problems, a finger dexterity test, and linear position preferences during the first 6 months resulted in no measureable changes. However, at 9 - 10 months post-irradiation, relative responses to cage-related stimuli ("prepotent" stimuli) significantly outweighed distracting uncontrolled auditory stimuli occurring outside the test room (Figure 15), suggesting decreased distractibility in the irradiated animals, which was confirmed 4 months later.

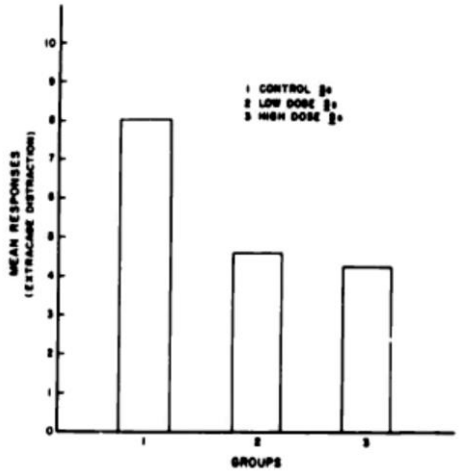

FIGURE 11

Mean responses to extracage distractions by the subjects of each of the three relative radiation dosage groups.

Figure 15. Reproduced from Melville et al. (1966). Decreases in responses to extra-cage stimuli indicating decreased distractibility. Dose groups were 0, 77-154 cGy (Low), and 312-613 cGy (High).

Food preferences of the monkeys underwent permanent changes 14 to 16 months after exposure, and by two years, a series of Wisconsin General Test Apparatus-based tests such as oddity reversal continued to show changes in the high-dose group consistent with reduced distractibility, leading, in turn, to enhanced test performance. At 3 years post-irradiation, a loss of visual acuity was detected in the high-dose group. At 78 months post-irradiation, reversal learning in a two-object discrimination problem showed enhanced performance (less distractibility) at the p=0.005 level of significance. Seven years after exposure, animals were tested for stability of behavior under conditions of social distraction when a female monkey at the estimated time of ovulation was presented as a distracting stimulus, and stability of behavior was disrupted except in the high-dose group.

Brown et al. (1962) and McDowell et al. (1959) reported on sixty-four rhesus monkeys (39 male and 25 female) of age 22 - 28 months in 8 groups that were subjected to irradiation during a nuclear weapons test at the Nevada Test Site and received from 1.91 to 4.5 Gy of mixed gamma-rays (~62%) + neutrons (~38%) [based on 1 rep = 0.93 cGy]. They were evaluated 11 months later using the WGTA test battery that used associations of food rewards with wooden block objects, spatial delayed response problems, patterned string tests, five-dot discrimination, and a version of object in place discrimination. Animals were subdivided into three dose groups, and the findings suggested that as doses increased, responses to a variety of tasks were degraded, with females generally showing better performance than males. Other examples of weapons test investigations, such as those by Pickering et al. (1958) with estimated doses from 2.2 to 11.8 Gy, also measured acute radiation sickness and LD_{50} but revealed the prompt onset of and sustained increase in nondirected visual activity (predominance of visual activity without apparent fixation), nondirected locomotor activity (e.g., bouncing, pacing, or swinging), object-directed activity (to cage parts or experimenter), and self-directed activity (responses to the subject's own bodies). These higher dose observations are less useful due to the associated severe morbidity.

There is still interest in the high-dose exposure regime in the context of radiological terrorism and countermeasure development. Thus, Hankey et al. (2015) conducted tests of a leukocyte growth factor drug (pegfilgrastim, Neulasta™) to mitigate acute radiation syndrome in 3 - 7-yr-old male rhesus macaques who were exposed to 7.50 Gy total-body irradiation (the $LD_{50/60}$) using 6 MV photons. While there was mitigation of hematologic parameters, the only behavioral observations were an improvement in activity and posture that were impaired by the radiation exposure at 60 days post-irradiation.

From a different perspective, there is concern regarding behavioral impairment associated with radiotherapy for head and neck tumors. Robbins et al. (2011) have led this area, and in a recent pilot study, three 6–9-year-old male rhesus macaques were whole-brain-irradiated with 40 Gy of 6 MV photons over 4 weeks in 8 fractions and tested for cognitive function using a delayed-match-to-sample (DMS) task 5 days/week for 4 months prior to irradiation and for 11 months after irradiation. A visual screen presented 2 to 6 clip art images in randomized positions and at randomized times, and the animals were required to identify previously presented images for a juice reward. Progressive post-irradiation cognitive impairment was observed beginning at one month using the 6-image (high cognitive load) test but not until 7 months using the two-image (low cognitive load) test. Figure 16 illustrates the cognitive decline. [^{18}F] deoxyfluoroglucose PET analysis comparing local brain metabolism 9 months post-irradiation vs. prior to irradiation indicated that mean cerebral glucose metabolism in the cuneate cortex and prefrontal cortex regions had decreased, indicating less glucose metabolism in these DMS task-associated brain regions.

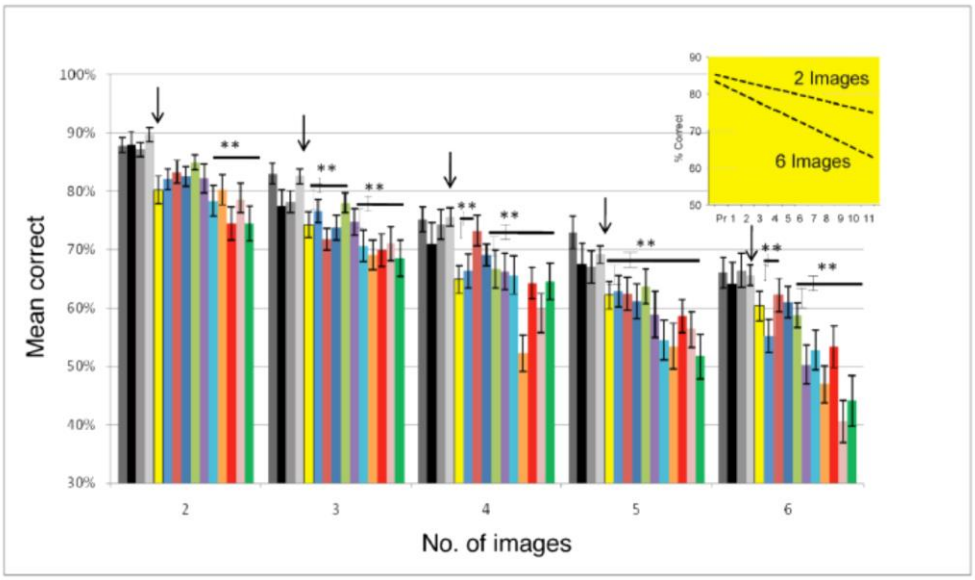

Figure 16. Fractionated whole-brain irradiation leads to chronic, progressive cognitive impairment. Each bar represents the mean percentage (±SEM) of correct trials, with two to six images summed over all animals, trials, and daily sessions for each month. Arrows indicate the start of irradiation. **P < 0.001; horizontal bars span months where asterisks apply. The inset shows a regression analysis of the average monthly performance of the three NHPs at low (two images) and high (six images) cognitive load. Reproduced from Figure 2 of Robbins et al. (2011).

Finally, there have been a few lower dose studies with non-human primates indicating behavioral effects at more space-like exposure levels. In a pilot study by Harlow (1962), a whole-body dose of 1.5 Gy of ^{60}Co-γ was administered to two mature rhesus monkeys as 5 daily fractions of 30 cGy each at 0.67 cGy/min. The results showed evidence of conditioned avoidance of a fruit drink (Kool Aid™) when doses reached 45 cGy and above that became progressively stronger over 5 weeks. In another study by Taylor et al. (1967), evidence was presented that monkeys can directly detect 9.4 cGy pulses of 300 kVp X-rays of duration 15 seconds (0.63 cGy/sec). Animals were trained in an operant responding test with variable ratio schedules involving lever pulling and food pellet rewards and then tested in a suppression trial with head-only irradiation terminated by an unavoidable foot shock. A suppression ratio of (T1 - T2)/T1 was determined, where T1 is the number of responses during 15 sec preceding the X-rays and T2 is the number of responses during the exposure. Suppression ratios as high as 0.8 were observed, but a mechanism for this response was not identified.

Taken together, these observations with their many limitations suggest that non-human primates are radiosensitive with respect to behavior and might show behavioral impairments after low-dose, charged-particle exposures using cognitively challenging tests adapted to the unique properties of the species.

E. Future Research Strategies- Recommendations of an Ad-Hoc Panel on CNS Research

The Space Radiation Program Element convened an Ad-hoc panel in 2012 to consider and make recommendations on CNS risks from ionizing radiation. Chaired by Dr. Walter Koreshetz, Deputy Director of the NIH Institute of Neurological Disorders, the panel's findings on CNS space research are summarized here:

"The National Aeronautics and Space Administration (NASA)-funded studies of animals exposed to high energy particles have demonstrated that some brain alterations can occur at total exposures that fall within the range of a prolonged human mission to outer space. These experiments raise the question of whether deep space radiation might cause changes in cognition that could affect astronaut performance during a long mission, as well as whether radiation exposure may increase the risk of accelerated onset of Alzheimer's disease, Parkinson's disease, cerebrovascular disease, or other neurodegenerative diseases. ... Studies to date have examined a wide spectrum of behavioral, pathologic, and physiologic changes in irradiated animals exposed to a variety of heavy ions at different energies and fluences. The experiments have been conducted for different purposes and by different groups and, thus, are not easily comparable. For these reasons, it is difficult to know whether they are tracking a common effect, whether the effects seen have been replicated, or whether they can be extrapolated to the human condition. Although these studies do not clear concerns for either short term effects on cognition or long term delayed risk of accelerated neurodegeneration, neither do the studies establish a definitive, clinically significant brain effect of high energy radiation within the expected range of exposure.

The panel identified a number of limitations in the evidence presented on CNS space radiation risk that need to be addressed to enable a more definitive determination of the CNS risk related to radiation exposure. To address these limitations, the panel made the following recommendations for future studies. 1) Identify quantifiable endpoints for the assessment of cellular, molecular, physiological, and behavioral changes and standardize these endpoints among

research groups. 2) Conduct more functional assays, to determine how radiation affects cell physiologic activity. 3) In addition to long term time points, include acute time points that will inform astronaut risk for cognitive dysfunction during space flight. 4) Create a limited and standardized set of HZE exposures to allow comparison and replication of data among research groups. 5) Promote tissue/sample sharing between CNS and carcinogenesis studies. 6) Continue primarily using rodent models, including studies of Alzheimer's disease (AD) and other neurodegenerative risks, with a long term goal of moving to a non-human primate (NHP) to assess cognitive risk to humans. Because of the current gaps in our understanding of the causes of neurodegenerative disease, even with these changes, the panel felt that a true estimate of the risk of accelerated neurodegenerative disease due to space radiation will be difficult to establish in the near time. However, a predictive risk model that estimates those acute exposures which have a reasonable likelihood of causing acute or subacute neurological impairment was considered feasible.

In considering a long term research strategy to quantitatively assess CNS risk from space radiation exposure, the panel recommended a 4-step process. 1) Definitively establish those pathological processes and behavioral correlates triggered by single dose high energy radiation in rodents. 2) Test the impact of chronic, fractionated exposures as compared to single dose high energy radiation at discrete and limited energies, doses, and time points. 3) Determine whether robust effects demonstrated in rodents are seen in the NHP. 4) Develop a set of experiments to test whether CNS effects suggested by work at the NASA Space Radiation Laboratory (NSRL) are indeed seen after exposure in deep space. This may include animal experiments but should certainly include a well-thought out evaluation of astronauts during and immediately after return from the first deep space missions.

Overall, the panel recommends that NASA adopt a more integrated research approach. The CNS space radiation research to date has been highly correlative and discovery-driven. This approach has helped lay a strong foundation of knowledge. In addition to further early stage discovery research, there is now a need, and the knowledge base, to mount a more coordinated research approach. For instance, NASA should consider developing a standardized set of radiation procedures at NSRL (i.e., exposures with standard range of fluency, energies, particles, and exposure timelines) that most closely represent the astronaut's exposure in deep space and establish those durations of deep space flight that would not be expected to pose short term safety concerns to the astronaut. NASA could achieve this integrated research approach with more NASA Specialized Centers of Research (NSCOR) on mission-critical topics. This strategy would ensure that NASA's human research program in CNS radiation risk makes tangible steps towards quantifying the CNS risk by 2020."

V. Adverse Outcome Pathways and Computer Modeling for Estimation of CNS Risks

A. Adverse Outcome Pathway Frameworks

Because human epidemiology and experimental data for CNS risks from space-like radiation are both limited, mathematical models of mammalian CNSs and their components will be essential tools for estimating the magnitudes and uncertainties of human risks. These models will be constrained by experimental data and organized according to mechanisms that play substantive roles in the pathophysiological processes underlying brain dysfunction and degeneration in both experimental models and humans. In toxicology, an organizing principle for understanding how undesirable consequences may develop from an environmental exposure is the *adverse outcome*

pathway (AOP). Ankley et al. (2010) used the definition: "An AOP is a conceptual construct that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome at a biological level of organization relevant to risk assessment". This approach can be applied to CNS risks. For example, Watanabe et al. (2011) provided the following definition and strategy for use of AOPs in a neurotoxicity context.

> "An adverse outcome pathway (AOP) is a sequence of key events from a molecular-level initiating event and an ensuing cascade of steps to an adverse outcome with population level significance. To implement a predictive strategy for ecotoxicology, the multiscale nature of an AOP requires computational models to link salient processes (e.g., in chemical uptake, toxicokinetics, toxicodynamics, and population dynamics)".

They illustrate the application of this process from exposures to a toxin that acts on glutamate gated ion channels to disrupt neuronal Ca^{++}. This leads to excitotoxicity, cell death, seizures, and impaired learning and memory. Development of this AOP required an iterative process to define a critically-reviewed, stressor-specific pathway, identification of key processes suitable for experimental evaluation, and strategies for model development. Radiation exposure can also be viewed as an environmental toxin exposure for which *in vitro* and *in vivo* endpoints give insight into the contributing key processes.

A similar framework has been helpful in organizing knowledge related to the development of cancer, where the key events in the adverse outcome pathway were designated the "hallmarks" of cancer (Hanahan and Weinberg 2011). In this conceptual framework, "The hallmarks of cancer comprise six biological capabilities acquired during the multistep development of human tumors. Underlying these hallmarks are genome instability, which generates the genetic diversity that expedites their acquisition, and inflammation, which fosters multiple hallmark functions."

Establishing critically-reviewed adverse outcome pathways for radiation-induced neuropathological processes should be a priority and would establish frameworks for developing predictive models of human risk. The evidence reported in section IV contains many examples of events and evaluation methods that should be organized into such a framework with guidance from existing systems biology knowledge of neurological diseases and incorporating existing models of neuronal processes.

Systems biology approaches (developed by research funded outside of NASA) have been applied to neurodegenerative diseases, including AD, and consider the biochemical and signaling pathways of importance in CNS disease pathophysiology. For example, Figure 17 shows a schematic of some biochemical pathways important in the development of AD. The description of the interaction of space radiation with these pathways would be an important approach in developing AOPs supporting predictive models of space radiation risks.

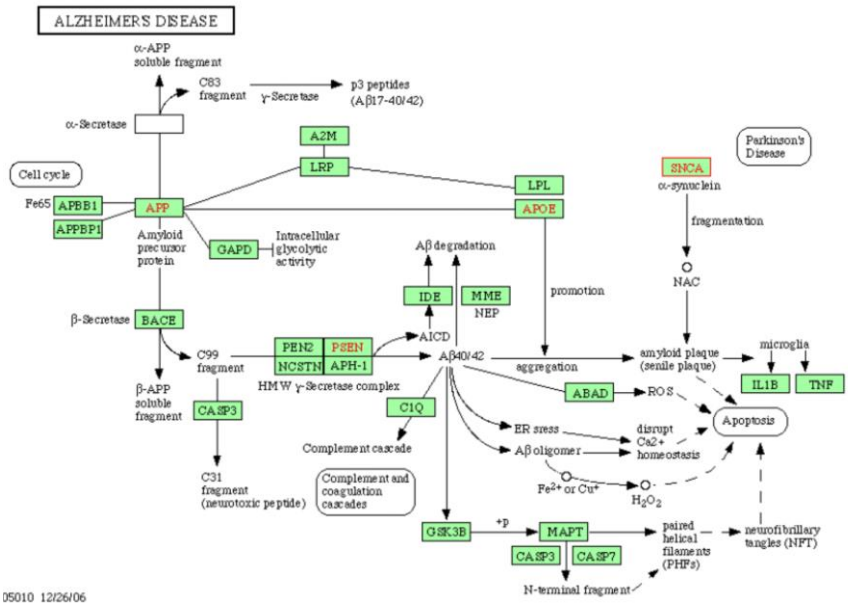

Figure 17. Molecular pathways important in Alzheimer's disease. From Kyoto Encyclopedia of Genes and Genomes (http://www.genome.ad.jp/kegg/pathway/ hsa/hsa05010.html)

Mizuno et al. (2012) have greatly extended and organized this type of information and constructed one of the first comprehensive maps of intra-, inter-, and extracellular AD signaling networks as a publicly available pathway map called "AlzPathway". This pathway map incorporates 1347 molecules and 1070 reactions in neurons, astrocytes, and microglial cells and relates them to their cellular localizations and functions in presynaptic and postsynaptic structures and the brain blood barrier. The AlzPathway map is accessible at http:// alzpathway.org/.

B. Models Applicable to Radiation-Induced CNS Responses

In order to have predictive value for risks, biological pathways and their outputs need to be organized into mathematical models. Approaches to modeling discrete disease processes such as amyloid deposition have been developed, such as the *in silico* biochemical model of Edelstein-Keshet and Spiros (2002) for senile plaques related to AD. They described biochemical interactions between TNF-α, IL-1β, and IL-6 and several important cell populations, including astrocytes, microglia, and neurons, and were then able to estimate kinetics of cell death based on plaque formation. However, to understand the effects of radiation exposure on the brain's overall information processing performance, models of neural networks linked to detailed electrical, biochemical, and anatomical parameters are needed. Computational neuroscience seeks to provide this modeling capability, and great strides have been made in the last decade. Brette *et al.* (2007) have reviewed the most commonly used, freely-available, open source and well-documented simulators and simulation environments presently available. These are used for analyzing detailed electrophysiological properties of spiking neural networks with realistic input parameters of neuron membrane properties, synaptic structure, neuron morphology, and connectivity. Perhaps the two most widely used simulation environments are based on the GENESIS™ and NEURON™ platforms, which can accept observational data from numerous databases, such as Neuromorph, CoCoMac, BioModels Database, and SenseLab (see Organization for Computational Neuroscience, http://www.cnsorg.org/model-database).

Perhaps the most comprehensive full-scale model of the rodent hippocampus, with over 10^6 neurons having accurate connectivity, neuron morphology, and electrophysiological properties, is that of Soltesz and collaborators (Schneider et al. 2012) developed in part with NASA funding. This model is based on the NEURON simulation environment and runs in a parallel computing setting. This model was used to compare predicted hippocampal CA1 region network firing statistics using input parameters from proton-irradiated versus control mice. The results, as seen in Figure 18, below, predicted that small radiation-induced differences in resting membrane potential and input resistance would lead to large differences in periodic pyramidal cell firing statistics (Sokolova et al. 2015). This model can also be used to test effects of altered neuron morphology or synaptic structure.

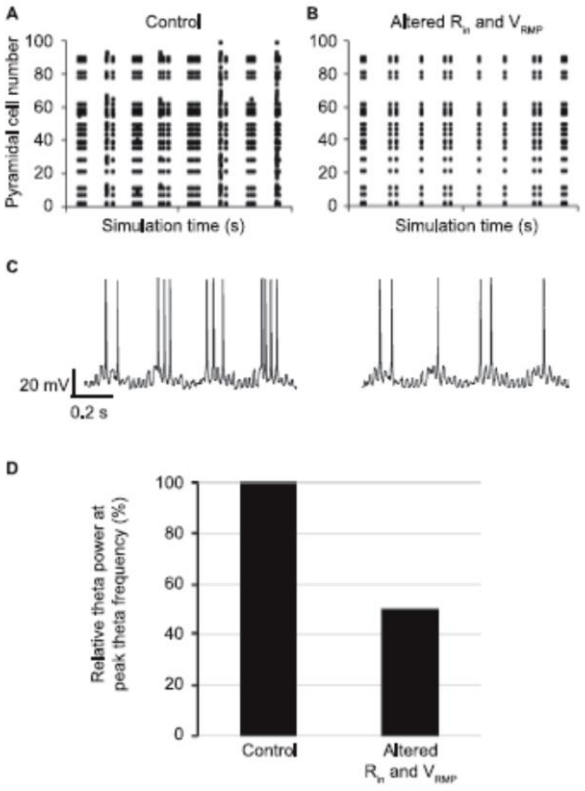

Figure 18. Incorporation of radiation-induced alterations into a computational model of the CA1 microcircuit containing 100 excitatory cells and four types of interneurons using the NEURON simulation environment. Panel A: Pyramidal cell firing statistics for the control condition after initial stimulation of 20% of the cells. Each dot represents a single action potential. Panel B: Pyramidal cell firing statistics after incorporation of radiation-induced changes in resting membrane potential and input resistance. Panel C: Voltage traces for pyramidal cell no. 1 under control (left side) and irradiated (right side) simulation conditions. Panel D: Relative theta oscillation power at the 4.0-Hz peak theta frequency. [Reproduced from Figure 6 of Sokolova et al. 2015].

To address the interactions of charged particles with CNS tissue, Cucinotta et al. (2014) combined data from neuron anatomy databases with models of charged particle track structure to determine the statistics of energy deposition in cellular compartments. This is particularly

important, as evidence suggests that many important targets of radiation in the CNS may include the complex cellular processes of neurons, rather than just the cell nucleus. The example in Figure 19 shows the interaction of a high-energy iron ion with granule cells of the DG.

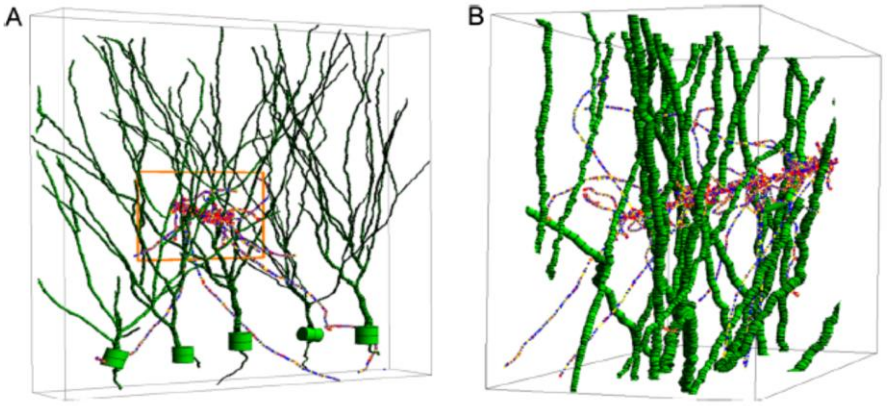

Figure 19. Model predictions of energy depositions from ^{56}Fe (200 MeV/u) particle tracks in mouse granule neurons. (Panel A) Track structure of energy deposition in a layer of 5 neuron cells. (Panel B) Energy deposition in the dendritic tree of a single neuron showing the spectrum of energy deposited, *e* in 20 ×20 ×20-nm voxels with blue, $e < 20$ eV; yellow, $20 < e < 100$ eV; and red, $e > 100$ eV. The diameters of dendritic branches are between ~1.4 and 2 μm. The dendrites are digitized as green connected cylindrical segments with topological neuron data as archived at NeuroMorpho.org (Parekh and Ascoli 2013). The rendered volume in these figures is 80 ×70 ×43 μm^3, with the neuron structures and particle tracks each represented by 20 ×20 ×20-nm^3 voxels. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) Reproduced from Figure 4 of Cucinotta et al. (2014).

In summary, comprehensive datasets and modeling techniques are now making it possible to interpret perturbations in neuronal structure and function at the network level, which can link experimental observations of isolated parameters to their impact on network performance. This will facilitate incorporation of experimental data from radiobiology investigations to frameworks describing pathways of acute and degenerative functional impairments.

VI. Risk in Context of Exploration Mission Operational Scenarios

A. Projections for Space Missions

Reliable projections of CNS risks for space missions cannot be made from the available data. Animal behavior studies indicate that HZE particles cause important detriments in rodent and neuronal cell culture models at space-relevant doses. However, the significance of these results for humans is not clear at this time. The use of non-human primates in experiments would hasten understanding of the significance of effects observed to date. Importantly, there have been only a relatively few HZE particle types tested, with no experiments performed above 1 GeV/u and very few testing the effects of slowing down or stopping particles with energies below 200 MeV/n. The latter experiments would involve very high LET particles, with the potential for results becoming

even more dissimilar from those derived from low-LET radiation with higher particle up to 1000 MeV/n. Other uncertainties include age at exposure, radiation quality, dose rate effects, and issues regarding genetic susceptibility to CNS risk from space radiation exposure. More research is required to estimate the CNS risks.

The use of dose and RBE is not sufficient to predict risk for GCR CNS risk assessment because there are no low-LET human data with which to scale effects. Estimates of fluence rates in tissues for different particle types are useful descriptive parameters of the physical environment and possible damage to the CNS. Table 1 below modified from Cucinotta et al. (2014) shows the number of particle hits per year for different GCR particle charge groups in different regions of the hippocampus under typical spacecraft shielding. Clearly, a large number of hits from HZE particles will occur behind typical shielding amounts, and, as noted earlier, delta ray exposures should not be ignored. Note that in these calculations, the reference location in the brain is the hippocampus, which lies relatively deep within the brain. Recent modeling also indicates that under light levels of shielding (such as in a spacesuit), there may be a significant dose to the cortical surface associated with very large SPEs.

Table 1. The number of GCR particle hits in the CA1, CA2/3, and dentate gyrus calculated using the HZETRN/QMSFRG model for average solar minimum conditions.

	Hits per Day with 10 g/cm^2 Shielding			Hits per Year
Fluence >Z^{*2}/β^2	CA1	CA2/3	Dentate Gyrus	Dentate Gyrus
All GCR	3.4×10^5	1.1×10^5	6.2×10^5	2.3×10^8
>100 (Z>10)	321	106	595	2.2×10^5
>250 (Z>14)	90	30	166	6.1×10^4
>500	38	13	71	2.6×10^4
>1000 (stopping ions)	16	5	30	1.1×10^4

B. Potential for Biological Countermeasures

The goal of space radiation research is to estimate and reduce uncertainties in risk projection models and, if necessary, to develop countermeasures and technologies to monitor and treat adverse outcomes to human health and performance relevant to space radiation for short-term and career, including acute or late CNS effects from radiation exposure. The need for the development of countermeasures to CNS risks is dependent on further understanding of CNS risks, especially issues related to a possible dose threshold and, if such a threshold exists, which NASA missions would likely exceed threshold doses. Based on animal experimental studies, antioxidants and anti-

inflammatories should be investigated as countermeasures for CNS risks from space radiation (Rabin et al. 2005). Diets of blueberries and strawberries were shown to reduce CNS risks after heavy ion exposure. Estimating the effects of diet and nutritional supplementation would be a primary goal of CNS research on countermeasures. However, the recent study from the Raber lab (2013) showed no protective effect from antioxidants such as α-lipoic acid in reducing early cognitive changes following doses of 0.1 to 1 Gy of Fe particles, but responses of signaling pathways may discriminate the different treatments. These results suggest that DNA damage may play an important role in modifying CNS responses and that high-LET radiation, especially at low to moderate doses, is less dependent on early oxidative stress responses to cause illicit detrimental effects.

A diet rich in fruit and vegetables significantly reduced the risk of several diseases. Retinoids and vitamins (A, C, and E) are probably the most well-known and studied natural radioprotectors, but hormones (such as melatonin), glutathione, superoxide dismutase, phytochemicals from plant extracts (including green tea and cruciferous vegetables), and metals (especially selenium, zinc, and copper salts) are also under study as dietary supplements for individuals exposed to radiation, including astronauts (Durante and Cucinotta 2008). Antioxidants should provide reduced or no protection against the initial damage from densely ionizing radiation such as HZE nuclei, as the direct effect is more important than free radical-mediated indirect radiation damage at high LET. However, there is an expectation that some benefits should occur for persistent oxidative damage related to inflammation and immune responses (Barcellos-Hoff et al. 2005). Some recent experiments suggest, at least for acute high-dose irradiation, that efficient radioprotection by dietary supplements can be achieved, even in cases of high-LET radiation exposure. There is evidence that dietary antioxidants (especially strawberries) can protect the CNS from the deleterious effects of high doses of HZE particles (Rabin et al. 2005). However, because the mechanisms of biological effects are different at low dose-rates compared to the high dose-rates characterizing acute irradiation, new studies on protracted exposures will be needed to understand the potential benefits of biological countermeasures.

Concern about the potential detrimental effects of antioxidants was raised by a recent meta-data study of the effects of antioxidant supplements in the diet of normal subjects (Bjelakovic et al. 2007). The authors did not find statistically significant evidence that antioxidant supplements have beneficial effects on mortality. On the contrary, they concluded that β-carotene, vitamin A, and vitamin E seem to increase the risk of death. Concerns are that the antioxidants may allow rescue of cells that still sustain DNA mutations or altered genomic methylation patterns following radiation damage to DNA, which can result in genomic instability. An approach to target damaged cells for apoptosis may be advantageous for chronic exposures to GCR.

C. Individual Risk Factors

Because human populations are not inbred like laboratory animals, there is considerable diversity in genetic background as well as nutrition and lifestyle differences that may affect sensitivity and reactions to radiation. Individual factors of potential importance are genotype and epigenetic profiles, prior radiation exposure, and previous head injury such as concussion. As discussed in section IV, age, sex, and species differences clearly affect outcome measures for radiation responses. Additionally, genetic variation at specific loci, such as the apolipoprotein E gene (ApoE), has been shown to modulate the effects of space radiation (Villasana et al. 2008). This particular gene is important, as it controls the age of onset of AD and the risk for atherosclerosis. Raber et al. (2015) further showed that there are differences in cognitive

impairment between C57Bl/6 inbred mice and hybrid B6D2F1 mice exposed to charged particle radiation. Performance on various behavioral tests has long been known to depend on rat strain, and it is also known that anatomical differences exist between strains. For example, Wistar rats have reduced granule cell projections to hippocampus CA3 compared with other strains (Ramirez-Amaya et al. 2001). Of particular interest are the results from Britten et al. (2014) and Davis et al. (2014) showing that cohorts of animals naturally stratify into high and low performers or groups that are sensitive or insensitive to radiation exposure with respect to high-level cognitive performance. Some of the uncertainty in measurements based on population averages might be reduced by considering the possibility of stratification in performance within test samples. This has implications for astronauts, who are a high-performing subset of humans.

D. Synergistic Effects of Spaceflight

The combined effect of space radiation exposure with other spaceflight factors on acute and late CNS adverse functional changes and neurodegenerative disease risks is unknown. Other spaceflight stressors contributing to behavior and cognitive risks include isolation, hostile/closed environment, distance from Earth, and altered gravity. These hazards are of concern because they contribute to psychological and physical stress or modified behavior (affect), sleep deficiency, altered circadian rhythm, hypercapnea, chronic inflammation, and altered immune, endocrine, and metabolic function. Related studies in the Behavioral Health and Performance Element of the Human Research Program are underway to further develop the evidence base for the effects of these spaceflight hazards on in-flight adverse cognitive or behavioral conditions through research on the International Space Station and Earth-based analogs (NASA SP-2009-3405, 2009).

VII. Gaps

Acute and late radiation damage to the CNS may lead to changes in motor function and behavior or neurological disorders. Radiation and synergistic effects of radiation with other space flight factors may affect neural tissues, which in turn may lead to changes in function or behavior. Data specific to the space flight environment must be compiled to quantify the magnitude of this risk using animal models and 2-dimensional or 3-dimensional cell culture models of human or other vertebrate cells. If this is identified as a risk of high enough magnitude, appropriate protection strategies should be employed. Research should be directed toward answering the following risk gap questions.

CNS – 1: Are there significant adverse changes in CNS performance in the context and time scale of space flight operations? If so, how is significance defined, and which neuropsychological domains are affected? Is there a significant probability that space radiation exposure would result in adverse changes? What are the pathways and mechanisms of change?

CNS - 2: Does space radiation exposure elicit key events in adverse outcome pathways associated with neurological diseases? What are the key events or hallmarks, their time sequence and their associated biomarkers (in-flight or post-flight)?

CNS - 3: How does individual susceptibility including hereditary pre-disposition (e.g. Alzheimer's, Parkinson's, apoE allele) and prior CNS injury (e.g. concussion, chronic inflammation or other) alter significant CNS risks? Does individual susceptibility modify possible threshold doses for these risks in a significant way?

CNS - 4: What are the most effective biomedical or dietary countermeasures to mitigate CNS risks? By what mechanisms are the countermeasures likely to work?

CNS - 5: How can new knowledge and data from molecular, cellular, tissue and animal models of acute CNS adverse changes or clinical human data, including altered motor and cognitive function and behavioral changes be used to estimate acute CNS risks to astronauts from GCR and SPE?

CNS - 6: How can new knowledge and data from molecular, cellular, tissue and animal models of late CNS adverse changes or clinical human data be used to estimate late CNS risks to astronauts from GCR and SPE?

CNS - 7: What are the best shielding approaches to protect against CNS risks, and are shielding approaches for CNS and cancer risks synergistic?

CNS - 8: Are there significant CNS risks from combined space radiation and other physiological or space flight factors, e.g., psychological (isolation and confinement), altered gravity (μ-gravity), stress, sleep deficiency, altered circadian rythms, hypercapnea, altered immune, endocrine and metabolic function, or other?

VIII. Conclusion

At this time, reliable projections for CNS risks from space radiation exposure cannot be made due to limited data on the effects of high LET radiation on the nervous system and the absence of epidemiological data for humans. The existing animal and cellular data show that space-like radiation can produce molecular, structural, functional, and behavioral effects at doses comparable to reference mission projections. If human responses closely resemble those in animal models, the possibility exists for impacts on mission operations and/or late degenerative changes. However, the significance of these results in terms of space flight operational performance or morbidity to astronauts has not been elucidated.

It should be noted that the studies to date have been carried out with relatively small numbers of young animals (usually <12 per treatment group); therefore, testing of dose responses and detection of potential threshold effects at the lowest doses have been limited. The roles of dose protraction, effects of combinations of radiation species, and ages of test subjects have not been studied adequately to date; however, work is in progress to provide a GCR simulation environment, and research solicitations are emphasizing the importance of using animals of ages comparable to those of the astronaut corp. An approach to extrapolate existing observations to possible cognitive changes, performance degradation, or late CNS effects in astronauts has not been discovered. Research on new approaches to risk assessment may benefit from concepts such as adverse outcome pathways. Computer simulations and systems biology approaches may be helpful in providing the necessary data and knowledge to evaluate the similarity between animal and human response mechanisms. Findings based on rodent models may need to be validated in higher species such as non-human primates. A vigorous research program will be required to solve these problems and must rely on new approaches to risk assessment and countermeasure validation because the unique properties of the CNS and its modes of impairment are intrinsically different than those associated with cancer risks.

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XI. List of Acronyms

AD	Alzheimer's disease
ApoE	Apolipoprotein E lipid binding protein
AOP	Adverse Outcome Pathway
BBB	Blood-Brain Barrier
BEIR	Biological Effects of Ionizing Radiation, expert panel report
BFO	Blood-forming organs
CA1	*Cornu Ammonis* region of hippocampus
C57BL/6	C57 black 6 (inbred laboratory mouse strain)
cGy	centiGray (=1 rad)
CNS	Central Nervous System
CTA	Conditioned Taste Aversion
DG	Dentate Gyrus field of hippocampus
DNA	DeoxyriboNucleic Acid
ED50	Dose where 50% of the population exhibits the effect (LD50 is similar but with lethality as the effect)
EGFP	Enhanced Green Fluorescent Protein
EPSP	Excitatory Post Synaptic Potential
FR	Fixed-Ratio schedule
GCR	Galactic Cosmic Rays
GeV	Giga-electron Volt
Gy	Gray (=100 rad, 1 J/kg absorbed dose, D)
HZE	High Charge (atomic number, Z) and Energy
IL	Interleukin
IQ	Intelligence Quotient
IR	Ionizing Radiation
ISS	International Space Station
keV/μm	kilo-electron Volt per micrometer of track length (common unit for Linear Energy Transfer, LET)
LEO	Low-Earth Orbit
LET	Linear Energy Transfer

LTP	Long-Term Potentiation
MeV	Mega-electron Volt
mGy	milliGray (=0.1 rad)
n	nucleon (sometimes u or amu is also used)
NCRP	National Council on Radiation Protection and Measurements
PELs	Permissible Exposure Limits
PSA-NCAM	PolySialic Acid-Neural Cell Adhesion Molecule
RBE	Relative Biological Effectiveness
ROS	Reactive Oxygen Species (free radicals such as $\bullet OH^-$ and $O_2\bullet^-$)
SEM	Standard Error of the Mean
SGZ	SubGranular Zone (neurogenic region of hippocampus dentate gyrus field)
SPE	Solar Particle Event
Sv	Sievert (= 100 rem) = Dose Equivalent, H (Dose in Gy x quality factor, Q)
TNF-α	Tumor Necrosis Factor-α
Z	Atomic number