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Radiation Exposures in Space and the Potential for Central Nervous System Effects (Phase II)

October 2018

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National Council on Radiation Protection and Measurements

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Preface

This report has been prepared at the request of the National Aeronautics and Space Administration (NASA). It is the second phase of a two-phase effort intended to provide guidance to NASA concerning the effects of space radiation exposures on the central nervous system of crew members, the implications for them and possible impacts on their missions. The first phase of this effort resulted in NCRP Commentary No. 25, *Potential for Central Nervous System Effects from Radiation Exposure During Space Activities Phase I: Overview*, which described the critical issues surrounding the potential short- and long-term consequences of space radiation on the CNS and laid the groundwork for a more comprehensive investigation, that is the basis of this report. This report summarizes the steps and approaches needed to more fully understand the risk of CNS effects following radiation exposures in space and provides guidance for risk management and radiation protection. The Committee has identified knowledge gaps regarding the implementation of a comprehensive and effective radiation safety program to protect astronauts against the potential for early and late CNS effects from space radiation.

This Report was prepared by Scientific Committee 1-24P2 on Radiation Exposures in Space and the Potential for Central Nervous System Effects (Phase II). Serving on Scientific Committee 1-24P2 were:

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John D. Boice, Jr.
President

Contents

1. Executive Summary

The National Council on Radiation Protection and Measurements undertook a two-phase study of the potential effects of radiation exposures in space on the central nervous system. The conclusions of the first phase, published as NCRP Commentary No. 25 (NCRP 2016), included that there is sufficient evidence of both acute and delayed radiation-induced CNS impairment to warrant a full report on the subject, that long-term medical follow-up of crew members to evaluate potential CNS changes is essential, that other sources of stress during space missions produce CNS effects which may interact with radiation induced effects, and that the mechanisms leading to CNS damage are likely completely independent of the mechanisms leading to cancer. However it is recognized that it is also conceivable that mechanisms common to cancer induction and CNS effects exist, including immune activation and dysregulation, and produce tissue-dependent consequences.

The potential for acute, during mission, and delayed, post-mission, effects due to the types of radiation encountered in space has been recognized for many years, but it has been difficult to determine if effects are operationally significant at the relatively low doses that occur in space. Side effects of medical exposures to the CNS include significant acute and delayed effects, but nearly all of these exposures are to low LET radiation at high dose rate and high dose. Experimental studies using small animals (mice and rats) initially produced mixed results, with some showing CNS effects but others indicating no CNS effects. As experimental techniques were refined, the probability of detecting a statistically significant effect of heavy ion irradiation at mission relevant doses increased. However, the experimental procedures remain complicated and time consuming and the available data are limited to relatively few radiation qualities and dose points. Furthermore, because of radiation delivery limitations all data are for

very high dose rate, exposures delivered in minutes, as contrasted with the low dose rate exposures in space which are extended over years. As a result, the conclusions, which can be drawn from data that often show unusual dose response relationships, are quite limited. Because of the high dose rates used and the complex dose response relationships obtained with small animal experiments and because of the high dose and lack of high LET exposures characterizing data for human response, any estimate of CNS risk made with the data currently available will have a very large uncertainty.

The committee conducting the phase two study was asked to address 8 specific questions and reached the conclusions which follow.

1. How should a "significant impairment" in performance be defined? What are the performance domains that could be significantly affected? What constitutes a "significant impairment" in the context of actual performance and operation?

Performance domains that could be significantly affected include attention, acquisition of new tasks, memory formation, memory retrieval, memory extinction, spatial navigation, ability to detect changes (novelty) in the environment under high stress conditions, and sensorimotor function. Additional behavioral alterations that can affect performance include depressive behaviors, anxiety, alertness, processing speed and executive function, and the ability to work well with others as part of the team, including interactions with ground control.

The level of impairment that is significant depends on the specific requirements of the mission and hardware being used. The ideal approach would be to determine the functional relationship between radiation exposure and risk to the mission and perform risk management procedures to minimize the total risk. This will result in different limits for radiation-induced

impairment depending on the magnitudes of other risks. Determination of the required functional relationship will require development and testing of biological response models for animals and humans, and will likely require many years. An interim approach to determining the boundary of "significant impairment" would be to determine the acceptable level of impairment due to a relatively well known chemical agent such as alcohol, and adopt that as the maximum acceptable level of radiation-induced impairment for the specific mission.

2. Do risk assessments for chemical toxicity, including neurotoxicity, provide guidance? Are the IARC evaluation schemes for chemical neurotoxicity of value in assessing health risks?

Risk assessments for chemical toxicity can provide useful guidance in terms of the adverse outcomes pathway (AOP) approach discussed in question 3 below.

The evaluation scheme of the International Agency for Research on Cancer (IARC) (http://monographs.iarc.fr/ENG/Classification/) is not of direct value for assessment of risks of behavioral and cognitive effects from radiation exposure of the CNS. The IARC evaluation scheme is designed specifically for classification of chemical and other agents according to the strength of evidence that they are capable of causing cancer and irrespective of their potency. Thus, it identifies agents as cancer hazards, but is not concerned with magnitude of risk.

3. Is the adverse outcome pathway approach used by EPA useful in the context of space radiation and CNS effects? What are the key events related to adverse outcomes in behavior/performance impairment that might affect mission and, conceivably, be related to late effects such as dementia?

The adverse outcome pathway (AOP) framework represents a conceptual linkage based on current knowledge of biological pathways that, when sufficiently disturbed, can lead to an adverse outcome that may justify regulatory action.

The adverse outcome pathway approach (AOP) is a useful framework for considering space radiation-induced CNS effects. Although developed for chemical-induced biological changes, the AOP framework could just as well be used to describe radiation-induced biological changes, as has been demonstrated for radiation cancer risk. In the context of space irradiation the molecular initiating events result from interaction of radiation with biological matter. The AOP framework offers a means to organize knowledge about relationships between the initial biological perturbation and an apical outcome. This framework could facilitate detailed characterization of the complex processes that lead from energy deposition by radiation to specific CNS impairments.

Relevant features of the AOP approach include description of a biological pathway that is agnostic to which physical/chemical stressors produce the initial biological alteration and description of the weight of evidence for the different portions of the biological pathway, thereby facilitating identification of knowledge gaps and indication of priority areas for future research. The majority of performance studies to date have been limited to identification of a single pathway. Unbiased studies involving large data sets might reveal other pathway changes following space irradiation that are pertinent to behavioral and cognitive performance during the mission, as well as risk to developing neurodegenerative conditions, including dementia, following the mission.

4. Are non-human primate (NHP) experiments necessary, and if so, how should they be considered?

A database focused on critical questions and confounding variables from a relevant animal model using NHP data may be needed to reduce what might otherwise be very large uncertainties in risk estimates. Well-designed NHP studies with large enough animal populations to answer specific targeted research questions would reduce the uncertainty, but it would probably require more than 10 years to produce significant results. Notwithstanding the challenges of conducting such studies, nonhuman primates would be valuable not only to confirm the findings from rodent studies on the risk of CNS effects, but ultimately also to confirm the effectiveness of potential pharmacological countermeasures for those effects.

5. Are the CNS effects, including the behavioral impairment domains, deterministic or stochastic? That is, assuming that there is sufficient evidence for concern, are there threshold levels below which the concern is minimal?

Most known CNS effects are considered to be deterministic, that is, tissue reactions with a threshold. The threshold depends on the sensitivity of measurements to changes in the tissue function and may be altered by additional stressors (such as sleep deprivation). A statistically significant effect does not necessarily imply operational significance. An individual may suffer impairment and still be within the normal range of individuals performing a specific task. Overall, the dose response curves reported to date for CNS effects of space irradiation in rodents have often been complex (Figs. 5.4 and 5.5). For example, while in some instances only the higher doses used showed significant effects, suggesting a safe threshold, in many other cases the opposite pattern was revealed, with the lower doses causing pronounced effects that were not seen at higher doses. Under such circumstances, it would be premature to suggest safe threshold

doses without additional studies confirming that doses below the threshold indeed do not cause any detrimental effects. It will be important for NASA to support reproducibility assessments for all studies, including those involving omics approaches, utilizing identical samples in different institutions.

6. How might space radiation "interact" with other aspects of a mission that would impair performance, both for the individual and the team, such as sleep deprivation, medications, zero gravity, constant close quarters, reduced communications, absence of windows to the world, and other?

A wide variety of features of the environment in space can produce behavioral and cognitive effects similar (or identical) to those produced by radiation. The effects could be independent, additive, or synergistic, depending on the features of the biological pathway for each cause; all may be expected to be involved in the variety of pathways for individual and team performance. Currently there is insufficient evidence to determine the nature of interactions. Sleep deprivation is an especially profound environmental challenge that may be expected to interact with measures of behavioral and cognitive performance. Increased CO_2 levels might also affect behavioral and cognitive performance.

7. What is the relative balance between the likelihood of neurobehavioral effects that would impair operational performance and adversely affect the mission, and the likelihood that serious neurodegenerative disease develop such as Alzheimer, Parkinson, Huntington, amyotrophic lateral sclerosis (ALS), and dementia?

As yet there are insufficient data to estimate the relative balance between in-mission and long-term health effects. It is likely that the biological pathways for the variety of effects, in-

mission and long-term, are different, potentially even in those instances for which the initial event in the pathways is the same. The rodent experimental data suggest that rather than the biological processes resulting in distinct early and late effects, there may be more of a continuum of effects. Even if neurobehavioral and/or neurocognitive effects during a mission are not detected, there may still be risks of developing late effects pertinent to neurodegenerative conditions.

8. True to all human tissues, the brain is equipped to respond rapidly to changes and then compensate for survival. Do compensation mechanisms exist that would influence the likelihood of getting to a level of significant impairment that would adversely affect performance and the mission?

If the CNS effects of interest are transient, fading on time scales of weeks, as is sometimes seen in rodent studies, this might suggest that at low dose rates the level of impairment during space missions may be reduced. Medical experience suggests that many delayed effects become progressive above a dose level; at low doses those effects generally disappear with time. On the other hand, while compensatory mechanisms might help to offset the earliest changes and related significant impairments, with continued challenges these compensatory mechanisms might not be able to prevent the occurrence of significant impairments during the mission. Sleep deprivation and alcohol exposure are good examples showing that there can be clear limits to what compensatory mechanisms can protect against. Based on the currently available data, there are insufficient grounds to assume that there would be compensatory mechanisms robust enough to prevent significant effects of space irradiation on neurobehavioral and/or neurocognitive performance.

The individual sections of this report include recommendations related to their particular areas of study. In addition, the committee wishes to highlight the following priority recommendations.

Recommendation 1:

The committee recommends establishing ongoing collaborations with the charged particle radiotherapy cancer centers globally to harness the world-wide data base of patients to enable follow-up of endpoints related to short- and long-term behavioral and cognitive effects potentially associated with charged-particle exposures to the CNS in this patient population.

Recommendation 2:

The committee reiterates the recommendations of the Phase I Report that life-long medical follow-up of crew members to evaluate potential CNS changes is essential.

- Comparison of the assessment results for exposed crew members with those for other non-exposed groups is essential (providing controls matched as closely as possible to exposed crew-members for follow-up studies).

- There is a critical need for long-term, regular follow-up of individuals who have flown in space, including appropriate assessments of potential detrimental CNS effects. While understanding that there are privacy and ethics concerns, a search should be made for a means to make relevant health information available to appropriate researchers**.**

Recommendation 3:

Priority should be given to development of methods for translation of results from studies in rodents, nonhuman primates, and other biological models, to humans. This should include identification of key molecular and tissue changes and pathways linking the early radiation damage to relevant short-term behavioral or cognitive changes and/or to late effects on behavioral or cognitive performance or altered risk of developing neurological conditions. This should also include elimination of dose rate as a potential confounder of the findings based on ground-based studies. The Adverse Outcome Pathway (AOP) approach may provide a helpful formalism to guide research, with emphasis being put on conservation between rodents and humans and on quantitative as well as qualitative relationships. For those pathways where there are major limitations comparing pathways in rodents and human, comparing the AOPs in nonhuman primates and humans will be critical.

These three priorities are challenging and will require substantial effort over several years. Recommendations that can be addressed on a shorter time scale include.

Recommendation 4

: **Support research on the combined effects of space irradiation and a limited number of other environmental challenges pertinent to astronauts during space missions (CO_2, crowding, etc.).**

Recommendation 5.

Support more studies comparing performance of females and males in space radiation studies.

2. Introduction

Radiation-induced CNS behavioral alterations and cognitive impairments are well known as side effects of radiation therapy. These radiation-induced CNS alterations are not only seen following radiation exposure of CNS tissue but also following only peripheral radiation therapy, which suggests, for example, that immune mediators might play an important role in indirectly affecting the CNS. Fortunately, these effects are noticeable in human populations only at relatively high doses. The complexity of the CNS radiation response is illustrated by the fact that hypofractionation, involving fewer fractions but with much higher doses each, seems to lead to less severe CNS effects than regular fractionation, involving more fractions with much lower doses per fraction. The observed benefit of hippocampal sparing during therapy, an approach based on rodent studies showing the sensitivity of the hippocampus to radiation, highlights the usefulness of animal models to predict risk to the CNS following radiation in humans.

The unique spatial and temporal distribution of energy deposition produced by high atomic number and energy (HZE) radiation, and the vivid experiences of astronauts observing "light flashes" during HZE irradiation (discussions between the NCRP committee members and individual astronauts at the Johnson Space Center), suggest that CNS damage might occur at relatively low doses of the types of radiation encountered in space. Consequently, NASA has supported a wide variety of research projects designed to determine if simulated space radiation can produce statistically significant neurobehavioral and neurocognitive effects in animal models, primarily rodent models.

Many, but by no means all, rodent studies have shown radiation-induced behavioral alterations and cognitive impairment, indicating changes in various CNS functions, at doses in the range that could be encountered during a deep space exploratory mission. Numerous

differences between the conditions in these rodent studies and human experience in space result in uncertainty about the actual risk space exploration will entail. These characteristics of animal studies include:

- Dose rates which are very high in most experiments.
- Short duration of the exposure, with the exception of the recently started chronic neutron exposure paradigm at Colorado State University in Fort Collins.
- Detection of changes in behavioral and/or cognitive performance of a task which is the result of either minimal or extensive training, learning of a task (acquisition) prior or after the exposure.
- Genetic factors, age at exposure to space radiation, time interval between exposure and testing, and sex of the animals involved in a particular study.
- Presence of environmental challenges other than space radiation in animal studies that differ from those effecting humans during space missions.

NASA requested and NCRP was contracted to initiate a two-phase study to determine if the existing evidence suggested that the radiation environment in space would result in operationally significant CNS impairment. Both acute effects, meaning that they occur during the mission, and late effects, those which would occur after the mission, are of concern. Anticipated after-mission effects include early onset or more severe forms of age-relative cognitive decline, mild cognitive impairment (MCI), and neurodegenerative conditions, including Parkinson's disease (PD) and Alzheimer's disease (AD). During mission, concerns include any neurobehavioral or neurocognitive change which would impact the behavioral and cognitive performance of individual astronauts and the crew's ability to successfully complete all aspects of the planned mission. The product of the first phase of this study, NCRP Commentary No. 25 (NCRP 2016)

indicated that there is a realistic possibility of such acute and delayed effects and recommended proceeding with a more detailed evaluation.

Evaluation of the operational significance of radiation-induced CNS effects is complicated by the fact that very similar, if not identical, neurobehavioral and neurocognitive changes are also induced by many other aspects of the space environment, including microgravity, prolonged isolation in very limited habitable space, sleep problems, elevated or variable levels of CO_2, communication delays, etc. The effects of many of these other environmental stressors can be clearly identified in the astronaut population, because the magnitude of the stress varies enough to correlate with neurobehavioral and neurocognitive changes. However, there is no evidence of how these other stressors interact with ionizing radiation, and no clear evidence of any "pure" radiation-induced CNS effect in crew members.

Subsequent sections of this report will describe:

- Background information including proposed mission profiles, current NASA radiation protection policy, a brief review of the pattern of energy deposition by space radiation, the concerns of astronauts and the public, and the charge to the study committee.
- What is known from observation of humans exposed to radiation during medical procedures or industrial situations and neurobehavioral and neurocognitive effects from other environmental stressors.
- Observations with rodents following simulation of space radiation at the Brookhaven National Laboratories.
- Observations relevant to mechanisms, including cellular neuronal structure and function, and molecular studies.

- Applicability of mechanistic models, including the adverse outcome pathway (AOP) method involving initiating events used by the Environmental Protection Agency, potential repair/compensation mechanisms, and considerations for extrapolation from rodents to humans, including the requirements that must be met for focused/limited non-human primate studies to improve human risk estimates.
- The connection between the physical characteristics of space radiation energy deposition and initiating events in an adverse outcome pathway, as described above, and ways of characterizing radiation exposure in order to correlate with CNS effects.
- Methods for characterizing during-mission risk due to radiation in the absence or presence of other environmental challenges in space, and behavioral, physical, nutritional, and pharmaceutical methods for mitigating risk.
- Methods for characterizing post-mission risk due to radiation in space, and behavioral, physical, nutritional, and pharmaceutical methods for mitigating risk.
- Summary of conclusions and recommendations.

3. Background

The ability of ionizing radiation to cause cell death, mutation, and malignant transformation at various doses is well known. However, some mechanisms responsible for carcinogenesis, including deletions or rearrangements in the DNA of a cell and modification or loss of genetic information in subsequent generations, are unlikely to be relevant to the central nervous system since most CNS cells do not divide after early childhood. Nevertheless, there has long been concern that the unique spatial distribution of radiation damage produced by high energy heavy ions, and other radiations encountered in space, might trigger significant damage in the CNS. High energy heavy ions are capable of producing significant radiation chemistry along their paths through many spatially correlated cells, and low levels of chemical changes in a cylindrical volume surrounding that path. It seems reasonable to assume that this damage might disrupt the function of the CNS which depends strongly on the spatial organization of neurons, synapses, and non-neuronal cells bi-directionally communicating with neurons. Consequently, there has been concern that radiation effects on the CNS might be a limiting factor for space exploration. In addition to direct effects of space radiation on the CNS, effects of radiation on the periphery might indirectly affect CNS function, for example involving primary effects on the gut or liver affecting the CNS through the gut-liver-brain axis, and/or primary effects on immune activation in the periphery.

3.1 Long Duration, High Dose Radiation Exposure Missions

3.1.1 Exploration Mission Profile Summary

As of 2017, NASA plans involve extending human presence in space through operations farther from Earth than previously achieved, with exploratory missions to Mars in the 2030s as a

long standing agency goal (NASA 2015). Private sector enterprises such as Space X are also entering the exploration domain, with Mars as a major target in their line of sight (Musk 2018). For NASA, achieving this goal involves plans for exploration missions (EMs) outside low-Earth orbit and will be accomplished through a phased approach beginning with a series of unmanned and manned exploration missions to the vicinity of the moon, with eleven EM profiles currently described (NASA 2015, Goodliff, 2016, Gerstenmaier 2017). Expanding activities into cis-lunar space will allow the agency to leverage on experience gained through years of research and mission operations on the International Space Station (ISS) in order to perform deep space operations with decreasing reliance on the Earth. Phase one EMs will include testing of the Space Launch System and Orion spacecraft followed by buildup of the Deep Space Gateway, a manned spaceport that will orbit the moon. In phase two, the Gateway will serve as a proving ground and waypoint for assembly and launch of the Deep Space Transport, the spacecraft that will support manned missions further into our solar system including to the vicinity of Mars.

3.1.2 Radiation Exposure Estimates for Exploration Missions

Radiation exposure to the crew for the EM scenarios will be mission specific, and dependent on multiple factors such as exact mission destination and duration, vehicle and habitat design specifications, and solar conditions. Highest galactic cosmic radiation (GCR) exposures occur during periods of solar minimum and lowest GCR exposures occur during solar maximum, but with a higher probability of a significant solar particle event (Cucinotta 2005, Slaba 2013, Simonsen 1991). The cis-lunar EM missions in the current NASA plan are outside low Earth orbit in free space. As such, these missions will not benefit from the protection provided by the

582 Earth's magnetosphere and atmosphere, while missions to the lunar surface will have protection
583 in 2 π directions by the lunar regolith (Clowdsley 2005).

584 Effective dose in free space, (weighted for cancer) resulting from a mixture of primary and
585 secondary particles, is estimated at approximately 1.7 mSv/day at solar minimum. This estimate
586 is consistent with the measured average GCR absorbed dose rate of taken by the Radiation
587 Assessment Detector (RAD) inside the Mars Science Laboratory spacecraft during its cruise to
588 Mars under conditions of low to moderate solar activity (Zeitlin 2013). It is noteworthy that solar
589 activity is currently unusually weak resulting in the deepest solar minimum and weakest solar
590 maximum in over 80 years with higher GCR dose-rates observed by CRaTER (Schwadron
591 2018).

592 Table 3.1 shows estimated absorbed dose (mGy) and effective dose (mSv) for EM designs
593 assuming typical spacecraft shielding at solar minimum. Planned Gateway missions range in
594 duration from approximately 20 to 40 days with total estimated crew absorbed doses ranging
595 from 12 to 24 mGy and effective doses varying from 35 to 70 mSv. Deep Space Transport EMs
596 range in duration from approximately 200 days to a final 400 day shakedown cruise in cis lunar
597 space with absorbed dose estimates from 120 to 240 mGy and effective dose estimates of 350 to
598 700 mSv at solar minimum. A 2 year mission to the vicinity of Mars would have an effective
599 dose of approximately 1300 mSv at solar minimum. Note, since the first Mars transit mission
600 will be entirely in deep space with no plans for landing or surface operations, the dose estimates
601 are in line with estimates for previously described conjunction class or opposition class Mars
602 design reference missions (Drake, 2009). Although the mission length is longer for the
603 opposition and conjunction class reference missions, the protection offered by the Mars planetary
604 body and atmosphere offer some reduction in the total dose to the crew compared to a continuous

flight in deep space. Dose rate estimates for the lunar surface are approximately half the dose rate for deep space with an additional contribution from neutrons produced by interaction of primary galactic cosmic rays with the lunar regolith (Slaba 2011). Finally, for comparison, the typical ISS exposures, where the Earth's magnetosphere attenuates lower energy GCR, absorbed dose rates are on the order of 0.3 mGy/day, and effective dose rates are approximately 0.6 mSv/day, depending on solar activity, and ISS altitude (Cucinotta 2008; Reitz 2005).

611 **Table 3.1. Multi-step Mission Plans for Mars with Associated Estimated Absorbed**

612 **Doses and Effective Doses**

Multi-step Plan for Mars - Exploration Mission (EM) Scenarios						
Mission		**Destination**	**Mission Duration**	**Number of Crew**	**Approximate Absorbed Dose (mGy)**	**Effective Dose (mSv)**
ISS*		LEO	6 months - 1 year	6 mos: 6 person; 1 year: 2 person	30 - 120	50 - 200
Deep Space Gateway^	EM2 - EM5	cis-lunar space	20 - 40 days	4 person crew & unmanned cargo missions	12 - 24	35 - 70
Deep Space Transport^	EM6 - EM10	cis-lunar space	200 - 400 days	4 person crew & unmanned cargo missions	120 - 240	350 - 700
Mars Flyby/ Opposition/ Conjunction**	EM11	Mars	up to 3 years	4 person crew	300 - 450	1000 - 1300
Lunar Habitat/Rover^^		Moon	up to 1 year	tbd	100 - 120	300 - 400

* dose estimated from dosimetry and flight experience; dependent on altitude and time in solar cycle

^ dose estimates are for solar minimum assuming average shielding (10 gm/cm^2)

** dose estimates are for solar minimum with average spacecraft shielding & a variety of mission parameters

613 ^^ estimates are for solar minimum and include additional contribution from albedo neutrons

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3.2 Current NASA radiation protection policy

3.2.1 Health Risks Associated with Space Radiation Exposure

The National Aeronautics and Space Administration's (NASA's) Human Research Program (HRP) has identified space-related health risks covering both short-term effects that may impact crew health, safety and performance in mission, as well as long term health consequences that may happen months to years following spaceflight. To ensure safe and productive human space exploration, the HRP performs the research necessary to characterize and establish an evidence base for these risks, to develop appropriate countermeasures, tools and technologies for risk mitigation and to set appropriate health and performance standards. The HRP's current research portfolio, strategies and evidence base are described in the HRP Integrated Research Plan (IRP) available online in the Human Research Roadmap, a managed tool used to convey these plans (https://humanresearchroadmap.nasa.gov/).

For health hazards associated with exposure to the radiation environment in space, four main areas of risk are recognized by the NASA HRP:

- Risk of Acute (In-flight) and Late (Post-flight) Central Nervous System Effects from Radiation Exposure (Nelson 2016)
- Risk of Acute Radiation Syndromes Due to Solar Particle Events (SPEs) (Carnell 2016)
- Risk of Cardiovascular Disease and Other Degenerative Tissue Effects from Radiation Exposure (Patel 2015)
- Risk of Radiation Carcinogenesis (Huff 2016)

3.2.2 NASA Human Health and Performance Standards for Radiation Protection

In order to limit crew exposure to hazardous conditions and maintain crew health within acceptable parameters, NASA has established standards for a number of the identified health and performance risks. An overview of the approach to risk management and process to develop and update health standards that is utilized by NASA is described in Kahn (2014). For radiation protection, human health standards inform the development of mission and vehicle specific design requirements and crew certification for flight. These standards are in the form of space permissible exposure limits (SPELs, or PELs) and include both short-term (30 days, 1 year) and career limits. These limits are in place to prevent both in mission and late, clinically or operationally significant adverse outcomes and/or limit risk to a level that NASA deems acceptable to the crew. Standards are reviewed and approved by the office of the NASA Chief Health and Medical Officer and are documented in NASA Standards 3001 Volume 1, Rev A. (NASA 2014). Standards are reviewed and updated on a 5 year cycle with the next review being scheduled for 2019.

A description from NASA Standards 3001 Volume 1 of the Space-Permissible Exposure Limits (SPEL) for Space Flight Radiation Exposure Standard is as follows: “Radiation sources in space consist of the galactic cosmic rays (GCR), trapped radiation, and solar particle events (SPEs). As missions progress to outside LEO and away from the protection of Earth’s magnetic shielding, the nature of the radiation exposures that astronauts encounter changes to include higher GCR and possible SPE exposures. SPEL for radiation have the primary functions of preventing in-flight risks that jeopardize mission success and of limiting long-term risks to acceptable levels based on legal, ethical or moral, and financial considerations. Both short-term and career exposure limits are applied using assessments of the uncertainties in projection models with the space radiation environment defined by the program. Uncertainties are related to

gaps in knowledge of biological effects of GCR heavy ions and the nature of SPEs. Although specific exposure limits are identified based on mortality risk, in all cases, decisions concerning vehicle, habitat, and mission design are made such that resulting crew radiation exposures are as low as reasonably achievable (ALARA). As an operating practice, ALARA is a recognized NASA requirement. However, at the current time, the large uncertainties in GCR risk projections prevent an effective ALARA strategy for shielding approaches to be developed. For SPEs, uncertainties are smaller, acute risks are of concern, and ALARA is possible".

From NASA Standard 3001, Volume 1, the space permissible exposure limits are as follows (with Tables 3.2 and 3.3):

1. Planned career exposure to ionizing radiation shall not exceed 3 percent Risk of Exposure-Induced Death (REID) for cancer mortality at a 95 percent confidence level to limit the cumulative effective dose (in units of Sievert) received by an astronaut throughout his or her career.
2. Planned radiation dose shall not exceed career and short-term limits as defined in table 3.2, Dose limits for Short-Term or Career non-cancer Effects (in mGy-Eq. or mGy).
3. Lifetime fatality risks for non-cancer circulatory and CNS diseases shall be limited as defined by career dose limits in table 3.2.
4. Exploration Class Mission radiation exposure limits shall be defined by NASA based on NASA-requested recommendations from the National Academy of Sciences, the Institute of Medicine, and the National Council on Radiation Protection and Measurements (NCRP).
5. In-flight radiation exposures shall be maintained using the as low as reasonably achievable (ALARA) principle.

Table 3.2. Dose Limits for Short-Term Career Non-Cancer Efects (in mGy-Eq. or mGy)

Note: RBEs for specific risks are distinct as described below.

Organ	30-day limit	1-Year Limit	Career
Lens*	1,000 mGy-Eq	2,000 mGy-Eq	4,000 mGy-Eq
Skin	1,500	3,000	6,000
BFO	250	500	Not applicable
Circulatory System**	250	500	1000
CNS***	500 mGy	1,000 mGy	1,500 mGy
CNS*** (Z≥10)	-	100 mGy	250 mGy

*Lens limits are intended to prevent early (<5 yr) severe cataracts, *e.g.*, from a solar particle event. An additional cataract risk exists at lower doses from cosmic rays for sub-clinical cataracts, which may progress to severe types after long latency (> 5 yr) and are not preventable by existing mitigation measures; however, they are deemed an acceptable risk to the program.

**Circulatory system doses calculated as average over heart muscle and adjacent arteries.

***CNS limits should be calculated at the hippocampus.

694 **Table 3.3 RBE for Non-Cancer Effects of the Lens, Skin, Blood-Forming –Organs, and**
695 **Circulatory Systems**

Radiation Type	Recommended RBE[b]	Range
1 to 5 MeV neutrons	6.0	(4-8)
5 to 50 MeV neutrons	3.5	(2-5)
Heavy ions	2.5[c]	(1-4)
Proton > 2 MeV	1.5	-

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697 [a] RBE values for late deterministic effects are higher than for early effects in some tissues and
698 are influenced by the doses used to determine the RBE.

699 [b] There are not sufficient data on which to base RBE values for early or late effects by neutrons
700 of energies <1 MeV or greater than about 25 MeV.

701 [c] There are few data for the tissue effects of ions with a Z>18, but the RBE values for iron ions (Z=26) are
702 comparable to those of argon (Z=18). One possible exception is cataract of the lens of the eye because
703 high RBE values for cataracts in mice have been reported.

704

Table 3.3, excerpted from the NASA Standard 3001 volume 1, is from NCRP report 132 (NCRP 2000), which recommended to adjust organ doses by an appropriate RBE to account for radiation quality, with dose limits for deterministic effects given in gray equivalents. These best estimates of RBE values are based on data from multiple endpoints and radiation qualities described in ICRP Publication 58 (ICRP 1990).

The cancer PEL limits the cumulative effective dose (Sieverts) received by an astronaut throughout his or her career in an age and sex dependent manner. Cancer is considered a stochastic risk, meaning the severity is independent of dose with no dose threshold considered so the standard is applicable to very small doses and the probability of occurrence increases with increasing dose. NASA's risk projection model provides a probability distribution function describing uncertainty in REID. The central value can be used as an estimate with the confidence level accounting for uncertainties, which currently vary between approximately150% to 300%, depending on the specific exposure type (GCR, SPE) and shielding. In contrast, the non-cancer risks are considered deterministic (tissue reactions), meaning both the probability of occurrence and the severity increases with dose above a threshold where clinical effects can be observed. These limits do not account for potential influence of age or sex. The short-term limits are imposed to prevent clinically-significant non-cancer health effects including performance degradation, sickness or death in-flight, as well as the possibility of in-flight CNS effects. Career dose limits for lens, circulatory system, and central nervous system are imposed to limit or prevent risks of degenerative tissue diseases (*e.g.*, stroke, coronary heart disease, neurodegenerative diseases, etc.) that occur post-mission. Since the Apollo era, NASA has received guidance on issues involving radiation protection from external agencies and boards with recommendations to NASA by the NAS Space Science Board (NAS/NRC, 1967; 1970) and

the NCRP (NCRP, 1989; 2000). Guidance has evolved over time to reflect new information on radiobiology, epidemiology, space environment and alterations in the make-up of astronaut crews. Current NASA PELs are based on guidance in NCRP Report No. 132 (2000) Radiation Protection Guidance for Activities in Low-Earth Orbit. Cucinotta, (2010) provides an historical perspective on NASA's short-term and career radiation limits. More recently, NCRP Commentary 23 Radiation Protection for Space Activities: Supplement to Previous Recommendations (2014) provides an in-depth review of the NASA space radiation PELs and associated evidence base, and includes commentary on the use of and applicability of the current space PELs for extended missions in LEO on the ISS and for exploratory missions outside LEO (NCRP, 2014). For the degenerative tissue risks, the NCRP 23 commentary states that "current dose limits for noncancer endpoints are reasonable for application to 1yr missions in LEO within the framework given by the Chief Health and Medical Officer (Williams, 2012). However, for exploration class missions, application of the current dose limits for non-cancer endpoints is less clear and needs additional review". For CNS, "caution must also be exercised with regard to consideration of CNS risks for exploration missions. Recent experimental data for HZE-ion risks obtained using animal models and human-derived culture models raise questions about the possibility of neurobehavioral effects at lower doses of HZE particles that may be relevant to exploration class missions. Additional research is needed to determine whether current dose limits for acute effects are sufficiently robust to ensure crew member safety during and after exploration missions".

For the CNS, the dose limits are preliminary and are based on experimental evidence from animal models. The CNS PELs correspond to doses at the region of the brain called the hippocampus and are set for time periods of 30 days or 1 year, or for a career with values of 500,

1,000, and 1,500 mGy, respectively. Note that since the RBE for CNS effects is currently unknown, a physical dose limit is utilized with an added requirement for particles with charge Z ≥ 10. NASA uses computerized anatomical geometry models to estimate the body self-shielding at the hippocampus to obtain exposure profiles that are intended to be representative of the whole brain for GCR exposures (Nelson 2016). The accuracy of the CNS PELs is especially important as NASA considers exploratory missions outside LEO where radiation exposures will be higher. Acceptable limits need to be considered in context of the whole body as indirect effects from exposure of other organ systems may impact the CNS. Likewise, as missions extend further into our solar system the contribution from additional stressors associated with the decreased reliance on Earth will play a larger role and must be considered.

3.3 Characteristics of Radiation in Space

The radiation environment in space depends strongly on location and time. In interplanetary space, beyond the influence of a planet's magnetic field, the radiation environment is composed of Galactic Cosmic Rays, GCR, Solar Event Particles, SEP, and secondary particles resulting from interaction of these primary radiations with the matter (the spacecraft, human body, etc.). Near a planet with a magnetic field, trapped radiations are added to this. On the surface of a planet, there may also be a significant contribution from secondary radiations produced in the planet's atmosphere and regolith. The characteristics of these radiations, and their spatial and temporal variations, have been reviewed in NCRP reports (NCRP 2000, 2006, 2014) and the Phase one report of the CNS effects study (NCRP 2016). The major features are summarized here:

- Most of the dose is delivered by high velocity charged particles, atomic nuclei, with a

small fraction delivered by electrons and photons. Neutrons can be a significant component of secondary radiation.

- GCR particles include all stable nuclei, approximately at the same ratio as the atoms in the universe, so protons dominate the fluence but nuclei up to iron contribute significant doses because their lower fluence is compensated by their higher stopping power.
- The GCR fluence is modified by the magnetic field created by the solar wind. At solar maximum, when the solar wind is greatest, the lower energy portion of the GCR spectrum is deflected away from the solar system and the absorbed dose rate is about half that at solar minimum.
- Solar particle events appear to occur randomly, but their probability is roughly correlated with sun spot activity.
    - The spectra and fluence of solar event particles vary widely from one event to another.
    - The fluence from impulsive events, probably related to sun spots, reaches a maximum in a few hours and decays away in a day or two. The particles are distributed over a relatively narrow range of angles, and follow the Sun's magnetic field lines, an Archimedes spiral.
    - Coronal mass ejections result in solar particles being accelerated by shocks. The particle fluence increases more slowly and decreases more gradually than with an impulsive event, typically lasting several days. The particles cover a wider range of angles, 2 or 3 steradians, and their directions are essentially isotropic by the time they reach the Earth's orbit.
    - Both types of solar events produce primarily protons with energies generally up to

a few hundred MeV, with very few particles heavier that helium.

- Secondary radiations include projectile and target fragments, often including neutrons
  - Target fragments produced by light ions, such as protons, have lower velocities and higher stopping powers than the incident radiation
  - Projectile fragments often have nearly the same velocity as the projectile that produced them, and lower stopping power due to lower Z.

The charged particles, GCR and SEP, which deliver dose in space, have energies on the order of 100 to 1000 times the energy of charged particles encountered in medical and industrial settings. Consequently, although the absorbed doses in space are large compared to the doses received by most terrestrial radiation workers, they are produced by relatively few charged particles. As a result, the energy deposition events in individual cells are generally widely separated in time. For example, typical cell nuclei are hit by GCR protons about 58 times per year (table 3.4) resulting in an average of 6 days between proton hits. Consequently, individual cells typically have adequate time to repair any repairable damage or to complete other responses to energy deposited. However, adjacent biological targets are often affected at nearly the same time since the ranges of most GCR particles are long compared to the size of biological targets such as cells. As a result, if there are interactions between irradiated targets, which alter the effectiveness of repair, the radiation in space may show some of the characteristics of high dose rate irradiation, even though the actual absorbed dose rate is quite low. The frequency of energy deposition events, produced by the primary cosmic ray particles, in some individual biological targets, neglecting the effects of delta rays, is indicated in Table 3.4. Eight micrometer diameter cell nuclei, 1 cm segments of 1 micrometer diameter axons, and synapses, assumed to be 10 nm

in diameter, are included in the table. Although the rates of interactions in individual objects are quite low, typically less than one event per year for the heavy ions interacting with cell nuclei, and much lower for smaller targets, they still amount to a large number of events per gram of tissue. Furthermore, though the occurrence of a charged particle track through a tissue is a random event, the occurrence of energy deposition in a biological target is not random, and is correlated with energy deposition in other targets along individual charged particle tracks. Since high velocity charged particles produce delta rays with significant ranges, many cells not traversed by the primary particle are hit by a delta ray. Consequently the number of targets affected per centimeter of cosmic ray track is often quite large.

In spite of the very large differences in fluence of protons and ions heavier than helium, protons and heavy ions make roughly equal contributions to the dose equivalent. This is because a single heavy ion delivers much more energy than a proton and because the quality factor applied to heavy ion dose is much more than 1.0. If the RBE of heavy ions for CNS damage is less than for cancer induction, different weighting factors might be appropriate for evaluating CNS risk.

Table 3.4. — Number of times an individual object is hit per year, the number of objects hit in 1 g of tissue per hour, and the number hit by a single track, for listed GCR particles at 1 AU during solar minimum (Straume et al 2017).

ion	Total Flux per cm^2-day	Cell nucleus[!]			1 cm of axon[!!]			Synapse[!!!]		
		Hits/y in each nucleus	No. hits per hour*	Hits per track*	Hits/y in each cm of axon	No. hit per hour**	Hits per track**	Hits/y in each junction	Number hit per hour***	Hits per track***
proton	3.19×10^5	58	25×10^5	190	11630	1.7×10^7	1300	9.0×10^{-5}	2.0×10^8	15000
carbon	8.91×10^2	0.17	7×10^3	190	32.5	4.7×10^4	1300	2.54×10^{-7}	5.5×10^5	15000
Silicon	1.23×10^2	0.023	1000	190	4.5	6.5×10^3	1300	3.5×10^{-8}	7.6×10^4	15000
Iron	8.22×10^1	0.015	640	190	3.0	4.4×10^3	1300	2.3×10^{-8}	5.1×10^4	15000

! 8 µm diameter sphere

!! 1 µm in diameter

!!! 0.01 µm diameter sphere

* In 1 g of tissue assuming 10 % of tissue is cell nuclei; 3.73×10^8 nuclei/g

** In 1 g of tissue assuming 10% of tissue is axons; 1.27×10^7 cm of axons/g

*** In 1 g of tissue assuming 1% of tissue is synapses; 1.91×10^{16} synapses/g

3.4 Phase 1 Report Recommendations

The conclusions of the phase 1 report were:

- Exploration-mission-relevant doses of HZE particles in animals, environmental exposure to other radiation insults, and clinical effects of therapeutic radiation exposure suggest that significant alteration in brain function can occur due to exposure to radiation. Taken together these findings warrant a detailed Phase II study leading to recommendations for future actions.
- The structure and function of the CNS results in damage mechanisms which are different from those for cancer induction.
- Other sources of stress during space missions may mask or add to any radiation-induced CNS effects.
    - Environmental factors, workload, and perceived risk may trigger CNS responses which are independent of, additive to, or synergistic with radiation-induced CNS effects that could affect behavior and impair performance
    - Attempts to mitigate effects of stress, such as use of pharmaceuticals to improve sleep, may result in additional CNS perturbation
- The nature of CNS damage leading to functional and cognitive effects is significantly different from that for cancer [cancer can be initiated through a wide range of mechanisms, often involving deoxyribonucleic acid (DNA) changes (mutagenesis), leading to uncontrolled clonal expansion] and therefore effects on the CNS may require a different approach to characterizing radiation exposure

- Functional and cognitive CNS effects can be induced by significant cell damage outside the nucleus.
- It is likely that many sequelae do not involve clonal expansion mediated by cell proliferation.

- Animal research addressing cell and molecular basis of damage and behavioral outcomes is needed to relate to both simple and complex behaviors relevant to the human risks.
  - Relevant animal or cellular models need to be defined based on the questions relevant to the human risks.
  - Animal studies should include pathological and morphological endpoints as well as behavioral outcomes including complex responses (executive functions).
  - A Phase II study should include analysis of any available primate data on CNS effects of radiation [e.g., as might come from the evaluation of radiation countermeasures in the Medical Countermeasures Against Radiological and Nuclear Threats program of the National Institute of Allergy and Infectious Diseases (NAIAD, 2012)].
- Methods for assessing the CNS damage and its possible impact on crew-member health and mission objectives need to be developed.
  - Life-long medical follow-up of crew members to evaluate potential CNS changes is essential.More research is needed to characterize the interaction of radiation exposure with other aspects of crew-member environment (e.g., sleep, lighting, exercise, and diet including micronutrients, supplements) and the pharmaceuticals or other methods used to mitigate these factors.

- Transgenic animal studies suggest that interindividual genetic differences may alter risk.
- Clinical studies with patients exposed to high-energy heavy-ion radiation, and occupational studies (e.g., workers exposed to polonium) are very limited but may be useful for evaluating tests for CNS function.

- Integration of data across all biological scales (subcellular damage through human behavior) and for all CNS endpoints should be emphasized.
- Pre- and post-flight imaging and biomarkers for CNS diseases, including tissue banking should be used to the fullest extent possible.
- Systems biology or medicine and animal mechanistic studies are generally considered independent disciplines and do not, in the present environment, inform each other conceptually. Steps should be taken to encourage interacting and integrative work at all levels of conceptualization.
- Comparison of the assessment results for exposed crew members with those for other nonexposed groups is essential (providing controls for exposed crew-member follow-up studies).
- Use of autonomic nervous system responses as indication of damage should be evaluated.
- Since mechanisms leading to functional and cognitive CNS effects may be different from mechanisms leading to cancer, which underlie current risk mitigation procedures, alternative approaches to risk management should be investigated.

The phase 1 report also makes the following recommendations:

A. Proceed with the Phase II Report as outlined in recommendation B.

B. The work of a Phase II committee should summarize and critically review the existing literature on each of 5 main topics and make recommendations for research and other directions needed within each topic. These topics, as clarified through discussions with NASA leadership, are:

1. Mechanisms for radiation damage in the CNS

- These are likely different from those for cancer because DNA damage and changes in cell proliferation may not be critical issues for induction of CNS damage where they are for cancer. Mechanisms for radiation damage and differences with the carcinogenic process will be evaluated..
- The structure and anatomy of the brain/CNS is quite different from epithelial tissues/organs in which most cancers develop; therefore, the interactions of charged particles tracks with cell/tissue structures may play different and quite complex roles in CNS compared to other tissues.
- The CNS is a complex environment of cell types and their interactions; hence, cell-cell interactions, the immune system and inflammation may play important roles in the development/expression of radiation-induced CNS damage and these are likely different from epithelial tissues..
- These important differences between CNS and epithelial tissues likely mean that the models that have been used for predicting cancer risk cannot be applied easily to CNS damage; new models and ways of thinking may be needed and approaches developed.

2. Experimental animal research

- This research needs to use endpoints that relate to both simple and complex

human behaviors, covering not just cognition and memory aspects, but including possible post-traumatic-stress-disorder-like changes.

- Development of biomarkers and bioindicators of detrimental outcomes in animals are needed that will also be applicable to and usable in humans/astronauts.
- It may be advisable to consider standardization of experimental radiation protocols - such as choices of charged particles and energies, doses and dose rates, as well as endpoints - so that data can be more readily compared among investigative teams and related to realistic space exposures.

3. Human data

- There are some sets of human data that might provide insight into charged-particle effects on the CNS (e.g., patients who have received brain irradiation for cancer or arterial venous malformations; occupationally exposed populations) should be explored.
- There is a critical need for long-term, regular follow-up of individuals who have flown in space, including appropriate assessments of potential detrimental CNS effects. Understanding that there are privacy and ethics concerns, an evaluation should be made for a means to make relevant health information available to appropriate researchers.

4. Integration of data across all biological scales (subcellular damage through human behavior)

- Systems biology or other approaches should be explored for integrating data across biological scales ranging from subcellular damage initiated by energy

963 distributions from particle tracks through cell responses and tissue changes to
964 cognition and behavior outcomes.

965 • The approaches need to integrate data from cells, experimental animals and
966 humans, including any epidemiology data available, and any relevant astronaut
967 data in particular, into a coherent model that will facilitate development of risk
968 projections, and accordingly, .risk management,

969 5. Interactions of radiation with other factors

970 • Radiation may interact with, or its effects be modified by, other factors in the
971 space microenvironment, such as hypogravity and altered oxygenation, etc..
972 This would also include space-flight factors such as stress, altered sleep
973 patterns, changes in exercise, diet etc. Little research has been conducted to
974 date on such potential interactions.

975 • Genetic susceptibility issues should be considered.

976

977 C. The Phase II effort should have access to expertise in the following fields:

978 • crew-member performance evaluation (Topics 3 and 5);

979 • CNS damage in animal models (Topics 1, 2, and 3);

980 • clinical manifestation of CNS damage (Topics 3 and 5);

981 • radiation physics and dosimetry (Topics 1 through 5);

982 • NASA plans and procedures (Topics 3 and 5);

983 • neuroendocrinology (Topics 2, 3, and 5);

984 • neurotoxicology (Topics 2, 3, and 5);

985 • CNS structure and function (Topics 2 and 3);

- space radiation biology and molecular biology (Topics 1, 2, 4, and 5);
- neurology (Topic 3);
- human behavioral science (Topics 3 and 5);
- human factors/interface design (Topics 3 and 5); and

In addition, the NCRP proposal posed 8 specific questions

1. How should a "significant impairment" in performance be defined? What are the performance domains that could be significantly affected? What constitutes a "significant impairment" in the context of actual performance and operation?
2. Do risk assessments for chemical toxicity, including neurotoxicity, provide guidance? Are the IARC evaluation schemes for chemical neurotoxicity of value in assessing health risks?
3. Is the adverse outcome pathway approach used by EPA useful in the context of space radiation and CNS effects? What are the key events related to adverse outcome in behavior/performance impairment that might affect mission and, conceivably, be related to late effects such as dementia?
4. Are non-human primate (NHP) experiments necessary, and if so, how should they be considered?
5. Are the CNS effects, including the behavioral impairment domains, deterministic or stochastic? That is, assuming that there is sufficient evidence for concern, are there threshold levels below which the concern is minimal?
6. How might space radiation "interact" with other aspects of a mission that would impair performance, both for the individual and the team, such as sleep

deprivation, medications, hypogravity, constant close quarters, reduced communications, absence of windows to the world, and other?

7. What is the relative balance between the likelihood of neurobehavioral effects that would impair operational performance and adversely affect the mission, and the likelihood that serious neurodegenerative disease develop such as Alzheimer, Parkinson, Huntington, amyotrophic lateral sclerosis (ALS), and dementia?
8. True to all human tissues, the brain is designed to respond rapidly to changes and then compensates for survival. Do compensation mechanisms exist that would influence the likelihood of getting to a level of significant impairment that would adversely affect performance and the mission?

The 5 main topics and 8 specific questions served as guidance for the committee in efforts to summarize and review existing literature, recommend future research, and provide guidance for risk management and radiation protection.

3.5 Possible CNS Effects of Space Radiation Exposure

As already mentioned, most cells in the CNS are non-dividing; thus, radiation effects in the brain, excluding the adult neurogenic areas, are anticipated to be different than effects in proliferating peripheral tissues. In particular, available data suggests that mechanistic effects underlying CNS risk fall more in the realm of persistent oxidative stress, inflammation and cellular senescence rather than apoptosis or genetic instability leading to carcinogenesis. Based on human and animal studies described in upcoming sections, potential space radiation effects in astronauts exposed to the deep space environment may include alterations in dendritic structures and synapses associated with changes in neural connectivity, alterations in cell function,

signaling and differentiation, and possible long-term effects on protein handling. Whether these changes arise from direct effects of radiation on the brain and nerve cells, or from changes in the microenvironment in or outside the brain mediated by cellular or systemic responses to radiation (*e.g.*, inflammation, gut-liver-brain axis, or cardiovascular effects), the concern is that they may impact CNS function. Particularly for NASA, CNS risks include alterations in the ability of crewmembers to carry out mission-relevant tasks (*e.g.*, increases in reaction time, reduced attention, detection of novelty in the environment, spatial navigation, or other mission-relevant forms of learning and memory) and increased risk to develop age-associated CNS disease (*e.g.*, stroke, Alzheimer's disease (AD), or Parkinson's disease (PD)) long after the mission is completed.

In considering risk to the CNS, it is important to note that with the exception of cataract development, which has been associated with astronaut exposure to radiation (Cucinotta, Manuel et al. 2001, Chylack, Peterson et al. 2009) and is a risk to the CNS as it interferes with behavioral and cognitive performance, there have been no confirmed associations between only space radiation exposure (*i.e.*, in the absence of other environmental challenges pertinent to astronauts during missions) and behavioral or cognitive changes or risk for earlier or more severe age-related cognitive decline, mild cognitive impairment (MCI), or neurodegenerative conditions such as PD and AD, in astronauts following the missions. There have been reported cases of PD, but in the relatively small pool of astronauts, it is hard to determine whether there might be increased risk. The lack of a control group that is matched in education and life style with the group of astronauts is a major challenge, not only in determining CNS risk but also in determining other risks, including cancer risk. For example, knowing that education and a healthy lifestyle are protective with regard to cancer and CNS risks, one could easily

underestimate the risks if the control group includes those in the general public that are not matched in education and/or lifestyle. This highlights the importance of controlled animal studies to determine CNS risk following space radiation. Although accumulating data is available for rodents that are subjected to components of space radiation at total doses relevant for space missions, the radiation exposure is generally given acutely, occasionally as a fractionated dose, but hardly as a chronic dose. In contrast to the animal space radiation data, the human data are described at much higher doses of low-LET radiation. Thus, it is not trivial to use the currently available animal space radiation data or human data to determine risk in the relatively modest sized populations of astronauts exploring the deep space environment for periods up to several years. This is further compounded by a relative lack of experimental evidence exploring the interactions of established environmental challenges/stressors, other than irradiation, such as sleep deprivation or isolation/confinement, on behavioral and cognitive performance.

4. Observations of Humans

Observations of the impact of radiation on cognitive function in humans come from two primary categories of data: incidental radiation exposure in the environment and therapeutic exposures to radiation as part of medical procedures. Data arising from environmental exposures are limited by the accuracy of quantitative information on dose and duration of radiation exposure. Therapeutic medical procedures employing radiation precisely quantify dose and duration administered, and often projected dose to the target organ. For this reason, this section will focus on side-effects of medical exposures. A significant caveat to this discussion is that medical exposures typically involve high doses over a relatively brief period of time. An additional caveat is that therapeutic radiation is administered to individuals with medical illness, not otherwise healthy individuals. In many circumstances, the illness the individual has developed prior to radiation exposure could impact cognitive and behavioral function independent of the effects of the radiation. Although data is limited, recent research suggests children diagnosed with Acute Lymphoblastic Leukemia (ALL) demonstrate elevated levels of myelin basic protein and glial fibrillary acidic protein in assays of cerebrospinal fluid collected at diagnosis, suggesting disease impact on brain white matter integrity prior to any treatment exposure (Cheung et al., 2018).

4.1 Side effects of medical exposures

Side effects of medical exposures can be categorized into acute- and late-effects, as well as direct and indirect effects. Acute effects begin near diagnosis and may progress throughout treatment exposure, and patients may partially or fully recover cognitive function. Late-effects are defined as cognitive problems present several years following diagnosis and treatment and

tend to be persistent. Although in some cases the late-effect may begin shortly after exposure to radiation, at that time they are generally subtle and they typically worsen or evolve over time. Direct effects of radiation exposure on brain function occur as a result of energy deposition in neurons, glial cells or cerebrovascular membranes. The process may begin relatively soon after radiation exposure and may progress with time. Indirect effects of radiation on brain function result from a secondary condition that arises in response to radiation exposure of other organs, with this secondary condition impacting brain function. Examples of indirect effects include pulmonary deficits associated with chest irradiation and hypothyroidism associated with neck radiation, both of which can lead to a secondary impact on brain function. Indirect effects take many years to manifest and are predictable as the secondary condition is present prior to the brain impairment. Generally, a relatively large dose of radiation is required to produce a secondary condition severe enough to result in brain impairment. Consequently, this review will focus on acute- and late-effects associated with direct cranial radiation exposure.

The type and amount of therapeutic radiation exposure is dictated by the specific diagnosis requiring such exposure and these diagnoses differ in juveniles compared to adults. Although both children and adults will receive therapeutic radiation for central nervous system tumors, children have also historically received prophylactic radiation exposure to the brain for treatment of ALL, the most common form of cancer of childhood. This cancer is relatively less common in adults, though when present adults will also receive prophylactic cranial radiation. Adults also receive prophylactic cranial radiation as part of treatment for lung cancer, which is a much more common cancer type. Given the different diagnoses common among children vs adults, literature from these populations will be discussed separately.

In addition to differences in the intensity of radiation exposure associated with cancer therapy and intensities likely to be experienced in astronauts, difference also exist in age at radiation exposure. Among those patients who receive prophylactic radiation for lung cancer, the average of onset is 70 years, much older than the average age of astronauts. Childhood leukemia is diagnosed at less than 21 years of age, younger than the average age of astronauts. However, as illustrated below, cognitive consequences of radiation exposure in both patient groups are similar and, thus, both juvenile and adult studies are important to consider in reference to effects of known doses of cranial irradiation.

4.1.1 Juvenile exposures

Existing literature on the effects of radiation exposure in children comes primarily from two diagnostic groups: treatment of central nervous system tumors and treatment of ALL. Although children who undergo bone marrow transplant also receive radiation to the brain, through total body irradiation, transplant recipients receive other procedures and develop other complications (e.g. graft *v*. host disease) which make interpretation of the limits available results difficult. The goal of radiation exposure for treatment for CNS tumors is to eliminate the cancer cells of the primary tumor, either as the primary treatment or following surgical removal of the tumor itself. The location of the tumor and the aggressiveness of the cancer will generally determine the type and dose of radiation administered. Tumors that are well-encapsulated may be treated with surgery only or surgery combined with focal radiation to the tumor bed. This focal radiation will often approach doses of 50 Gy, typically delivered in fractions of 1.8 Gy. More invasive or aggressive tumors may also be treated with surgery and radiation focused on the tumor bed, but will generally also be treated with whole brain or craniospinal irradiation to eliminate cancer

cells that may have moved to regions distal to the tumor bed and are at risk for developing additional tumor sites. Roughly 24 or 36 Gy whole brain or craniospinal irradiation has historically been used, again administered in fractions of 1.8 Gy.

Prospective studies examining the effects of medical doses of cranial radiation for CNS tumors suggest acute- and late-effects. Although subtle decline in processing speed may be observed immediately following irradiation, cognitive impairment typically evolves over the course of several years. In a prospective longitudinal study of brain integrity in 146 children treated for medulloblastoma (93 with average risk disease treated with 23.4 Gy craniospinal irradiation, 53 with high risk disease treated with 36 Gy craniospinal irradiation; both groups also treated with boost to the posterior fossa reaching a total of 54 Gy) compared to 72 healthy controls, magnetic resonance imaging (MRI) and neuropsychological testing was conducted at baseline (within a few weeks of completing therapy) and annually over 36 months (Glass et al.,2017). No difference in frontal white matter volume or cognitive performance was observed at baseline. Over the course of the 36-month follow-up, frontal lobe white matter increased in controls, showed no change in average-risk patients and declined in high-risk patients. Slowed processing speed in the patients became apparent at the 36-month follow-up, with no difference between average- and high-risk groups. White matter integrity, as measured by diffusion tensor imaging, was impacted throughout the brain and was associated with the decrease in processing speed at the 36-month follow-up. Children treated with focal radiation for low grade gliomas also experience a gradual decline in cognitive function. Among 78 such survivors followed from diagnosis over 60 months and treated with 54 Gy focal radiation, gradual decline in spelling and reading occurred over the five-year interval (Merchant et al., 2009). However, this decline was

moderated by age at diagnosis, such that those diagnosed at 12 or more years of age displayed no significant change over time, as displayed in Figure 4.1.

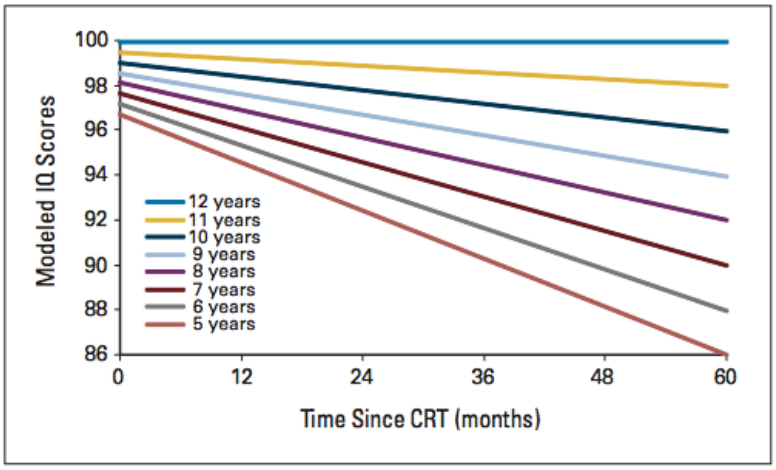

Fig.4.1 Regression based model of change in spelling from diagnosis to five years post diagnosis. From Merchant et al, *Journal of Clinical Oncology*, 2009.

The impact of radiation on cognitive function appears progressive not only during the years immediately following therapy, but may also progress during adulthood. In a long-term follow-up study of cognitive outcomes in 224 adult survivors of childhood CNS tumors (mean time from diagnosis 17.7 years), 71 survivors treated with only focal radiation to the tumor bed (mean dose of 54 Gy) demonstrated a 40% increased risk for attention and processing speed problems and a 90% increased risk for memory problems compared to 63 survivors of CNS tumor treated without radiation (Brinkman et al., 2016). The addition of a mean dose of 35 Gy whole brain radiation increased the risk for problem in attention, processing speed and memory to 72%, 45% and 193%, respectively, above that seen in the group without whole brain radiation exposure.

Although relatively rare, cranial radiation increases risk for development of meningiomas. Among over 4000 adult survivors exposed to cranial radiation as children, the cumulative incidence of meningioma was 4% after a mean interval of 20 years (Bowers et al., 2017). Cumulative cranial dose of 20-29.9 Gy radiation was associated with a 60% increased risk compared to those who received radiation doses less than 20 Gy. Further, 20% of those with meningiomas reported significant neurologic sequela.

Given many of the difficulties associated with separating the effects of radiation from a space occupying CNS tumor, radiation therapy in childhood ALL may be a better model to examine the effects of radiation exposure. As stated above, cranial radiation in ALL is given prophylactically to prevent cancer cells that may have entered the cerebrospinal fluid from turning into secondary brain tumors. Although prophylactic radiation treatment of the brain is rarely used today in ALL therapy, much research has been completed with children treated in the 1980's through the early 2000's. Radiation doses began with 24 Gy cranial (in fractions of 1.8

Gy) and shifted to 18 Gy (again in fractions of 1.8 Gy) before being replaced with intrathecal chemotherapy regimens.

Early studies demonstrate delayed onset of cognitive impairment following cranial irradiation in children with ALL. In a multi-institutional study with 203 children diagnosed with ALL and treated with a cumulative dose of 18 Gy (10 fractions of 1.8 Gy) cranial irradiation (n=129) or chemotherapy only, those treated with cranial irradiation demonstrated lower intelligence at a mean time of 6.4 years following diagnosis (Jankovic et al., 1994). However, differences were only apparent for those less than three years of age at diagnosis. Among 77 children treated with 24 Gy (n=28), 18 Gy (n=23) or no cranial irradiation (n=26) evaluated during therapy, off therapy and more than 48 months off therapy, no change in intelligence was noted, though reading, spelling and mathematics skills steadily declined in all groups (Mulhern et al., 1992). However, in a study comparing specific cognitive abilities among 25 children treated with 18 Gy cranial irradiation to 32 children treated with high-dose chemotherapy and 22 children with very high-dose chemotherapy, and tested when more than five years from diagnosis, only those children treated with cranial irradiation demonstrated memory and learning problems (Spiegler et al., 2006). One study examined cognitive outcomes in children treated with 18 Gy cranial irradiation using conventional fractionation (fractions of 1.8 Gy, n=71) to those who received hyperfractionation (fractions of 0.9 Gy twice daily, n=54) (Waber et al., 2004). At more than five years post diagnosis, no significant group differences were noted on measures of intelligence, academics or verbal learning, though the hyperfractionation group demonstrated modestly better performance on measures of visual-spatial processing.

As with the pattern observed in children with CNS tumors treated with cranial irradiation, the cognitive effects of radiation in children with ALL appear to be progressive with time from

diagnosis. Among 567 adult survivors of childhood ALL tested when more than 10 years from diagnosis, processing speed, attention, memory and executive function problems were observed in those treated with 18 Gy (n=167) and 24 Gy (n=186) compared to those treated with chemotherapy only (Krull et al., 2013). Compared to population norms, those treated with 24 Gy demonstrated an 11-fold increased rate of processing speed problems, a 15-fold increased rate of memory problems, and a 16-fold increased rate of executive function problems. For those treated with 18 Gy cranial irradiation, increased rates of problems were 8-fold, 9-fold and 11-fold for processing speed, memory and executive function, respectively. These adult survivors also demonstrate reduced cortical thickness in multiple areas, reduced white mater volume, which corresponds to processing speed, and smaller hippocampi (Edelmann et al., 2014; Schuitema et al., 2015).

Compared to children treated with chemotherapy only, who themselves have increased rates of problems, risk for problems with intelligence, academics and memory decline with age, while risk for executive function problems increase with time from diagnosis, as displayed in Figure 4.2. This increased risk with time from diagnosis may suggest accelerated cognitive aging (Schuitema et al., 2015).

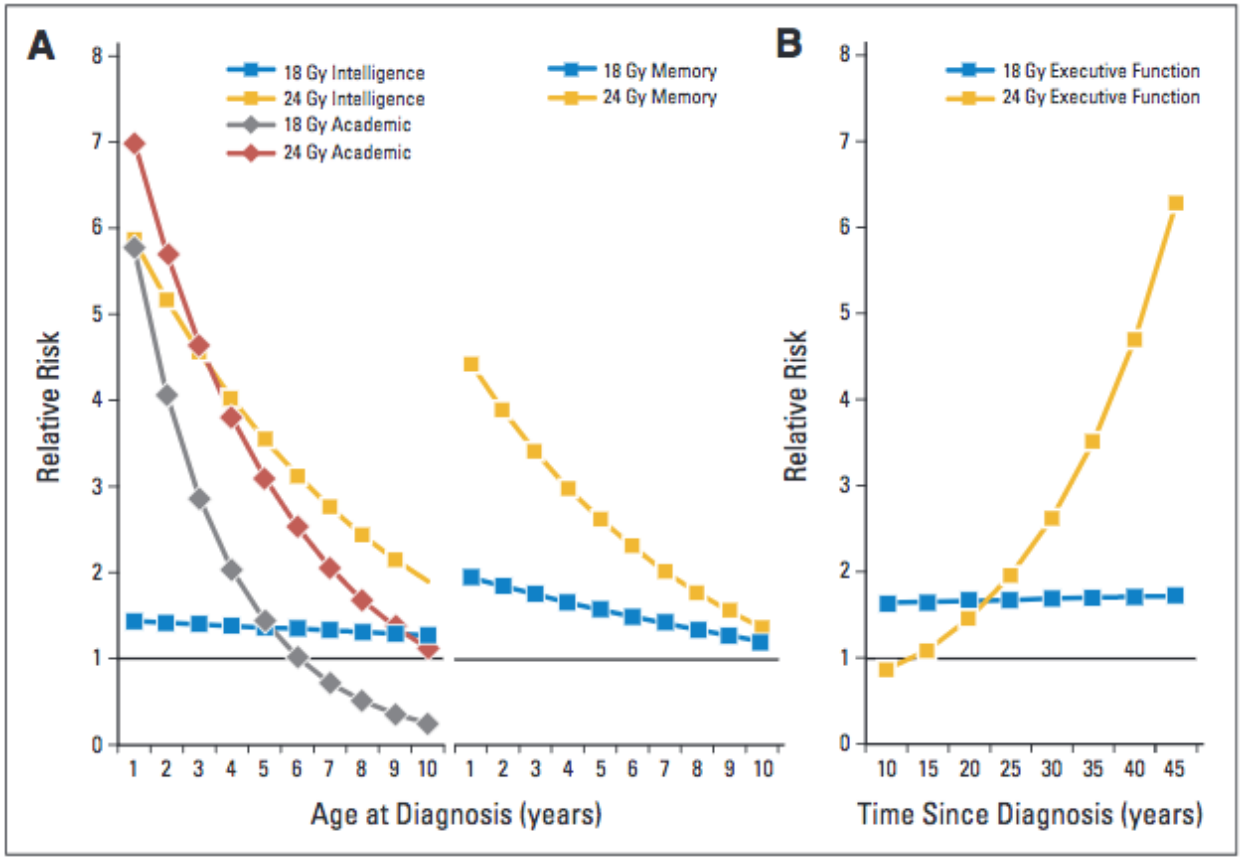

Fig. 4.2. Relative risks of cognitive problems as a function of age at diagnosis (left panel) and time since diagnosis (right panel) for adult survivors of childhood ALL exposed to 18 or 24 Gy whole brain irradiation. The reference line at relative risk of 1 is a group exposed to chemotherapy only. From Krull et al, *Journal of Clinical Oncology*, 2013.

Increased risk of cognitive problems following cranial irradiation also appears higher among females, particularly those treated when less than six years of age (Kadan-Lottik et al., 2010). Among survivors treated with 22.5 Gy cranial irradiation for ALL and tested 25 years following diagnosis, 50% of females and 15% of males demonstrated impaired working memory (Schuitema et al., 2015).

Cancer survivors treated with cranial radiation are also at increased risk for structural brain abnormalities. Compared to siblings, risk for cerebrovascular accidents is increased in a cranial radiation dose-response fashion. Among 2202 children treated with cranial irradiation, 54 had overt strokes, 8.5 times more common than survivors treated without cranial irradiation and community controls (El-Fayech et al., 2017). Hazard ratios for overt stoke are reported at 5.9 for cranial radiation doses of 30-49 Gy, and 11.0 for doses >=50 Gy (Mueller et al., 2013). Cranial radiation >= 50 Gy is also associated with recurrent stroke, with 21% of those having a 1st having a 2nd (Fullerton et al., 2015). Cerebral microbleeds appear to be more common than overt stroke. The cumulative incidence of cerebral microbleeds in 149 pediatric CNS tumor patients was reported to be 48.8% at 5 year follow-up (Roddy et al., 2016). Children who received whole brain radiation had a 4-fold higher rate of cerebral microbleeds compared to those with only focal radiation. Cerebral microbleeds were also associated with poorer neurocognitive performance.

4.1.2 Adult exposures

A wide array of radiotherapy treatments (RT) are used in cancer treatment in adults, in some cases solely restricted to RT, and in others combined with chemotherapy treatment and/or surgical resection. The following considers what is known from use of RT in CNS or in regions

in proximity to the CNS on cognition; this includes empirical findings in CNS cancers treated with whole brain radiation therapy, non-CNS cancers treated with prophylactic cranial irradiation either with or without hippocampal sparing techniques, and in head and neck/nasopharyngeal cancers in which radiation may be incidentally delivered to otherwise healthy CNS tissue at lower doses. The relevance of the cognitive effects of RT used in cancer treatment to the anticipated effects of radiation exposure outside of low-earth orbit is limited given a number of factors that complicate interpretation. Radiation treatments in the cancer setting use far higher doses of radiation in fractionated amounts and at a very different exposure schedule compared to what is expected in deep space, which may include episodic exposures to HZE particles unlike in the clinical setting. Treatments are often combined with chemotherapy treatments or radio-sensitizers that may have independent or multiplying effects on the CNS and cognition. In the case of treatments for CNS cancers, CNS structural pathology is already evident due to primary tumors, metastatic disease, and/or prior resection(s). Finally, survival times are far shorter for the cancers discussed here (months to several years), compared to what would be expected for crew members following mission completion, making very late effects difficult to quantify or anticipate. Table 4.1, which includes selected, relevant empirical work on RT CNS exposure and cognitive effects, is displayed.

1283 TABLE 4.1— Late cognitive effects of radiation in adults (cancer table)

Article	Gy/Fraction	Dx/Tx (N)	Time-points	Outcomes
Prophylactic Cranial Irradiation (PCI) – NSCLC/SCLC				
Gondi, et al. [13]	36 Gy/24 36 Gy/18 30 Gy/15 25 Gy/10 Prior chemotherapy	No PCI (90) PCI (209	Longitudinal baseline to 6 months	PCI<No PCI HVLT Learning $p < 0.0001$ HVLT Delayed Recall $p < 0.04$ Quality of Life $p < 0.0001$ (Raw change scores not available)
		No PCI (62) PCI (138)	Longitudinal baseline to 12 months	PCI<No PCI HVLT Learning $p < 0.0001$ HVLT Delayed Recall $p < 0.005$ Quality of Life $p < 0.0001$ (Raw change scores not available)
Le Pechoux, et al. [31]	25 Gy/10 36 Gy/18 Prior chemotherapy	25 Gy (197) 36 Gy (119)	Longitudinal baseline, 6, 12, 24, 36, 48 months	Decline across both groups -Clinician rated Intellectual deficit -Clinician rated Memory deficit
Hippocampal Sparing WBRT – CNS and Non-CNS Cancers				
Tsai, et al. [21]	25 Gy/10 30 Gy/10-12	Lung/CNS (24)	Longitudinal baseline to 4 months post-tx	Cut-points by dose and volume exposed associated with cognitive function (Word List Memory) Bilateral hippocampus (only significant) Max ≤12.6 Gy: Preserved 10(83.3 %), Decline 2(16.7 %) Max >12.6 Gy: Preserved 3(25.0 %), Decline 9(75.0 %) D10% ≤8.81 Gy: Preserved 9(75.0 %), Decline 3(25.0 %)

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	>8.81 Gy	4(33.3 %)	8(66.7 %)	
D50%	≤7.45 Gy	9(75.0 %)	3(25.0 %)	
	>7.45 Gy	4(33.3 %)	8(66.7 %)	
D80%	≤6.80 Gy	9(75.0 %)	3(25.0 %)	
	>6.80 Gy	4(33.3 %)	8(66.7 %)	
D100%	≤5.83 Gy	9(75.0 %)	3(25.0 %)	
	>5.83 Gy	4(33.3 %)	8(66.7 %)	
Left hippocampus (only significant)				
Max	≤12.41 Gy	10(83.3 %)	2(16.7 %)	
	>12.41 Gy	3(25.0 %)	9(75.0 %)	
D10%	≤8.75 Gy	9(75.0 %)	3(25.0 %)	
	>8.75 Gy	4(33.3 %)	8(66.7 %)	
D40%	≤7.70 Gy	9(75.0 %)	3(25.0 %)	
	>7.70 Gy	4(33.3 %)	8(66.7 %)	
D50%	≤7.48 Gy	9(75.0 %)	3(25.0 %)	
	>7.48 Gy	4(33.3 %)	8(66.7 %)	
D80%	≤6.73 Gy	9(75.0 %)	3(25.0 %)	
	>6.73 Gy	4(33.3 %)	8(66.7 %)	
D100%	≤5.73 Gy	8(66.7 %)	4(33.3 %)	
	>5.73 Gy	5(41.7 %)	7(58.3 %)	

Gondi, et al. [19]	30 Gy/10 Prior chemotherapy	CNS (100) Control (208)	Longitudinal at baseline, 2, 4, 6 months	<9 Gy to total hippocampus/ maximal dose to hippocampus <17 Gy HS-WBRT 7% relative decline in delayed recall from baseline to 4 months WBRT 30% relative decline
Lin, et al. [20]	30 Gy/12 Prior chemotherapy	Mixed Non-CNS (3) CNS (22)	Longitudinal at baseline and 4 months post-initiation	40% isodose surface Significant decline in verbal recall. All other domains exhibited no significant change.

Nasopharyngeal Head and Neck Cancers

Tang, et al. [32]	68-76 Gy/34-38 Chemotherapy (12)	Nasopharyngeal RT-Radiation Inj (46) RT (46)	Cross-sectional at an average of 6 years	Estimated dose to CNS 70–73 Gy Average performance in both groups met criteria for impairment based on the Montreal Cognitive Assessment (MOCA)

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Gan, et al. [30]	60 Gy/25 64 Gy/20 70 Gy/35 Chemotherapy	Head and neck RT (6) RT/CT (4)	Longitudinal pre-treatment and 9 to 35 months post-treatment	Estimated dose to CNS <1 – 46.99 Gy) 9/10 exhibited decline from pre- to post-treatment with memory most affected. Severity of memory impairment associated with dose to temporal lobes. No difference between RT alone versus concurrent RT and chemotherapy treatment.
Hsiao, et al. [29]	70-72 Gy/35-40 Chemotherapy	Nasopharyngeal RT/Chemo (26) IMRT (4)	Longitudinal pre-treatment and 12 to 26 months post-treatment	Estimated dose to CNS 34 Gy +/- 6.5 No temporal lobe necrosis found. >36 Gy versus <36 Gy was associated with poorer cognition

	Pre-tx	Post-tx	p Value
Attention	6.93	6.93	0.999
Concentration	8.67	8.07	0.095
Orientation	17.57	7.60	0.832
Long term memory	9.40	9.20	0.560
Short-term memory	10.25	9.68	0.020
Language abilities	8.92	8.48	0.023
Visual construction	9.50	9.47	0.942
Verbal fluency	8.17	7.30	0.001
Abstraction/judgment	9.57	9.20	0.144

Lam, et al. [27]	66 Gy/27-44	Nasopharyngeal RT-Radiation Inj (40) RT (20) Controls (19)	Cross-sectional on average 5 years post-treatment	Both patient groups exhibited lower verbal learning and memory performance. Dose dependent relationship on executive function tasks
Meyers, et al. [28]	50-68 Gy/25-34	Paranasal sinus RT (19) Pre-treatment (9)	Cross-section 20 months to 20 years post-treatment	Mean irradiated brain volume was 245 cc, ranging from 10 to 574 cc Dose (>60 Gy) associated with Recall score. No association with volume. No effect of subsequent chemotherapy

	Pre-tx	Post-tx	%Imp
Storage	38.7	29.9	58
Retrieval	24	16.2	69
Recall	8.7	4.7	80

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Hua, et al. [26]	64-72 Gy/38-42 No chemotherapy	Nasopharyngeal RT (27) Pre-treatment (28) Controls (35)	Cross-sectional at average of 1.7 years following treatment	Estimated dose to CNS 63-84 Gy (M = 71 Gy) In 27 patients (93%), the basal frontal lobe was definitely included in the radiation field (M = 52 Gy).

	HC	PT	RT	Sig.
Figure Memory	7	7	3	$p < 0.05$
Event Recall	39	38	37	$p < 0.05$
Word Learning	54	51	49	$p < 0.05$
Mental Control	5	4.68	4	$p < 0.05$

Lee, et al. [37]	350 cGy mean tumor dose three per week to 59.5 Gy over 6 weeks (range 55-63 Gy)	Nasopharyngeal RT (16) Pre-tx controls (21)	Cross sectional at 5.5 years (2.5 – 10 years)	Estimated dose to CNS 52.8 Gy (range 47-57 Gy).

	TX	Ctrl	Sig.
Information	6.68	8.85	$p < 0.05$
Comprehension	7.6	9.5	$p < 0.05$
Similarities	6.25	8.85	n.s.
Coding	5.6	7.5	n.s.
Block Design	7.8	9.7	$p < 0.05$
Verbal IQ	8.31	9.53	$p < 0.05$
Performance IQ	89.1	99.0	$p < 0.05$
Full Scale IQ	84.7	96.7	$p < 0.05$
Rey Fig. Copy	87.6	96.6	n.s.
Rey Recall	38.4	37.1	$p < 001$
Log. Memory	16.8	21.5	$p < 005$
Log.Memory	12.9	16.9	n.s.
Assoc. Learning	11.3	12..3	n.s.
Subj.Memory	44.8	21.0	$p < 005$

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4.1.2.1 Whole Brain and Prophylactic Cranial Irradiation. Fractionated whole brain radiation therapy (fWBRT) is used in both metastatic disease and in conditions in which multiple tumors are present or are not candidates for surgical resection. Due to existing CNS involvement in which multiple tumors are potentially present, the isolated effects of therapy itself versus existence and progression of disease are difficult to distinguish. WBRT applies an external beam to the whole brain in fractionated doses, i.e., multiple treatments of lower dose radiation to achieve a total dose at completion (30 Gy in 10 fractions). Radiation effects have been classified as acute, early-delayed, and late-delayed injury (Tofilon and Fike, 2000; Greene-Schloesser et al., 2012). Acute effects are less common with contemporary treatments, whereas reversible, early-delayed effects (1-6 months following treatment) may include underlying demyelination and somnolence. Late-delayed injury (>6 months post-treatment) includes demyelination, vascular abnormalities, and white matter necrosis (Greene-Schloesser et al., 2012). As a result of late-delayed effects to the CNS, longer-lasting and potentially irreversible cognitive dysfunction is identified in this time-frame. Later effects may be predicted by the volume of radiated tissue, radiation dose, age, combined chemotherapy treatment, and vascular risk factors (Correa and Root, 2016). The pattern of cognitive deficits associated with whole brain radiation therapy is generally diffuse, and specifically associated with declines in learning and retrieval of new information, executive dysfunction, inattention, and psychomotor slowing (Weitzner and Meyers, 1997; Wefel et al., 2004; Crossen et al., 1994; Archibald et. Al., 1994; Salander et al., 1995; Scheibel et al., 1996; Taphoorn and Klein, 2004). Underlying mechanisms that have been considered in late-delayed effects include vascular damage in the form of thickening of vessel walls, dilation, and effects on endothelial function that together lead to ischemic changes and resulting white matter damage. Also considered is the effect of radiation on several CNS cell

types including: damage to oligodendrocyte progenitor cells leading to disrupted myelin production and resulting white matter damage; activation of astrocytes leading to increased inflammatory response and disrupted blood-brain barrier integrity; and chronic activation of microglia leading to chronic inflammatory processes and oxidative stress in the CNS (Greene-Schloesser et al., 2012).

4.1.2.2 Prophylactic Cranial Irradiation. Similar to WBRT, prophylactic cranial irradiation (PCI) applies an external beam to the whole brain to treat potential occult tumor cells that may have metastasized in cancers that commonly spread to the CNS (e.g., small cell and non-small cell lung cancer), but in absence of proven evidence of metastasis. PCI therefore provides some window onto the effects of radiation exposure absent primary CNS structural pathology at similar doses to those used in WBRT (24 – 36 Gy in 10 – 24 fractions). PCI has been found to be associated with cognitive declines in the Radiation Therapy Oncology Group (RTOG) 0214 randomized clinical trial (Gore et al., 2011), with significant declines in learning and memory in the PCI treated group compared to observation (Sun et al., 2011), and declines in self-reported quality of life (Gondi et al., 2013b). Similar findings were reported in relation to PCI in a European cohort (Slotman et al., 2009). Given the findings of significant cognitive effects of WBRT and PCI, attempts to reduce radiation dose to areas supporting learning and memory, with a particular focus on bilateral hippocampi, have been introduced.

4.1.2.3 Hippocampal Sparing Radiotherapy. Previous animal studies have found that external radiation may have a specific effect on hippocampal neurogenesis in radiation exposed non-human animals (Rola et al., 2004b; Raber et al., 2004). The translation of

hippocampal radiation exposure to human-level learning and memory performance has been confirmed in prospective studies examining varying doses of hippocampal radiation exposure, with greater hippocampal exposure predicting lower learning and memory performance both in children (Redmond et al., 2013) and adult patient groups (Gondi et al., 2013a). These results also highlight the contribution and importance of preclinical rodent studies for the development and optimization of treatment strategies in humans. Gondi et al. (2013a) reported that an EQD2 to 40% of the bilateral hippocampi >7.3 Gy significantly affected delayed recall performance. Hippocampal sparing PCI or WBRT applies a conformal external beam with the aim of targeting areas outside of hippocampal and medial temporal compartments. A benefit of intentionally sparing hippocampal areas was found in the RTOG 0933 trial, which contrasted traditionally treated WBRT historic controls with patients undergoing hippocampal-sparing WBRT (hs-WBRT) for brain metastases (Gondi et al., 2014). The authors reported learning and memory decline of 7% at four months in the HS-WBRT group compared to 30% decline in historic controls. Similar results were reported for a mixed group of PCI and WBRT treated patients with hippocampal sparing treatment, finding an association of verbal learning and memory with dose escalation to hippocampus and generally preserved cognitive functioning at follow-up (Lin et al., 2015). Tsai et al. (2015) studied the effects of hippocampal sparing in a mixed group of CNS and lung cancer patients undergoing WBRT. Since the effectiveness of hippocampal sparing is variable with varying doses to hippocampus and varying proportion of hippocampal volume exposed, the investigators were able to quantify cut-points for both dose and volume that were associated with decline in cognitive function. They reported paired volume and dose levels cut-points

(volume/dose) for bilateral hippocampus in EQD2 values of 0%/12.6 Gy, 10%/8.81 Gy, 50%/7.45 Gy, 80%/5.83 Gy similar to the levels reported by Gondi et al. (2013a) of 40%/7.3 Gy to be significantly associated with immediate recall performance, with specific effects of left hippocampus volume and dose on verbal learning.

4.1.2.4 Stereotactic Radiosurgery. Efforts have been made since the introduction of radiation therapies to more focally target radiation treatments to affected tissue and in so doing to spare healthy tissue. Stereotactic radiosurgery (SRS), in which multiple, weaker external beams converge on the intended target while sparing areas outside the target, has been used to this end. A handful of recent studies have investigated cognitive outcomes, tumor progression and overall survival between SRS alone or combined with WBRT. In a recent study documenting the effects of SRS versus SRS/WBRT on cognitive function (Brown et al., 2016), improved cognitive outcomes were found in learning and memory performance with increased tumor progression in the SRS group but with no significant difference in survival. Similar results were found in a smaller study that contrasted cognitive outcomes between SRS and SRS/WBRT, with the study stopping early due to the significant decline in total recall with the addition of WBRT to SRS treatment (Chang et al., 2009). In contrast, two studies (Aoyama et al., 2006; Aoyama et al., 2007) found relatively better functioning when treatments were combined, although cognitive assessment was restricted to only mental status testing and therefore may have been insensitive to higher order cognitive decline.

4.1.2.5 Radiation Treatments in Head and Neck Cancers. Radiation therapy is used in head and neck/nasopharyngeal cancers to deliver radiation to isolated tumors with close proximity to CNS.

Treatment of upper esophageal, base of the tongue, and nasopharyngeal tumors as well as other head and neck cancers may result in lower doses of scatter radiation to inferior/basal areas of the brain including inferior temporal and frontal regions. In an early study of RT effects in nasopharyngeal patients, Lee (1989) found wide-spread cognitive differences between treated and untreated patients, including a 10-12 point difference in Full Scale IQ, Performance IQ and Verbal IQ, as well as significant differences in verbal learning and visual memory with an estimated dose to CNS of 52.8 Gy. Similar findings have been reported in subsequent studies, including declines in memory and attention (estimated CNS dose = 65-84 Gy) (Hua et al., 1998; Lam et al., 2003), and dose dependent relationships (>36 Gy; >60 Gy; dose to temporal lobes) (Lam et al., 2003; Meyers et al., 2000; Hsaio et al., 2010; Gan et al., 2011). These findings are exhibited both in groups with significant temporal lobe necrosis as well as in groups without structural pathology related to radiation exposure.

While the literature is suggestive for more intense and time-limited doses of radiation to CNS structures and associated cognitive changes, as noted above, its applicability to what may be expected in longer duration, mission-relevant exposures is limited. Unsurprisingly, larger doses of radiation to critical structures, specifically to the hippocampus, result in larger declines or differences in cognitive function in longitudinal and cross-sectional studies. Of most relevance to the association of lower doses of radiation with cognitive function, although still within briefer time-frames and higher doses, is the work of Gondi et al., (2013a) and Tsai et al., (2015). Gondi et al. (2013a)reported that a biologically equivalent dose in 2-Gy fractions (EQD2) to 40 % of the composite structure of bilateral hippocampi less than 7.3 Gy was significantly associated with neurocognitive preservation as indicated by performance on a measure of verbal delayed recall (decline considered at -1.5 z change from pre- to post-

treatment). Tsai et al., (2015) reported that EQD2 values of 0, 10, 50, 80 % irradiating the composite hippocampal structure with <12.60 Gy, <8.81, <7.45 Gy and <5.83 Gy respectively were significantly associated with neurocognitive preservation as indicated by a verbal recall measure (preservation indicated by decliners versus improvers).

Cerebrovascular abnormalities also occur in survivors of adult-onset cancers treated with cranial irradiation. Among 27 patients diagnosed with CNS tumors (31-80 years old) and treated with 54 Gy, vascular injury in the hippocampus was identified as soon as one-month post radiation exposure (Farjam et al., 2015). No differences in memory function were apparent at that time, though memory gradually declined to a significant reduction by 18 months post-diagnosis. Radiation to the neck region is also associated with increased stroke risk. In a retrospective population-based study in Ontario, Canada, among over 14,000 adults treated for neck carcinomas, 8% of those treated with radiation therapy alone (n=4475) experienced an ischemic stroke, and their risk for stroke was 70% higher than that in survivors treated with surgery along (n=3120) (Arthurs et al., 2016). This increased risk appears associated with stenosis of carotid arteries that were within the radiation field (Bashar et al., 2014). However, this change appears to develop over time. Among survivors of head and neck cancer treated with 66 Gy radiation to the neck region, no significant change was observed in carotid intima-media thickness for the first 2-years post therapy (Wilbers et al., 2014) though increased thickness was observed in 58% of the survivors after 6 years (Wilbers et al., 2015).

Hypothyroidism is a relatively common occurrence following radiation therapy for head and neck tumors. In a cohort of 241 patients treated for head or neck carcinoma, 53% developed clinically significant hypothyroidism (Alba et al., 2016). Among 188 adult head/neck cancer patients, cumulative incidence of hypothyroidism was 21.1% at 1 yr and 36.4% at 2 years

(Fujiwara et al., 2015). Lower incidence of hypothyroidism was identified when radiation dose to the neck was <30 Gy, though there was apparently no association with the volume of the thyroid gland spared. Hypothyroidism is associated with cognitive impairment in non-cancer populations[1] even when the hypothyroidism is subclinical (Ergur et al., 2012; Martino and Strejilevich, 2015).

Medical consequences of radiation exposure have also been identified in adults exposed to non-medical sources of radiation. Recently, an epidemiological study of nuclear plant workers with mean cumulative dose, of primarily low LET radiation, of 25.2 mSv delivered over several years found an association between increased mortality due to cerebrovascular disease and dose (Gillies, 2017).

4.2 Other radiation exposures

Most literature on outcomes of medical radiation exposure involves examining survivors treated with photons. Most recently protons have been used as these particles have Bragg peaks that permit better concentration of the radiation intensity at the tumor location, with improved sparing of healthy brain tissue. An example is shown in Figure 4.3. The benefit of the proton beam is not in the efficiency of treating the cancer, but rather the control and localization of the beam such that healthy tissue is spared (Harrabi et al, 2016).

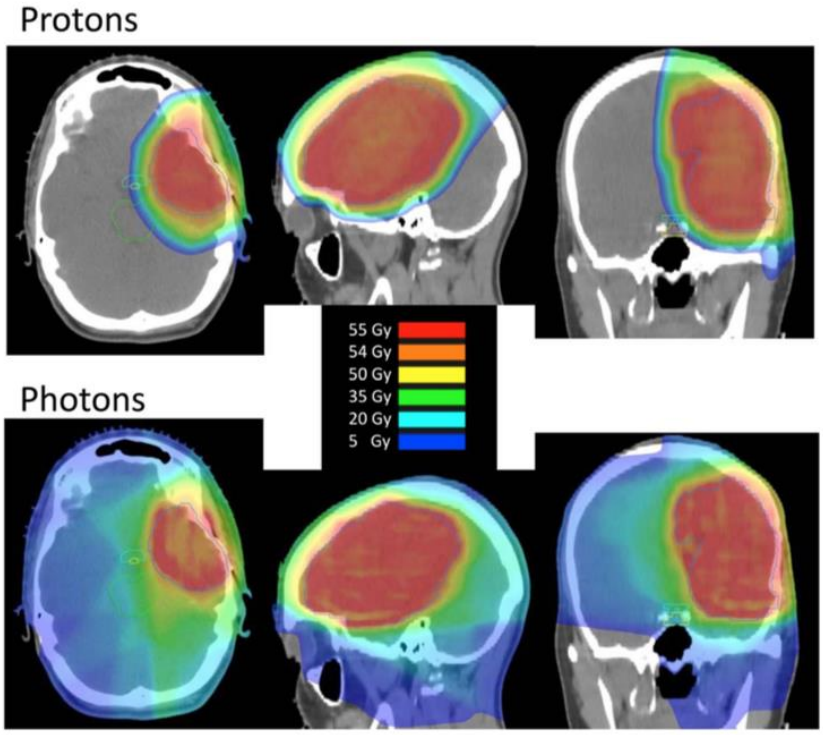

Fig. 4.3. Radiation intensity profiles.Note that protons' Bragg peaks permit better concentration of the radiation intensity at the tumor location, with improved sparing of healthy brain tissue.

4.2.1 Exposure to Protons and Heavier Ions

There is not a long history of use of proton therapy and, thus, long-term outcomes are limited. Among 150 survivors of CNS tumor treated during childhood (n=90 with proton, n=60 with photon; both groups treated with 54 Gy to the tumor bed and roughly half of each group also received 23 Gy craniospinal irradiation), no change in intelligence over a five year interval from diagnosis was observed in the group treated with protons, though the group treated with photons demonstrated an average decline of 1.1 IQ pts per year (Kahalley et al., 2016). Among the subgroups of patients who received the craniospinal irradiation (either proton or photon), no change in IQ slope was observed. Among 260 CNS tumor patients (n=30 children) treated with protons (n=176) or carbon ions (n=84; n=36 with photon and carbon ion boost), with median follow-up of 12 months, no neurocognitive problems were identified in either group (Combs et al., 2013). At longer follow-up, children (n=60) treated with protons for CNS tumor do appear to demonstrate a decline in processing speed from pre-therapy to 2.5 years post-therapy (Pulsifer et al., 2015). Response to proton beam exposure appears to have a similar age sensitivity as that seen following photon exposure. In 32 children treated for CNS tumor with protons (mean dose of 52.2 Gy to tumor bed), no decline in intelligence was observed over a period of 4.5 years, with the exception of those who were less than 7 years old at diagnosis (Greenberger et al., 2014). Among 20 survivors of CNS tumor treated during adulthood with 54 Gy focal proton therapy (1.8 Gy per fraction), who were tested at baseline and annually for 5 years, no decline in cognitive function was observed from baseline (Sherman et al., 2016).

Given the improved control associated with proton beams, children treated for standard-risk medulloblastoma, which involves 54 Gy exposure to the tumor bed and craniospinal radiation of 18-27 Gy, demonstrate a three-fold reduced risk of hypothyroidism if treated with

proton compared to those treated with photons (Eaton et al., 2016). This reduced risk is presumably associated with reduced exposure to the thyroid gland.

Between 1954 and 1993 charged particle radiosurgical treatments of selected cranial targets using protons and helium beams were used to treat tumors that are either partially or completely encircling the brain stem of spinal cord, pituitary tumors, arterial venous malformation and a number of other cranial tumors (Fabrikant et al., 1991; Levy et al., 1991; Levy et al., 1990; Steinberg et al., 1990) at the University of California Lawrence Berkeley Laboratory (LBNL). For tumor therapy, although the target doses at the tumor sites are comparatively large (upwards of 40 Gy and above), nevertheless, the fact that these doses are administered as daily fractions and that, by nature of the particle beams, the neighboring cells in the brain could be exposed to low doses makes the CNS findings from these patients relevant to this study.

By dividing the target volume into two or more portions and using a combination of beams, a reasonably homogeneous irradiation of the target volume can be obtained while protecting critical CNS structures from over-irradiation (Castro et al., 1989). Using this technique, a variety of tumors abutting the brain stem and spinal cord, including chordoma, chondrosarcoma, meningioma, osteosarcoma and metastatic tumors were treated. Results showed significant local control rate (62%) and the incidence of serious complications has been acceptable (13%). The median follow-up is 20 months with a range of 6-90 months. The authors concluded that charged particles can be safely and effectively used to irradiate lesions encircling the brain stem or spinal cord with lower risks for complications when compared with low-LET irradiation.

Stereotactic protons or helium radiosurgery of the pituitary gland using several plateau portions of the narrow beams in several intersecting arcs, was conducted at LBNL (Lawrence, 1957; Linfoot et al., 1963), Harvard Cyclotron Lab/Massachusetts General Hospital (Kjellberg et al., 1968), and Burdenko Neurosurgical Institute and the Leningrad Institute of Nuclear Physics in Russia. The maximum dose to the tumor range from 30 to 50 Gy mostly often delivered in four fractions over 5 days. Other than hormonal insufficiency, complications in the pituitary tumor patients treated with helium-ion plateau radiosurgery were relatively few. These include seizures because of limited temporal lobe injury, mild or transient extraocular nerve palsies and partial visual field deficits, possibly due to low out-of-field exposures. Postmortem histopathological evaluations of progressive delayed sequelae of the pituitary irradiations were conducted in 15 patients who died 3 – 15 yrs after receiving helium particle radiation at LBNL between the years 1975 – 1981 (Woodruff et al., 1984). Patients in this cohort received 50- 135 Gy of fractionated exposures. At autopsy in all patients, the radiation field was clearly demarcated by the abrupt aplasia of spenoidal bone marrow in the bone of the sella turcia surrounding the pituitary gland. All patients had advanced and generalized atherosclerosis as a complication of either diabetes or hypertension accompanying acromegaly. Microscopic sections through the temporal lobes, the thalamus, the optic nerves and other CNS structures showed no radiation-induced lesions implying that, even if the surrounding normal tissues were exposed to lower doses, there were no detectable histopathological lesions present at autopsy.

Over 400 patients with symptomatic inoperable intracranial arteriovenous malformations (AVMs) were treated with stereotactic helium-ion Bragg peak radiosurgery at LBNL in a collaborative program with Stanford University Medical Center and the University of California Medical Center at San Francisco (UCSF) (Fabrikant et al., 1985). Focused accelerated helium

ions beams were stereotactically-directed into the brain to obliterate abnormal shunts with generally little or no neurovascular or parenchymal injury to adjacent critical brain structures. Initially, radiation doses range from 45 to 35 GyE but these doses were reduced to under 10 Gy under special circumstances. The fields of AVM exposure vary from <4 cm^3 up to >70 cm^3. Treatment plans showed that the dose to the critical normal brain structures adjacent to the AVM is considerably less than the dose to the target volume because falloff to 10% of the central dose occurs within 4 – 6 mm and is within 2 – 3mm along the lateral margins of the beam. The dose drop-off to 10% of the target dose brings the dose down to ≤ 1 Gy to sections of the normal tissues, the dose relevant to long duration space missions. Cohorts of more than 250 patients were followed for up to 10 yrs after irradiation (Steinberg et al., 1990). Based on long-term CT and MRI of all patients, and PET and radioisotope scanning of selected patients, together with extensive clinical neurological follow up, this procedure produced good results with reduced morbidity and no treatment-related mortality. Seizures improved in 63% of patients, headaches in 68% and neurological deficits in 27%. Progressive neurological deficits stabilized in 55%. Complications that occurred 4 to 26 months after treatment could be classified as white-matter changes or vasculopathy. Treated patients demonstrated delayed changes in brain anatomy and function that occur months to years after radiosurgery suggested that the underlying mechanism of the brain's delayed reaction involve complex perturbations in cerebrovascular and metabolic function. Such changes include hemodynamic changes, blood-brain barrier disruption and vasogenic edema, metabolic suppression and parenchymal necrosis. These findings imply that cerebral endothelial cells, oligodendroglia and astrocytes are involved in the radiation-induced injury to the brain (Lo and Fabrikant,1991; Lo et al.,1989). In general, long-term survival benefit was observed in this large group of stereotactic helium-ion Bragg peak radiosurgery

AVM patients. However, limited evaluations of neurological defects using the limited techniques available at the time suggests that some residual effects may persist due possibly to scattered particle radiation to parts of the brain that were not directly in the target exposure areas.

4.2.2 Other Effects of Exposure

Recently, an epidemiological study of nuclear plant workers with mean cumulative dose, of primarily low LET radiation, of 25.2 mSv delivered over several years was shown to increase mortality due to cerebrovascular disease {Gillies et al., 2017}

4.2.3 Exposure to Neutrons

There are no published reports of cognitive or central nervous system outcomes following neutron therapy.

4.2.4 Health Risks Following Chronic Exposure to Brain Tissue from High-LET Radiation in Radiation Workers

Ingested or inhaled intakes of radionuclides that decay by emitting alpha particles can pass the blood-brain barrier and expose brain tissue for many years (Boice et al. 2018a, 2018b; Leggett et al. 2018, 2019; NCRP 2018). Such exposure is more uniform across brain tissue and differs from alpha particle exposure that might occur from bone-seeking radionuclides that deposit in the cranium. Currently, an NCRP scientific committee (SC 6-12) with colleagues at the Oak Ridge National Laboratory are developing biokinetic models for estimating brain dose directly following the intake of radionuclides and not using the current indirect approach that considers dose to all soft tissue and apportions the dose to brain based on weight or other considerations (Leggett et al. 2018, 2019; NCRP 2018),

Postmortem radiochemical analysis of tissues has shown that intakes of radium deposit in the brain and result in alpha particle exposure to brain tissue (Schlenker et al. 1982).

Radiochemical analyses also have detected brain depositions for other radionuclides such as plutonium (James et al. 2003, 2007), americium (McInroy et al. 1985), uranium (Avtandilashvili et al. 2015; Kathren and Tolmachev 2015), and polonium (Nathwani et al. 2016). This research is made possible because of the generosity of radiation workers who many years ago donated their bodies for scientific research to the United States Transuranium and Uranium Registries (Filipy and Russell 2003; Tolmachev and McComish 2016; Tolmachev et al. 2011; USTUR 2018). Within the MPS brain dose from alpha particles have or will be determine for intakes of polonium at Mound (Boice et al. 2014), radium at Mallinckrodt (Ellis et al. 2018) and perhaps at Fernald (Silver et al. 2013), uranium at several facilities such as Rocketdyne (Boice et al. 2011) and Oak Ridge, and plutonium at Los Alamos, Rocky Flats, and Hanford among others. A suggested dose-response has bee seem among Mound workers with intakes of polonium and mortality from dementia, Alzheimer's and other forms of motor neuron disease (Boice et al. 2018a); however a similar response was not seen for workers at Mallinckrodt exposed to radium but at much lower dose levels (Golden et al. 2019a).

Limitations to these evaluations include the different dose distributions within the brain and the energy depositions from alpha particle emitters compared with that of high-energy heavy ions experienced in space (Boice 2017, 2019; Boice et al. 2018a, 2018b). Nonetheless, there is no other human analog that provides information on possible health risks from high-LET radiation to brain tissue. The mouse models provide valuable insights into effects of space radiation on the brain, but the mouse has considerable limitations as a laboratory model for the human brain. Further, the dose delivery in experimental studies is brief and of the order of minutes compared with the years it will take for a mission to Mars. The current research within the MPS thus will add to the collection of evidence, though imperfect, needed to inform radiation

protection guidance against the possible effects of galactic cosmic radiation on brain function (NCRP 2016).

High-level cognitive performance is critically important during space missions, which can involve environmental, physiological and psychological stressors (Basner et al. 2015; NCRP 2016; Moore et al. 2017). Radiation exposure to GCRs is a possible stressor that may affect brain function (NCRP 2016). NASA has supported the development of tests that assess multiple domains of neurocognitive functions: Cognition Test Battery and WinSCAT (Moore et al. 2017) which have been administer to astronauts. Within the MPS it might be possible to evaluate cognition among radiation workers who have had continuous brain tissue exposure to alpha particles for up to 40 years after the intake of plutonium or other radionuclides. Many workers today voluntarily come back to national laboratories and provide urine samples. Over 1,000 workers could be identified with known radiation doses to brain who conceivably might be contacted and asked to complete a modified cognitive test similar to ones taken by astronauts on the International Space Station or elsewhere.

4.2.5 Recommendations

It is recommended that NASA implement a program to foster collaboration with the charged particle radiotherapy centers globally to harness the world-wide data base of patients and conduct follow-up on behavioral and other CNS-associated endpoints in this patient population. Due to the favorable depth-dose characteristics of particle beams and their utility in radiotherapy, the patient populations that were either treated or are currently undergoing therapy are increasing both in the U.S and internationally. Of these, results from head and neck cancer patients can provide data of use to NASA. Therapeutic target doses to the cancer tissues are much higher than the estimated doses in space, but the lower scattered doses to the neighboring

normal tissue may be in the dose ranges that are relevant to NASA. Some neurocognitive function tests in longitudinal and cross-sectional studies are included in the follow-up evaluations in these clinical protocols. However, additional space-relevant endpoints may be needed to understand particle radiation induced effects in the CNS.

It is also recommended that NASA monitor developments in the area of airline crewmember health. Crewmembers are exposed to variable air quality, disrupted sleep and elevated radiation levels, primarily neutrons. Recent data demonstrates crews have higher prevalence of cancer (McNeely et al, 2018) and may be at increased risk for cognitive impairment and brain white matter abnormalities (Reneman et al, 2016). Although this literature is too premature to make solid conclusions, enhanced research efforts could provide useful data that generalizes to astronaut experiences.

Initiate clinical studies of workers exposed to polonium, radium, plutonium, uranium and americium. This population is limited but may be useful for evaluating tests for CNS function as well as mortality from dementia or other motor neuron diseases that might be associated with high-LET alpha particle dose to brain tissue following years of exposure.

4.3 Interaction and Potentially Synergistic Effects of Cosmic Radiation with Other Neurobehavioral and Physiological Stressors in Spaceflight.

In order to identify overlapping mechanisms of injury and potential discriminatory biomarkers, this section summarizes physiological, neurobehavioral and psychological conditions and stressors in spaceflight that may be synergistic or antagonistic with the risk of acute and late adverse central nervous system (CNS) effects of cosmic radiation on behavioral, cognitive, physiological, psychiatric, and sensorimotor functions in spaceflight. A better

understanding of these synergistic effects could aid in efforts to enhance human radiation resistance for deep space exploration (Cortese et al., 2018).

4.3.1 Other Stressors in Spaceflight.

Astronaut exposure to cosmic radiation can interact with multiple factors that contribute to stress in spaceflight. Astronauts can be exposed to additional spaceflight stressors that include the type and tempo of mission operational demands (e.g., workload, work complexity/difficulty; equipment reliability); loss of a crewmember(s); degree of confinement in mission; concerns about environmental exposures (e.g., CO_2;); challenges managing basic biological needs in mission (e.g., obtaining adequate sleep quantity/quality, food quantity/quality); coping with discomfort, pain, as well as other challenges to health and wellbeing in mission; interpersonal conflicts in mission (e.g., interactions with crewmembers and/or mission control); and astronaut concerns relative to family members and other matters on Earth.

4.3.1.1. Stress, Memory, and Hippocampus. Both acute and chronic stressors, alone and combined, adversely affect hippocampal dendritic morphology (Chen et al., 2010; Alfarez and Krugers, 2003), and multimodal stressors have been associated with severe memory deficits (Maras et al., 2014). There is also considerable evidence from studies in rodents that cosmic radiation can adversely affect the brain's hippocampus, which plays important roles in the consolidation of information from short-term memory to long-term memory and in spatial memory critical for navigation. Studies of cosmic radiation exposure in mice have identified neurobehavioral deficits that include reductions in exploratory behavior (Raber et al. 2016a; Allen et al 2015b), deficits in spatial, episodic, and recognition memory (Parihar et al 2016), reduced rates of fear extinction and elevated exploration anxiety (Kiffer et al 2015; Parihar et al 2016), and cognitive decrements in learning and memory (Parihar & Limoli 2013). Cosmic

radiation exposure is also associated with reductions in dendritic spine density, complexity and altered spine morphology along medial prefrontal, cortical, and hippocampal neurons that mediate neurotransmission of behavioral tasks (Parihar et al., 2016; Raber et al., 2016a; Allen et al., 2015; Kiffer et al., 2018; Parihar & Limoli, 2013). Impaired behavioral performance of individual animals has been reported to correlate significantly with reduced hippocampal spine density (Parihar et al 2015a).

4.3.1.2. Stress, Negative Affect and Right Prefrontal Cortex. Research on the neurobiology and physiology of stress and negative affect in healthy human adults has identified key neurobiological mechanisms subserving stress-related negative affective reactions. Neuroimaging studies have found that psychological stress can induce negative emotions and reduced vigilance, and the ventral right prefrontal cerebral cortex (RPFC) plays a key role in the central stress response. For example, the ventral RPFC along with right insula/putamen and anterior cingulate show sustained activation after task completion in healthy adults reporting a high stress level during tasks. Variations of baseline cerebral blood flow (CBF) in the ventral RPFC and right orbitofrontal cortex were found to correlate with changes in salivary-cortisol level and heart rate caused by undergoing stressful tasks (Wang et al., 2005).

The ability to regulate negative affect and thereby enable effective responses to stressful experiences involves engagement of prefrontal cortex (PFC) and amygdala brain regions, which influence the hypothalamic-pituitary-adrenal (HPA) axis (involved in the release of cortisol). Neuroimaging studies in human adults reveal that increasing negative affect results in activation of ventral lateral, dorsolateral, and dorsomedial regions of PFC and in amygdala activation. However, decreasing negative affect in human adults is associated with higher ventromedial

prefrontal cortex (VMPFC) activity and lower amygdala signal, with more normative declines in cortisol over the course of the day (Urry et al., 2006).

4.3.1.3 Stress From Social Isolation, Confinement and Crowding. Prolonged social isolation and restrictive confinement of rats has been reported to result in increased irritability and increased aggressive behavior (Welch et al., 1974; Palinkas *et al*., 2004). The most extensive space-simulation study of prolonged human confinement and social isolation was the MARS 500 study, in which behavioral, psychological, and physiological reactions were evaluated in a multinational crew of 6 healthy adult males confined in a 550m^3 chamber for 520 days during a high-fidelity simulated mission to/from Mars. Substantial inter-individual differences were observed in the psychological responses of crewmembers to the prolonged mission confinement and isolation (Basner et al., 2014). Crewmembers had elevated cortisol levels, increased lymphocyte amount and heightened immune responses during the mission, suggesting that prolonged isolation and confinement acted as chronic stressors and resulted in poorly controlled immune responses (Yi et al. 2014).

The two crewmembers who had the highest ratings of stress and physical exhaustion during the 520-day mission also accounted for 85% of the perceived conflicts (Basner et al., 2014), which suggests there are differential inter-individual neurobehavioral and neurobiological vulnerabilities to affective dysregulation and social stress during prolonged confinement simulating space flight.

4.3.2 Circadian Biology and Cosmic Radiation.

Humans, like all animals on Earth are biologically and behaviorally regulated temporally by an endogenous molecular clock that coordinates circadian (~24h) rhythmicity in cellular and physiological functions throughout the body, as well as behavioral alertness, cognitive capability,

and emotional regulation. The molecular clock is a biochemical oscillator that cycles with a stable phase and synchronizes to solar time. The mammalian circadian pacemaker is in the suprachiasmatic nucleus (SCN) of the hypothalamus, which neurobiologically regulates sleep and circadian rhythms (Saper et al., 2005). The SCN also controls peripheral physiological oscillators distributed throughout the body (Müller et al., 2015). It regulates human sleep–wake timing at an endogenous circadian period averaging 24.18h, that can be entrained to Earth's 24h period by certain synchronizers (i.e., zeitgebers), the most important being an ambient light-dark cycle of 24h in which the light contains the necessary blue portion of the photo spectrum (i.e., 446-477 nanometers) needed for daily entrainment. Absent this photo spectrum, circadian entrainment of biological functions and behaviors will not occur, and sleep and performance will be disrupted. For example, in the Earth-based Russian 520-day simulated round-trip mission to Mars, the simulated spacecraft facility had fluorescent lighting, which lacked the blue wavelength spectrum essential for circadian entrainment. As a result, chronically disrupted sleep-wake timing and increased sleep disturbances persisted in four of the six crewmembers throughout the mission (Basner et al., 2013a). One of them developed a persistent sleep onset insomnia with ratings of poor sleep quality, which resulted in chronic partial sleep deprivation, elevated ratings of daytime tiredness, and frequent deficits in behavioral alertness.

The endogenous circadian clock works by the expression of genes in SCN neurons that serve to activate or inhibit in a cyclical pattern over the course of a day. These molecular oscillations involve a negative feedback loop formed when the protein product of a gene turns off production of more protein (this discovery was awarded the 2017 Nobel Prize in Physiology or Medicine). Daily oscillations of the SCN molecular clock are communicated physiologically throughout the body to coordinate circadian rhythmicity in other tissues. Circadian regulation has

a fundamental role in cellular functions (e.g., disruption of core circadian clock genes can erode health and may lead to cancer (Gery et al., 2006)).

Importantly, spaceflight beyond Earth orbit poses a number of significant challenges to circadian biology, including potential exposure to cosmic radiation, which has been shown to damage the circadian clock and produce a phase advance in endogenous circadian biology, in a dose- and time-dependent manner (Oklejewicz et al., 2008). Cosmic radiation-induced circadian phase advances have been observed in liver, adrenal and pancreas (Müller et al., 2015), which could disrupt physiological functions and degrade both health and neurobehavioral capability in spaceflight. There is also evidence that the cellular response to DNA damage from cosmic radiation varies as a function of the endogenous circadian phase of the molecular clock to cosmic radiation exposure (Corrà et al., 2017). Therefore, interactions between exposure to cosmic radiation and endogenous circadian phase should be accurately characterized in studies of the effects cosmic radiation in animals and humans, in order to ensure accurate prediction and mitigation of the neurobehavioral consequences of exposure to cosmic radiation.

Circadian rhythmicity and homeostatic sleep drive interact neurobiologically (Saper et al., 2005; Borbély et al., 2016) to produce effective waking neurobehavioral, cognitive, and sensory-motor functions, and healthy physiological regulation. Cosmic radiation effects on circadian and sleep neurobiology in spaceflight could have serious adverse consequences for astronauts' health and behavioral capabilities. Biomarkers of endogenous circadian phase and circadian period in spaceflight are needed to identify this risk. There are a number of physiological measures that could serve as circadian-phase biomarkers in spaceflight (e.g., monitoring of core body temperature, melatonin secretion, or molecular clock dynamics). A biomarker to detect perturbation of circadian biology by cosmic radiation would ideally be used

when there is environmental circadian entrainment via blue-enriched (i.e., 446-477 nm) light in the photon spectrum, which is the most potent region for synchronizing circadian rhythms of sleep and waking during spaceflight (Brainard et al., 1988; Mottram et al., 2011).

4.3.3 Sleep Duration, Sleep Quality, Stress and Physical Exhaustion in Spaceflight.

Adequate daily sleep duration, like daily circadian entrainment, is an essential requirement for optimal cognitive and neurobehavioral functions and healthy physiological functions on Earth and in prolonged spaceflight, where sleep is often reduced for reasons not well understood (Dinges et al., 2016). Cognitive and affective functions and their neurobiological substrates are especially vulnerable to the effects of inadequate sleep. In addition to disease prevention, scientific studies in animals and humans consistently find that adequate daily sleep is essential for vigilance, learning and memory. For example, sleep after motor learning promotes the formation of postsynaptic dendritic spines on a subset of branches of individual layer V pyramidal neurons. With adequate sleep, new spines form on different sets of dendritic branches in response to different learning tasks and are protected from being eliminated when multiple tasks are learned. Neurons activated during learning of a motor task are reactivated during subsequent non–rapid eye movement (NREM) sleep. Disrupting this neuronal reactivation prevents branch-specific spine formation (Yang et al., 2014; Restivo et al., 2009). These and many other findings indicate that sleep has an essential role in promoting learning-dependent synapse formation/strength and maintenance on selected dendritic branches, which contribute to memory storage and cognitive functions (Raven et al., 2018). Importantly, inadequate sleep has an immediate and profound effect on cortical and hippocampal dendritic spines concurrent with compromise of motor endurance and spatial memory (Wang et al., 2005). Inadequate sleep also has adverse effects on concentration, attention and memory in both rodents

and humans (Havekes et al., 2016). This suggests that adequate daily sleep duration in space could be a key countermeasure for the loss of hippocampal dendritic spines due to cosmic radiation.

Both reduction in astronaut sleep duration on ISS, and astronauts' self-ratings of poor sleep quality on ISS during 6-month missions, were found to be significantly associated with decrements in their psychomotor vigilance test (PVT-B) performance of alertness and attention, and with increases in their perceptions of stress and physical exhaustion during the missions (Dinges et al., 2016). Satisfying daily human sleep need via adequate sleep duration, sleep stage physiological consolidation and good sleep quality, is essential for maintaining effective neurobehavioral functioning in spaceflight, including all aspects of cognitive performance, mood and appetite regulation, nociception, as well as critical physiological functions (e.g., glymphatic clearance of brain metabolites (Xie et al., 2013)). A review 1,266 scientific publications on sleep duration and health found that chronically sleeping <7h per day (on Earth) was associated with a range of health risks including cardiovascular and cerebrovascular diseases, obesity and metabolic dysregulation, cancer, depression, diabetes, and all-cause mortality risk (Watson et al., 2015a,b), as well as altered metaboloic levels in blood, inflammation, and loss of muscle tissue (Cedernaes et al., 2018). Obtaining adequate daily sleep in space is therefore critical to astronaut health functioning, especially during long-duration missions.

However, obtaining adequate sleep in space flight has been a challenge to date. Two prospective studies involving a total of 6,123 daily sleep durations in spaceflight at low Earth orbit, recorded either with wrist actigraphy (4,014 days) or with electronic diary (2,109 days) in N=115 astronauts, during STS shuttle missions and ISS missions, found that sleep duration in spaceflight consistently averaged less than 7h/day and most often averaged 5h-6.5h/day in-flight

(Barger et al., 2014; Dinges et al., 2016). In spaceflight, astronauts averaged ~2h less sleep per day than they obtained postflight on Earth. Nearly half of the astronauts obtained ≤6h sleep per day on ISS, which was associated with reliable deficits in Psychomotor Vigilance Test (PVT-B) performance inflight (Dinges et al., 2016; Basner et al., 2011). Sleep times logged in by N=21 astronauts during 6-month ISS missions revealed that sleep duration was significantly longer on weekends inflight than on weekdays, suggesting astronauts experienced a "sleep debt" in spaceflight that was periodically dissipated on weekends (Dinges et al., 2016). Sleep medications (i.e., hypnotics) have been used to facilitate astronauts' sleep deficits in space (Wotring, 2015). However, the use of hypnotic medications can pose a risk to astronauts' performance capability if there is an emergent event in spaceflight (Johnston et al., 2015).

4.3.4 Cumulative Neurobehavioral Deficits from Chronic Sleep Restriction.

The sleep-restricted PVT-B performance deficits found in astronauts who acquired 6h sleep or less per day on ISS (Dinges et al., 2016) are consistent with Earth-based laboratory experiments showing that chronic restriction of sleep to 4h/day, 5h/day, or 6h/day results in escalating sleep-dose-response cognitive performance deficits in a range of neurobehavioral functions, including decreases in alertness, instability of vigilant attention, delayed psychomotor response times, slowed cognitive processes, reduced emotional expressiveness, increases in working memory errors, and increases in operational errors and accidents. These performance deficits, and associated health risks, became markedly worse as sleep restricted to <7h per day continues across multiple days without extending sleep to >7h to allow recovery from the escalating sleep debt (Dinges et al., 1997; Van Dongen et al., 2003; Belenky et al., 2003; Lim & Dinges, 2008; Lim & Dinges, 2010a; Minkel et al., 2011; Mollicone et al., 2008, 2010; Basner et al, 2013b; Watson et al., 2015a,b).

Although the neurobiological bases of the cumulative increases in performance deficits across days of inadequate sleep have not been established, arterial spin labeling (ASL) perfusion functional magnetic resonance imaging (fMRI) in healthy adults has shown that performance on the PVT activates a right lateralized fronto-parietal attention network in addition to the basal ganglia and sensorimotor cortices. Regional cerebral blood flow (CBF) decreases in this network correlated with fatigue-related performance decline as a function of PVT time-on-task (Lim et al., 2009). Moreover, resting regional CBF in the thalamus and right middle frontal gyrus prior to task onset was predictive of subjects' subsequent PVT performance decline (Lim et al., 2009), suggesting that resting CBF quantified by ASL perfusion fMRI may be a useful indicator of performance and a potential biomarker of the level of fatigue or fatigue-vulnerability in the neural attentional system.

PVT vigilant attention performance (Lim & Dinges, 2008; Basner et al., 2011) has proven to be a highly sensitive neurobehavioral assay for identifying the impact of sleep loss in humans (Basner et al., 2015). PVT performance has also been found to be a sensitive performance assay for the effects of ionizing radiation in rats (Davis & Hienz, 2014; Davis et al., 2016a,b), thereby providing a translational paradigm for comparing the effects of these two spaceflight risk factors on basic neurobehavioral functions without contamination by learning and aptitude.

NASA recently completed development and validation of a neuroimaging-based cognitive performance test battery (i.e., “Cognition”) for use in spaceflight (Basner et al., 2015). It consists of 15 unique forms of 10 neuropsychological tests that cover a range of cognitive domains, including emotion processing, spatial orientation, and risk decision-making. “Cognition” includes tests known to engage specific brain regions and/or networks, as evidenced

by functional neuroimaging, which makes the battery useful for identifying biomarkers for the effects of ionizing radiation relative to other spaceflight conditions. Normative data on the "Cognition" test battery have been established, as have validity, sensitivity, feasibility, and acceptability.

4.3.5 Biomarkers for Phenotypic Differential Vulnerability to Spaceflight Stressors.

Biomarkers are needed for the substantial phenotypic differential vulnerability of individuals to the stressors conditions in prolonged spaceflight. Cognitive vulnerability to sleep loss, which varies greatly among healthy adults (Tkachenko and Dinges, 2018), including astronauts (Dinges et al., 2016), illustrates why such biomarkers are needed. As an example of one approach to this problem, microarrays and bioinformatics analyses were used by Uyhelji et al. (2018) to explore candidate gene expression biomarkers associated with phenotypic differential vulnerability to neurobehavioral impairment to sleep loss (N=11 healthy adults undergoing 62h of total sleep loss). A total of N=212 genes changed expression in response to sleep loss (most exhibiting down-regulation), while 28 genes were associated with neurobehavioral deficits in PVT performance. Results supported previous findings associating total sleep loss with the immune response and ion signaling, and revealed novel candidate biomarkers such as the Speedy/RINGO family of cell cycle regulators relative to inter-individual PVT performance vulnerability to sleep deprivation (Uyhelji et al., 2018).

4.3.6 Immune and Inflammatory Responses to Sleep Deprivation and Confinement.

The biological mechanisms underlying astronauts' reduced sleep duration and/or reduced sleep quality in spaceflight, as well as their subjective ratings of elevated stress and physical exhaustion in spaceflight (Dinges et al., 2016), have not been adequately identified. However, these responses to spaceflight can have biological consequences that may interact with the

effects of cosmic radiation (Schaue et al., 2015). A common pathway by which reduced sleep duration and poor sleep quality in spaceflight could promote feelings of stress and physical exhaustion is via neuroendocrine and neuroimmune mechanisms. Sleep deprivation is a neurobiological and physiological stressor (McEwen, 2006). Neuroendocrine stress systems (i.e., the autonomic sympatho-adrenal system and the hypothalamic-pituitary-adrenal axis) show temporary increases in activity during sleep deprivation and sleep restriction (Meerlo et al., 2008). Experiments have shown that acute sleep deprivation on Earth is associated with both elevated resting cortisol release and with an exaggerated cortisol response to a stressor indicative of elevated HPA axis responses in healthy adults (Minkel et al., 2014). Moreover, sleep deprivation in humans has varied effects on the immune system. A partial night of sleep can reduce natural killer and cellular immune responses in humans (Irwin et al., 1996), while a night of total sleep deprivation can activate morning levels of cellular and genomic markers of inflammation, including IL-6, TNF alpha (Irwin et al., 2006), and IL-17 (Cedernaes et al., 2018).

Sleep deprivation may not only have a direct activating effect by itself, but overtime it may also affect the reactivity of these systems to other physiological stressors and challenges, including cosmic radiation. For example, prolonged crew confinement in the 520-day simulated Mars mission resulted in elevated cortisol levels, increased lymphocyte amount and heightened immune responses, suggesting that prolonged isolation acted as a chronic stressor that triggered leukocyte phenotype changes and poorly controlled immune responses (Yi et al., 2014). Studies of healthy adults restricted to 6h sleep per night, which is not uncommon in spaceflight (Barger et al., 2014; Dinges et al., 2016), found increased levels of circulating IL-6 (Vgontzas et al., 2004). Sleep restricted to 4h per night for 5 nights resulted in increased lymphocyte activation and the production of proinflammatory cytokines including IL-1, beta IL-6, and IL-17, which

regulates host defense and inflammatory diseases, remained elevated after 2 nights of recovery sleep. This suggests that long-term sleep restriction in spaceflight may lead to persistent changes in the immune system, and to increased production of IL-17 which, together with C-reactive protein (CRP) may increase the risk of developing cardiovascular diseases.

Experimental sleep deprivation studies in healthy adults have found that total sleep deprivation resulted in leukocytosis and increased natural killer cell activity (Dinges et al., 1994). Total sleep loss also produced significant increases in plasma levels of sTNF-αRI and IL-6, messengers that connect the nervous, endocrine, and immune systems (Shearer et al., 2001). Plasma levels of CRP, an acute-phase reactant indicative of inflammation that increases following IL-6 secretion, have also been found to be elevated during sleep restriction and sleep deprivation (Meier-Ewert, et al., 2004), which may be a risk factor for cardiovascular health in spaceflight.

4.3.7 Sleep Deprivation and the Effects of Cosmic Radiation.

There is recent evidence that sleep deprivation may potentiate the effects of cosmic radiation. A study of N=16 healthy adults undergoing 39 hours of total sleep deprivation followed by a well-validated social stress test, assessed DNA damage in blood cells irradiated *ex vivo* (Moreno-Villanueva et al., 2018). Sleep deprivation alone or in combination with psychological stress did not induce significant increases in DNA damage, and radiation-induced DNA damage decreased significantly in response to sleep deprivation. However, DNA damage increased to baseline levels when combined with psychological stress. The study reported that individuals who were cognitively more vulnerable to sleep loss also had more radiation-induced DNA strand breaks before sleep deprivation, which the authors concluded suggested a greater sensitivity to DNA damage from environmental stressors (Moreno-Villanueva et al., 2018).

It is unknown to what extent cosmic radiation may affect the functions of sleep directly or indirectly, but if there are effects, they likely will involve disruptions of the neural circuity of sleep, as well as the humoral regulation of sleep, particularly immune regulation.

4.3.8 Carbon Dioxide (CO_2) Effects.

CO_2 levels in the closed environment of a spacecraft can be elevated via respiration, and contribute to physiological and cognitive deficits (Basner et al., 2018), as well as physical symptoms (Law et al. 2014), depending on the degree of exposure. Increased CO_2 blood concentrations elicit a number of physiological responses triggered by a pH-induced stimulation of central and peripheral chemoreceptors, including increases in heart rate and minute ventilation, cerebral arterial vasodilation, and central nervous system (CNS) arousal (Langhorst et al., 1983; Brian, 1998; Guyenet et al., 2010; Guyenet & Bayliss, 2015). Shortness of breath, headaches, and dizziness are early symptoms of exposure to increased CO_2 concentrations, while higher concentrations can induce sweating, chest pain, anxiety, and ultimately convulsions, unconsciousness, and ultimately death (Law et al., 2014).

Build-up of CO_2 in the blood (i.e., hypercapnia) due to hypoventilation results in respiratory acidosis. There is evidence from rodent studies that extracellular acidosis associated with several pathological conditions in the CNS may significantly diminish long-term potentiation (LTP) increases in synaptic strength, and thus negatively affect physiological processes that utilize LTP, such as memory consolidation (Velíšek, 1998). Extracellular acidosis-induced synaptic depression is likely to result from an inhibition of presynaptic Na+ conductance, thereby decreasing the amplitude of action potentials in individual afferent fibers or the number of afferent fiber activation to stimuli, and then indirectly affecting the signaling processes contributing to neurotransmitter release (Hsu et al., 2000).

During his 1-year mission on ISS, astronaut Scott Kelly noted his experiences and those of the crew relative to CO_2 exposure (e.g., headaches, irritability), especially when the carbon dioxide removal assembly (CDRA) was down or undergoing maintenance (Kelly, 2017). These experiences included a sense of a lack of sharpness, periodic headaches, irritability, difficulty concentrating on what he was doing, and deficits in his cognitive performance. He noted that difficulty concentrating on ISS is what most astronauts refer to as "space brain" and that over time you can "work through the symptoms, which can vary based on CO_2 levels, vestibular symptoms, sleep quality, and probably other factors too" (p. 226, Kelly, 2017).

Astronaut Kelly was also concerned that the loss of a "Progress" resupply ship to ISS could result in the loss of the crew via CO_2 asphyxiation, and that when the CDRA was repaired there was a boost in crew morale. He noted that CO_2 was not a problem when he was the only one on the US side of ISS. CO_2 was also noticeably lower when he wore his space suit, which had a more efficient CO_2 scrubber. He noted in his book that after his return to Earth, NASA agreed to manage CO_2 at a lower target level, and get better versions of the carbon dioxide scrubbers (Kelly, 2017).

4.3.9 SPACECOT Study: Head-down Tile Plus CO_2

The combined effects of simulated microgravity and elevated levels of CO_2 on cognitive performance was assessed via a randomized, double-blind, crossover trial (SPACECOT), in 6 healthy males (mean ± SD age: 41±5y) by Basner et al. (2018). Subjects were exposed to 0.04% (ambient air) and 0.5% CO_2 concentrations during 26.5-h periods of -12° head-down tilt (HDT) bed rest with a 1-wk washout period between exposures. Subjects performed the 10 tests of the "Cognition" test battery before and on average 0.1, 5.2, and 21.0 h after the initiation of HDT bed rest. HDT in ambient air induced a change in cognitive performance response strategy, with

increased response speed (+0.19 SD; P = 0.0254) at the expense of accuracy (+0.19 SD; P = 0.2867), resulting in comparable cognitive efficiency. The observed effects were small and statistically significant for cognitive speed only. However, even small declines in accuracy can potentially cause errors during mission-critical tasks in spaceflight. Unexpectedly, exposure to 0.5% CO_2 reversed the response strategy changes observed under head-down tilt in ambient air. This was possibly related to hypercapnia-induced cerebrovascular reactivity that favors cortical regions in general and the frontal cortex in particular, or to the CNS arousing properties of mildly to moderately increased CO_2 levels. There were no statistically significant time-in-CO_2 effects for any cognitive outcome (Basner et al., 2018). The small sample size and the small effect sizes are major limitations of this study and its findings. The authors caution that the results should not be generalized beyond the group of investigated subjects until they are confirmed by adequately powered follow-up studies.

4.4 Future Direction and Recommendations

1. Promote analyses of results from head and neck cancer patients as they can be of interest to NASA. While therapeutic target doses to the cancer tissues are much higher than the estimated doses in space, lower scattered doses to the neighboring normal tissue may be in the dose ranges that are relevant to NASA. Due to the favorable depth-dose characteristics of particle beams and its utility in radiotherapy, the patient populations that were either treated or are currently undergoing therapy are increasing both in the U.S and internationally.

2. Collaborate with the charged particle radiotherapy cancer centers globally to harness the world-wide data base of patients to enable follow-up on behavioral and other CNS-associated endpoint in this patient population.

5. Observations with rodents

5.1 Inbred and outbred animals

For assessment of CNS risk as a result of space irradiation, inbred animals, crosses of inbred mice to generate F1 or F2 mice, stocks with very limited genetic heterogeneity, or outbred animals without analysis of the genetic factors of the individual animals, are mostly being used. It is critical to include both inbred and outbred animals in assessments of CNS risks, as both models allow answering distinct questions regarding risk that cannot be easily answered or cannot be answered at all at this moment in both inbred and outbred animal models. In general, CNS radiation effects in genetically heterogeneous populations would better model genetically diverse human populations. However, the degree of genetic heterogeneity of an outbred colony depends on the history of the colony, varies dramatically, and typically there is no genetic quality control. Although genetic variation is more relevant to the genetic diversity in humans and will be valuable to determine the role of genetic risk factors in the radiation response, genetic variation in outbred animals makes it very challenging to assess overall effects of irradiation due to the large individual variation in performance levels. Even if a longitudinal design is being used, it can be challenging to determine risk when baseline performance is so variable between animals. Obviously, it will be easier to detect space radiation-induced impairments if baseline performance or performance in sham-irradiated animals is robust and easier to detect an improvement in performance when baseline performance or performance in sham-irradiated is relative modest. In addition, to determine the role of epigenetic mechanisms in the radiation response, it will be extremely challenging to distinguish genetic from epigenetic effects when there is a large individual variation in behavioral and/or cognitive performance. Although this is not always fully appreciated, the choice of using inbred or outbred animals should depend on the

space radiation question being asked and both are required for a complete and valid risk assessment. In addition, there are practical and budget limitations that need to be considered as more outbred animals might be required due to the individual variability. For example, a power analysis for detecting a 4-min difference in sleeping time under barbiturate anesthetics, revealed that for a power of 80% and a 5% significance level 12 inbred mice per group or 180 outbred mice per group would be required (Chia, Achilli et al. 2005). Within the limitations of budget, it might not be feasible to use outbred animals and to maintain sufficient power to determine a radiation dose response curve for CNS-related outcome measures in a given study. In addition, it is hard to determine and model risk understanding that there is a wide variation in performance at baseline that might critical modulate the radiation response and the impact of environmental challenges in general on behavioral and cognitive performance. For example, the analyses of the effects sleep deprivation on performance in astronauts illustrate the individual susceptibility in the ability of sleep deprivation to affect individual behavioral and cognitive performance; while some astronauts are resilient to detrimental effects of sleep deprivation on behavioral and cognitive performance, other astronauts are highly sensitive. A similar heterogeneity in CNS response would be expected following space radiation exposure and is very much in line with the concept of cognitive reserve. This is important to keep in mind when modeling approaches are being developed and used to determine risk to individual astronauts, the team, and ultimately the success of the mission. Typically, the modeling is already challenging enough and requires several assumptions. It is not trivial how baseline performance differences and individual risk factor that modulate the radiation response can be easily incorporated. Similarly, sex differences in the radiation response are typically not being considered in the modeling of CNS risk to radiation. One potential approach could be to consider modeling risk in high and low risk

individuals and females and males separately. The approach of separately analyzing and comparing performance in low and high performance has been pursued in the context of pathways and biomarkers pertinent to cognitive performance in rodents (Benice, Rizk et al. 2006, Benice and Raber 2009) and nonhuman primates (Haley, Kroenke et al. 2011b, Haley, McGuire et al. 2011a) in aged animals and, more recently, in rodents following radiation (Britten, Jewell et al. 2017). Although this seems a reasonable practical approach, it is recognized that for some performance there is really a wide spectrum more than two distinct ranges in performance and as a result the division in two performance groups is rather artificial and might make it challenging to model risk for intermediate performance levels.

In contrast to inbred animals, outbred animals, and in particular advanced heterogeneous stocks with high-throughput single nucleotide polymorphism (SNP) genotyping, would be ideal for study space radiation-genotype relationships and genotype-phenotype relationships involved in radiation effects on the brain. An example of an advanced heterogeneous stock is the HS/Npt colony that was developed in 1991 as a tool to investigate complex genetic traits (Demarest, Koyner et al. 2001). The eight inbred strains that were combined to generate this colony were chosen for their diversity in a number of behavioral traits. The mice also capture a significant amount of the genetic diversity that is available in *Mus musculus*. The mice are now at G_{70} which means the genetic map has expanded approximately 35-fold. The genome of each mouse can be scanned with markers to identify changes. Subsequently, fine mapping might lead to a narrowed set of genes regulating the phenotype. Since the organization of genes on chromosomes is highly similar between mouse and humans, mapped quantitative trait loci (QTL)s in the mouse often maps them in the human genome as well. So, for generating a thorough understanding of effects of space irradiation on the brain both inbred and outbred animals are valuable.

In one large-scale unique study, the effects of HZE ion irradiation on the brain were assessed in the genetically heterogeneous HS/Npt population, by irradiating mice at 8-12 weeks of age with 0.4 Gy of 240 MeV/n ^{28}Si or 600 MeV/n ^{56}Fe ions or 3 Gy of ^{137}Cs gamma rays, or sham irradiating them and three months later weighing and testing them for contextual (hippocampus-dependent) and cued (amygdala-dependent) fear conditioning (see below for the description of these tests) (Raber, Belknap et al. 2014a). Each mouse was genotyped for 77.8K SNP markers by GeneSeek (Lincoln, NE). tested. About 40K markers were identified with sufficient genetic diversity (minor allele frequency above 5%). The mapping resolution was in the 200 kb to 2 Mb range for this HS mapping population. The QTL analysis was based on close to 40 behavioral and cognitive phenotypes for radiobiological endpoints of interest related to performance in the hippocampus-dependent contextual and hippocampus-independent cued fear conditioning tasks. The analysis revealed large differences in mean and variance in virtually all ~40 individual behavioral phenotypes between the 600 and 1200 cohorts. Radiation effects did not lead to overt changes in behavioral or cognitive performance. However, one important finding is that the identity of the genetic loci (QTLs) that influence behavior shifts depending on radiation type. The large number of phenotypes measured (>40) are not independent but display significant dependencies/correlations. A major finding, which is also a feature of the analysis approach, is the fact that all phenotypes fall in the category of highly polygenic traits, which means that numerous (hundreds) of genes/loci contribute small amount to traits that are nevertheless significantly driven by genetic factors. This is concordant with other finding in literature (best example is human height). Moreover, this seems to be the emerging consensus coming out of the GWAS studies. Biological pathways/mechanisms in the form of Gene

Ontology categories were identified associated with the groups of genes/loci emerging from the analysis.

Rat strains commonly used for space radiation studies include Sprague-Dawley, Wistar, Long-Evans, Fisher-344, Lewis, and WAG/Cmcr/Rij. WAG stands for Wistar Albino Glaxo. Adding the CMCR happened in about 2005 when the animals were registered in the rat genome database and by that time were separated by enough generations from the original that a distinction was required. Rij stands for Rijswijk, the city in the Netherlands where the TNO institute bred these animals. The WAG/Cmcr/Rij strain is of interest in the context of long-term space missions such as those planned to Mars, as WAG/Rij rats exhibit depression-like symptoms. These symptoms include: 1) decreased investigative activity in the open field test; 2) increased immobility in the forced swimming test; 3) decreased sucrose consumption and preference (anhedonia); 4) adopting passive strategies in stressful situations, helplessness, and submissiveness; and 5) inability to make choice and overcome obstacles. In addition, there are alterations in serotonin and dopamine levels in the brain, which are typical for depressed patients (Sarkisova and van Luijtelaar 2011).

Mouse strains commonly used for space radiation studies include C56BL/6 and C56BL/6 x DBA F1 (B6D2F1). It is important to recognize that there are dramatic strain differences in performance on behavioral and cognitive tests in mice (Paylor, Baskall et al. 1993, Paylor, Tracy et al. 1994, Klapdor and Van der Staay 1996, Crawley, Belknap et al. 1997, Owen, Logue et al. 1997) and rats (van der Staay 2002, van der Staay, Schuurman et al. 2009). For behavioral and cognitive studies, the sham-irradiated animals in the selected animal model need to be able to perform the test well so that an effect of space irradiation can be detected. However, it is conceivable if the performance is very good that it might be harder to detect an effect of

irradiation than if the performance is adequate to show an effect. For consideration of cognitive outcome measures, it is important to exclude potential differences in behavioral, sensory, or motor abilities that might affect performance on cognitive tests. For example, difference in response to tone or shock are important to consider in interpretation of cognitive paradigms such as active or passive avoidance learning and memory and fear conditioning. Rearing and housing conditions and motivational differences can affect behavioral and cognitive performance as well and therefore must also be considered.

5.1.1 Mutants

In addition to wild-type animals, mutant animals are being used to assess the effects of space radiation on the brain. These mutants include several animal models. One group includes animals lacking specific genes, either conventionally through brain development or inducible/conditional later in life, whose transcripts are thought to be important in mediating the radiation response. Examples include mice deficient in an isoform of superoxide dismutase (Raber, Villasana et al. 2011b, Huang, Zou et al. 2012, Zou, Leu et al. 2013) the chemokine receptor C-C chemokine receptor type 2 (CCR2) (Belarbi, Jopson et al. 2013, Raber, Allen et al. 2013a), or apolipoprotein E (Acevedo, McGinnis et al. 2008a). A second group includes mutant models involving the expression of a human gene, for example human catalase in the mitochondria (MCAT) (Olsen, Johnson et al. 2013, Parihar, Allen et al. 2015a). In studies in which conventional knockout or transgenic animal models are being used, a mechanistic or mitigating question is typically being asked and the potential developmental effects as a result of the gene deficiency or overexpression need to be considered. A third group of animal models involves the replacement of mouse gene with a human one. An advantage of such a model is that the human gene can be expressed under control of a mouse promoter, providing physiological control and levels of the gene product

without potential competition of mouse and human proteins in the same animal. An example of such an animal model are the human apolipoprotein E (apoE) targeted replacement mice that express human apoE isoforms, which differ in risk to develop age-related cognitive decline, Alzheimer's pathology, and post-traumatic stress disorder (PTSD), under control of the mouse apoE promoter (Villasana, Benice et al. 2011, Haley, L et al. 2012, Yeiser, Villasana et al. 2013, Villasana, Weber et al. 2016). A fourth group of animal models are models of conditions or diseases pertinent to long-term risks of the brain to space irradiation. For example, animal models containing dominant negative genes of Alzheimer's disease, either alone (Vlkolinsky, Titova et al. 2012) or combined (Cherry, Liu et al. 2012), are being used. With regard to the 4th group, the question is typically whether space irradiation causes an earlier age at onset and/or worsens the severity of the CNS condition or disease.

5.1.2 Sex differences

Sex has a profound effect on brain function under physiological and pathological conditions and on susceptibility and severity of various neurological conditions (McNemar and Stone 1932, Gray 1971, Gray and Lalljee 1974, Einon 1980, Forloni, Fisone et al. 1986, Gaulin and Fitzgerald 1986, Hobson 1987, Heinsbroek, Feenstra et al. 1988, Lipa and Kavaliers 1990, Juraska 1991, Persaud 1991, Galea and Kimura 1992, Halpern 1992, Kimura 1992, Corder, Saunders et al. 1995, Galea, Kavaliers et al. 1996, Kolb and Cioe 1996, Perrot-Sinal, Kostenuik et al. 1996, Collins and Kimura 1997, Dufouil, Ducimetiere et al. 1997, Farrer, Cupples et al. 1997, James and Kimura 1997, Epting and Overman 1998, Fugger, Cunningham et al. 1998, Moffat, Hampson et al. 1998, Monleón and Parra 1998, Postma, Izendoorn et al. 1998, Curry, Karnaoukhova et al. 1999, Gur, Turetsky et al. 1999, Lebowitz and Brown 1999, Maguire, Burgess et al. 1999, Postma, Winkel et al. 1999, Pryce, Lehmann et al. 1999, Pellis 2002, Frick

and Gresack 2003, Jonker, Eriksson et al. 2003, Easton, Norton et al. 2004, Guo, Wu et al. 2004, Morris, Jordan et al. 2004, Postma, Jager et al. 2004, Colton, Brown et al. 2005, Lacreuse, Kim et al. 2005, Raber, Inman et al. 2005, Clinton, Billings et al. 2007, Devor, Gilad et al. 2007, Morris, Jordan et al. 2008, Huang, Smith et al. 2010, Lin, Choi et al. 2011, Lebron-Milad, Abbs et al. 2012, Zuloaga, Johnson et al. 2014). There are also sex differences in regulation of the immune function (Ngo, Steyn et al. 2014, Klein and Flanagan 2016) and cardiovascular function (Denton and Baylis 2007, Kollar and Ostadal 2013, Wheatly, Snyder et al. 2014), both of which can affect brain function. Therefore, sex needs to be considered as a biological variable (Bale and Epperson 2017, Miller, Marks et al. 2017) in assessing CNS risk of space irradiation as it is conceivable that space irradiation might also have differential effects on the brains of female and male astronauts. Female astronauts have participated in space missions since 1963, and the role of sex differences in effects of space irradiation on the brain are being considered. Up to this point, most radiation studies have used males while a limited number of radiation studies have involved only (Acevedo, Tittle et al. 2008, Dayger, Villasana et al. 2011, Rabin, Carrihill-Knoll et al. 2013) female animals (Acevedo, McGinnis et al. 2008) or both male and female animals (Villasana, Poage et al. 2008, Rabin, Shukitt-Hale et al. 2010, Villasana, Rosenberg et al. 2010, Villasana, Benice et al. 2011, Haley, L et al. 2012, Kronenberg, Gauny et al. 2018).

5.2 Descriptions of behavioral and cognitive endpoints

5.2.1 Behavioral and cognitive endpoints relate to both simple and complex human behaviors (including possible post-traumatic-stress-disorder-like changes)

For assessments of CNS risk to space irradiation, both short-term effects, as described earlier and defined by NASA as those occurring during the mission, as well as long-term effects, defined as those occurring following the mission, are relevant. In addition to radiation exposure,

there are other environmental challenges/stressors astronauts are exposed to in the isolated, confined, and extreme environment during a mission that might interact with the effects of space irradiation in a synergistic fashion or cause additive effects. This extreme environment includes microgravity and its effects on sensorimotor function and central reinterpretation of vestibular information, and spatial disorientation. Ultimately, for assessment of risk to individual astronauts and the whole crew with regard to success of the mission, all the environmental challenges the astronauts are exposed to should be included in the model. Spatial disorientation includes inversion illusion, paradoxical sensation of being gravitationally upside down even when visually upright, height vertigo caused by viewing the earth beneath, and visual reorientation illusion of surfaces nearest to the feet (seem like floor) and parallel to the body (seem like walls). Visual acuity is affected as well. Other aspects of the environment include sleep loss, circadian desynchronization, work overload, and elevated carbon dioxide levels. As a vasodilator, elevated carbon dioxide levels increase cerebral blood flow, which in turn is associated with the probability of headaches. This might impair decision making (Satish, Mendell et al. 2012), as well as altering nitric oxide and carbon monoxide levels in brain (Garthwaite 1991, Artinian, Ding et al. 2001).

Although in this report we focus on effects of irradiation on the brain, we realize that there are environmental stressors involved with testing animals that are irradiated at BNL, including stress during transport to and from the NSRL facility where the animals are irradiated. This transport, when documented in detail, might not be a big concern, as astronauts are also exposed to multiple stressors. In addition, we recognize that for most studies performed so far acute single irradiation doses, combined mixed irradiations, or fractioned doses have been used while during a space mission there will be chronic exposure. There is a current effort to determine

effects of chronic neutron exposure on CNS and cancer-related outcome measures in rodents in a dedicated building on the campus of Colorado State University in Fort Collins but the results from these studies are not available yet to be included in this report.

CNS effects of space irradiation include both behavioral and cognitive alterations. Behavioral alterations include hallucinations, stress-related symptoms, depressive-like symptoms, apathy, increased irritability, increased impulsivity, and reduced sleep (both REM and non-REM sleep are affected). For a recent evidence report about risk of adverse cognitive or behavioral conditions and psychiatric disorders, please see (Slack, Williams et al. 2016). Cognitive alterations include reduced reaction time, reduced attention, reduced recognition of the environment and affected spatial navigational abilities. Behavioral alterations can affect cognitive performance as well. For example, psychomotor function and cognitive speed are affected by sleep. The studies on the behavioral health of astronauts revealed profound individual vulnerabilities and resiliencies, indicating the involvement of genetic and epigenetic factors in these effects. Individual behavioral and cognitive differences will be further discussed below in 5.1.4.

Various behavioral and cognitive tests typically used in space radiation studies involving animal models assess behavioral and cognitive measures relevant to those in humans during and following space missions. Thus, although the tests used in animals might not resemble those used in the clinic, what is being tested and the outcome measures are relevant to both humans and animals. In addition, there have been efforts to develop specific tests that are somewhat similar in animals and humans, although different strategies might be used in animals and humans performing these tests. Examples include a test of object recognition (Novel Image Novel Location (NINL) (Raber, Inman et al. 2005, Raber 2015a), a test spatial navigation (Memory

Island) (Raber, Inman et al. 2005), the Cambridge Neuropsychological Test Automated Battery (CANTAB) using a touchscreen test for rodents (Bussey, Holmes et al. 2011), and a rodent version of the human psychomotor vigilance test (Davis, Roma et al. 2016). A potential disadvantage of the latter test is the involvement of multiple trials (36 trials per session for 50 sessions) over 60 days and food restriction at 85% free-feeding body weight in some reported experimental paradigms (Ninthianantharajah, McKechanie et al. 2015).

Common behavioral and cognitive tests used in animal models (Van Meer and Raber 2005) to assess effects of irradiation are described below, along with effects of space irradiation on behavioral and cognitive outcome test measures. In order to include pertinent space radiation CNS data not yet published, Dr. Raber solicited this information from NASA-funded colleagues in April of 2016 and again in January and March of 2017. We thank our colleagues for sharing this information. Realizing that with regard to unpublished the researchers who generated them might not agree with us including their unpublished data as part of this NCRP report, we limited our review to published studies and unpublished studies for which the authors provided explicit consent for their inclusion in the report. We did not include a complete overview and discussion of the effects of X-Rays and ^{137}Cs or other gamma irradiation in animals, as they are less relevant and reviewed elsewhere (Obenaus, Mickley et al. 2012). (Obenaus, Mickley et al. 2012). In addition, most gamma irradiation in animal studies did not involve radiation exposure at and transportation to and from BNL. The additional environmental conditions make it hard to directly compare space radiation studies involving exposures at BNL with gamma irradiation exposure performed elsewhere. It should also be noted that even when space and gamma radiation exposure are performed at BNL, the environmental conditions are differed. The space radiation exposures all occur in the NSRL building and involve transportation of animals to and

from that building and housing of animals at the NSRL building. Up to this point, gamma irradiation of animals at BNL occurred at one of two location, neither of them involving transportation or housing similar to what animals would experience when receiving space radiation or sham-irradiation in the NSRL building. Therefore, future efforts are warranted to plan for gamma radiation of animals in the NSRL building as well. However, as those studies are very valuable to consider and compare with effects of X-Rays and ^{137}Cs or other gamma irradiation on the brain reported in human studies, we selected particular studies for this important cross-species comparison pertinent to assessment of CNS risk in humans based on animal studies.

5.2.1.1 General health and housing conditions. Animals must be in good general health for assessments of behavioral and cognitive performance. Therefore, assessments of space irradiation effects include inspection of general health of the animals. Careful monitoring and maintaining of the optimal temperature and humidity and maintaining the same housing condition and diet is important (for a more detailed review of general mouse health and how it might affect behavioral and cognitive performance, please see (Crawley 2000, Van Meer and Raber 2005)). With the space radiation-relevant doses being used at BNL, typically no adverse effects on general health are seen.

In reviewing the currently available space irradiation rodent CNS data, we realize that as historically there has been a transition over time from studies involving relatively higher to lower radiation doses, changes in the particles being predominantly used (*e.g.*, from more ^{56}Fe ions and protons in the earlier studies to more studies with other particles, and exposures to more than one particle in sequential beam exposure studies in more recent studies). In addition, while the earlier studies might have involved anesthesia and brain-only irradiation, the more recent studies

typically all involve whole-body exposures without any anesthesia during the exposure. This is especially important to consider as it is increasingly clear that space radiation might either directly affect the CNS or indirectly, for example via the involvement of effects of space irradiation on immune mediators in the periphery, primary effects on the gut affecting the brain through the gut-liver-brain axis, or effects on the vasculature in the periphery affecting cerebrovascular blood flow in the brain. Further, most space radiation studies involved only males, few females, and even fewer both sexes. Finally, while the earlier studies mostly involved animals that were about two-month-old at the time of exposure, more recent studies typically involved animals that were about six-month-old or older at the time of exposure. A strength of this richness in experimental space radiation exposure paradigms that has been used is that there is better coverage to base CNS risk on than if a single or only a few space radiation exposure paradigms would have been used. However, this richness comes with a price; it is harder to compare and briefly summarize results across studies. This across studies comparison in turn is important to consider for assessing CNS risk. Consistent with the goal to assess CNS risk, we have used behavioral and cognitive tests and pertinent biomarkers sensitive to radiation exposure and most widely used in the various space radiation studies.

5.2.1.2 Sensorimotor function. Motor function is mediated by several structures, starting in the cortex, brain stem and spinal cord, and terminating in skeletal muscle. The rotorod test, involving a rotating rod, is often used to assess motor function and balance (Jones and Roberts 1968, Rustay, Wahlsten et al. 2003). In some experimental paradigms, the speed of the rod is kept constant. In others, animals start out walking on the rotorod at a low rotation speed, and are trained to remain on the rotorod while the speed gradually increases. The nature of a potential rotorod impairment can be analyzed further by conducting follow-up tests assessing either

muscle strength (for example using the wire hang test) or balance (for example using the balanced beam or inclined screen tests) (Pullela, Raber et al. 2006).

5.2.1.3 Circadian activity levels in the home cage. In the CNS, radiation exposure can affect the cortex, a structure critical for behavioral and cognitive performance. Behavioral alterations reported in astronauts that can affect cognitive performance include reduced sleep (Slack, Williams et al. 2016). For example, psychomotor function and cognitive speed are affected by sleep. Therefore, it is important to include assessments of effects of space irradiation on depressive behaviors and circadian activity levels.

5.2.1.4 Exploratory behavior in a novel environment and measures of activity and anxiety. During space missions, astronauts are exposed to novel environments and anxiety-provoking stimuli. Anxiety is a normal response to threatening situations and has important protective effects (Hohoff 2009). However, some individuals display an excessive anxiety response, which might be an indication for an anxiety disorder. The open field test is a common test to assess general exploratory behavior, motor activity, and measures of anxiety in rodents (Candland and Nagy 1969, Wilson, Vacek et al. 1976, Prutt and Belzung 2003). Besides activity measures, measures related to exploratory activity in the more anxiety-provoking center areas are being assessed. In nonhuman primates, behavior in a play room has also been used to assess both exploratory behavior and measures of anxiety (Haley, McGuire et al. 2011b).

In contrast to one open area, the elevated plus maze and elevated zero maze contain closed areas and more anxiety-provoking open areas. The plus maze consists of a perpendicular cross that is elevated above the floor, in which the sides of one axis are walled off. Less anxious animals will venture more on to the open areas, and poke their heads more over the edges of the open areas (Lister 1987, Lister 1990). In the elevated plus maze, the mouse needs to turn around

in the open arms in order to return to the closed arms. This turn is highly anxiogenic and might constitute an unnatural behavior for a mouse. In the elevated zero maze (Kulkarni, Singh et al. 2007), the mouse does not need to turn around in the open areas to enter the closed areas.

In the open field and elevated plus and zero mazes described above, mice can avoid the anxiety-provoking areas. There are also tests of anxiety involving unavoidable anxiety-provoking stimuli. An example of such a test is 'acoustic startle' (Parham and Willott 1988, Meloni and Davis 1998). Animals are placed in a sound-attenuated box on a sensing platform, where they are subjected to acoustic stimuli consisting of white noise. These stimuli will startle the animal, resulting in a full-body flinch recorded by the sensing platform. The acoustic startle threshold is defined as the lowest sound intensity level that elicits a body flinch. Less anxious animals might display higher thresholds than control peers and/or reduced amplitudes of acoustic startle responses.

In Fig.5.1 (left circle), the lowest doses showing significant radiation effects are indicated. When 3-month-old human apoE2, apoE3, and apoE4 male mice received brain-only ^{56}Fe ion irradiation (1 or 2 Gy), mice irradiated with 2 Gy showed higher activity levels in the open field than sham-irradiated mice or those irradiated with 1 Gy (Yeiser, Villasana et al. 2013).

Fischer-344 male rats, which were 2, 7, 12, and 16 months of age at the time of irradiation, were exposed to brain-only ^{56}Fe ion irradiation (1000 MeV/n, 0.5–2 Gy) and tested 1 or 2 months later in the elevated plus maze (Rabin, Carrhill-Knoll et al. 2007a). ^{56}Fe ion irradiation increased measures of anxiety; the dose increasing levels of anxiety was a function of age at the time of irradiation and lower in the rats that were irradiated at 7 and 12 months of age (0.5 Gy) than in those irradiated at 2 months of age (2 Gy) (Rabin, Carrhill-Knoll et al. 2007a).

Two-month-old Sprague-Dawley rats were irradiated (head only) with Helium (1000 MeV/n, 0.01-0.1 Gy) and behaviorally and cognitively tested 10 days to 3 months later (Rabin, Carrihill-Knoll et al. 2015a). The lowest dose (0.01 Gy) affected performance in the elevated plus maze, novel location recognition, but not novel object recognition, and operant responding on an ascending fixed-ratio reinforcement schedule (Rabin, Carrihill-Knoll et al. 2015b).

5.2.1.5 Depression tests; forced swim and tail suspension tests. The Porsolt forced-swim test (Borsini and Meli 1988, Yates, Panksepp et al. 1991, Can, Dao et al. 2012) and tail suspension test (Steru, Chermat et al. 1985, Mayorga and Lucki 2001) measure behavioral despair in rodents, and are generally used to assess depressive-like behaviors. The forced swim test involves a cylinder filled with water in which the animal has no other option but to swim. The water level does not allow the animal to rest on its tail, or escape the cylinder by climbing out. Primary measures are the time floating and the time spent fighting or trying to escape. The percentages fighting or floating are derived from the primary measures. In the tail suspension test, the animal tries to move up to escape. The time an animal remains immobile is scored as a measure of depressive-like behavior.

Behavioral performance in the Porsolt forced-swim test was assessed in 4-6 month-old female and male B6D2F1 mice two month following exposure to mixed beam space irradiation, consisting of 20 percent 250 MeV ^{16}O ions, 20 percent 240 MeV/n ^{28}Si ions, and 60 percent 1 GeV protons at 25, 50, or 200 cGy (Kronenberg, Gauny et al. 2018). Mice irradiated with 50 cGy showed increased depressive behavior in the forced swim test.

5.2.1.6 Emotional learning and memory; passive avoidance, contextual and cued fear learning and memory, and fear potentiated startle. The passive avoidance test involves a two-compartment chamber with a connecting door (Beatty, Gregoire et al. 1973). The animal receives

a slight foot shock when it enters the preferred dark compartment and latency to re-enter in subsequent training or memory trials is assessed. In contextual and cued fear learning and memory an environment (context) or cue (for example a tone) are combined with an aversive stimulus (for example a foot shock) and learning and memory is assessed (Fanselow and Kim 1994, Gerlai 1998, Maren 2001). Based on the animal tests, translational fear conditioning tests in humans are being used (Milad, Igoe et al. 2011). In fear potentiated startle, the acoustic stimulus response used in the acoustic startle response is potentiated by including a foot shock (Missig, Ayers et al. 2010).

In Fig. 5.1 (right circle), the lowest doses showing significant radiation effects are indicated. C57BL/6J wild-type male mice and those lacking the chemokine receptor CCR2 were used to compare the effects of ^{56}Fe ion irradiation (600 MeV/n, 0.25 Gy) in the contextual fear conditioning test. In the absence of shocks, irradiation of mice at 3 months of age enhanced habituation to the environment in wild-type, but not CCR2 mutant, mice three months after exposure, suggesting that lack of CCR2 was associated with reduced cognitive performance (Raber, Allen et al. 2013a). Irradiation reduced contextual fear memory in both genotypes equally (Raber, Allen et al. 2013a,b). Following a higher dose of ^{56}Fe ion irradiation (600 MeV/n, 0.5 Gy) in 2-month-old C57BL/6J mice, exploratory behavior in the new environment of the conditioning chamber was reduced following irradiation (Raber, Allen et al. 2016a). In addition, the increase in spine density in the CA1 region of the hippocampus and enclosed blade of the dentate gyrus increased following cognitive testing in sham-irradiated, but not irradiated, mice (Raber, Allen et al. 2016a).

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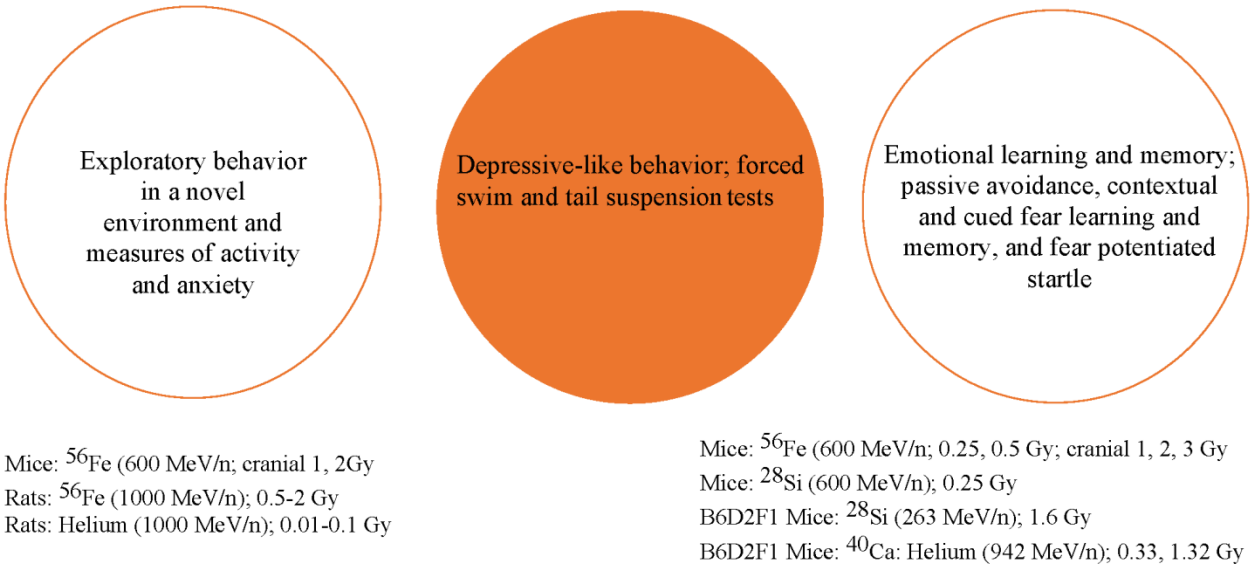

Fig. 5.1. Left circle. Lowest doses showing significant effects of space irradiation on exploratory behavior in a novel environment and measures of anxiety and activity. **Right circle.** Lowest doses showing significant effects of space irradiation on emotional learning and memory. The data for mice and rats are indicated separately.

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When 3-month-old C56BL/6J male mice received ^{56}Fe ion irradiation (600 MeV/n, 0.5 Gy), they showed increased freezing levels 24 hours after training, and these freezing levels during the memory test correlated with cells expressing the immediate early gene Activity-Regulated Cytoskeleton-Associated Protein (*Arc*), which can be used to assess neuronal stability and network stability (Guzowski, McNaughton et al. 1999, Guzowski, Lyford et al. 2000, Guzowski, McNaughton et al. 2001), in the enclosed blade of the dentate gyrus (Raber, Allen et al. 2013b). When 3-month-old C56BL/6J male received brain-only ^{56}Fe ion irradiation (600 MeV/n, 1 Gy), irradiated mice that did not receive shocks during training showed reduced contextual fear memory and less Arc-expressing neurons in the free blade of the dentate gyrus than sham-irradiated mice, suggesting reduced hippocampus-dependent spatial habituation learning (Raber, Rosi et al. 2011a). However, irradiated mice that did receive tone-shock pairings showed enhanced contextual freezing but a reduced percentage of Arc-expressing neurons in the enclosed blade and changes in Arc expression correlated negatively with freezing in mice that received shocks during training (Raber, Rosi et al. 2011a).

Sex differences in effects of brain-only ^{56}Fe ion irradiation (600 MeV/n, 1, 2, or 3 Gy) on contextual fear memory were measured three months after exposure (Villasana, Rosenberg et al. 2010). Hippocampus-dependent contextual fear conditioning was impaired in female mice but improved in male mice following ^{56}Fe ion irradiation; there was significance at 2 Gy but a clear trend was seen at 1 Gy as well (Villasana, Rosenberg et al. 2010).

Enhanced contextual fear memory and enhanced synaptic plasticity in the CA1 region of the hippocampus was seen 3 months following ^{28}Si ion irradiation (600 MeV/n, 0.25 Gy) of C57BL/6J male mice at 3 months of age; this cognitive effect was dose-dependent and not seen

at 1 Gy (Raber, Rudobeck et al. 2014b). In contrast, when 6-month-old B6D2 F1 female and male mice were irradiated with ^{28}Si ions (263 MeV/n, 0.4, 0.8, 1.6 Gy), contextual fear memory was impaired at the 1.6 Gy dose; at lower doses, trends towards impaired contextual fear memory were seen but they did not reach significance (Raber, Marzulla et al. 2015d). Although the energies and doses used were different in these two studies, these data highlight the importance of genetic background in modulating the effects of space irradiation.

When 6-month-old B6D2 F1 female and male mice were irradiated with ^{40}Ca ions (942 MeV/n, 0.165, 0.33, 1.32 Gy), three months following exposure there was an effect on baseline motion (prior to the first tone during training) at a dose of 0.33 and 1.32 Gy in males and at 1.32 Gy in females (Raber, Weber et al. 2016c). In addition, the response to the shock during training was altered in females only at all three doses (Raber, Weber et al. 2016).

Recently, the effects of ^{4}He ions (250 MeV/n; linear energy transfer (LET) = 1.6 keV/μm; 0, 0.21, 0.42 or 1.68 Gy) on behavioral and cognitive performance of B6D2F1 mice three months following irradiation at 4-6 months of age were assessed (Raber, Torres et al. 2018). In the passive avoidance test, mice received a slight foot shock in a dark compartment and latency to re-enter that compartment was assessed 24 hours later. In contrast to sham-irradiated mice and mice irradiated with 0.21 or 0.42 Gy who showed a higher latency on day 2 than day 1, the latency to enter the dark compartment in mice irradiated with 1.68 Gy was comparable on both days. Interestingly, ^{4}He ion irradiation, at 0.42 and 1.68 Gy, reduced the levels of the dendritic marker microtubule-associated protein 2 (MAP-2) in the cortex but not the hippocampus. Thus, while reduced levels of MAP-2 in the cortex might have contributed to the altered performance in the passive avoidance test it does not seem sufficient to do so.

5.2.1.7 Object recognition. The novel location and novel object recognition tests assess the ability of animals to recognize a change in their environment by preferentially exploring the novel location or object and is often used as it seems remarkable sensitive to detects effects of radiation on cognition (Ennaceur, Neave et al. 1997, Benice, Bader et al. 2008, Ennaceur 2010, Antunes and Biala 2012, Raber 2015a). As mentioned earlier, translational object recognition tests are being developed and used in humans (Raber 2015a).

In Fig. 5.2 (left circle), the lowest doses showing significant radiation effects in object recognition are indicated. Three-month-old C57BL/6J wild-type male mice irradiated (whole body) with ^{56}Fe (600 MeV/n, 0.1, 0.2, or 0.5 Gy) showed impaired novel object recognition 0.5 month after exposure but no alterations in contextual or cued fear memory (Haley, Yeiser et al. 2013). Cherry et al irradiated 3-month-old APPswe/PS1dE9 bigenenic male mice on a mixed C3H C57BL/6J background with ^{56}Fe (1000 MeV/n, 0.1 or 1 Gy) and observed reduced contextual fear memory 9 months later in the mice who had received the 1 Gy dose, while object recognition was impaired at both doses (Cherry, Liu et al. 2012). At 1 Gy, the mice showed increased plaque pathology and ICAM-1 expression 6.5 months after exposure (Cherry, Liu et al. 2012). Female APPswe/PS1dE9 bigenenic mice irradiated with 1 Gy did not show altered fear conditioning, but did show impaired novel object recognition 7 months after exposure without a radiation-induced increase in amyloid plaque deposition (Cherry, Liu et al. 2012).

Sprague-Dawley male rats, cranially irradiated with ^{56}Fe (1000 MeV/n, 0.5-2 Gy) at 4 months of age, showed impaired novel object recognition 2-3 months following irradiation at 0.8, 1, and 1.5 Gy, but not following irradiation at 0.5 or 2 Gy (Rabin, Carrihill-Knoll et al. 2009). A diet containing 2% fruit extracts and started two weeks prior to irradiation prevented the impairment in object recognition seen following 1.5 Gy (Rabin, Carrihill-Knoll et al. 2009).

Using the same radiation conditions in female ovariectomized Sprague-Dawley rats, treated with or without Capsules containing 17β-estradiol three days prior to exposure, novel object recognition was not affected by ^{56}Fe ion irradiation (Rabin, Carrihill-Knoll et al. 2013). Sprague-Dawley male rats, cranially irradiated with ^{56}Fe ions (600 MeV/n, 0.25 Gy) or ^{16}O (600 MeV/n, 0.05 Gy) 2-4 hours after training, showed impaired novel object recognition 17 hours after exposure (Rabin, Polose et al. 2015c).

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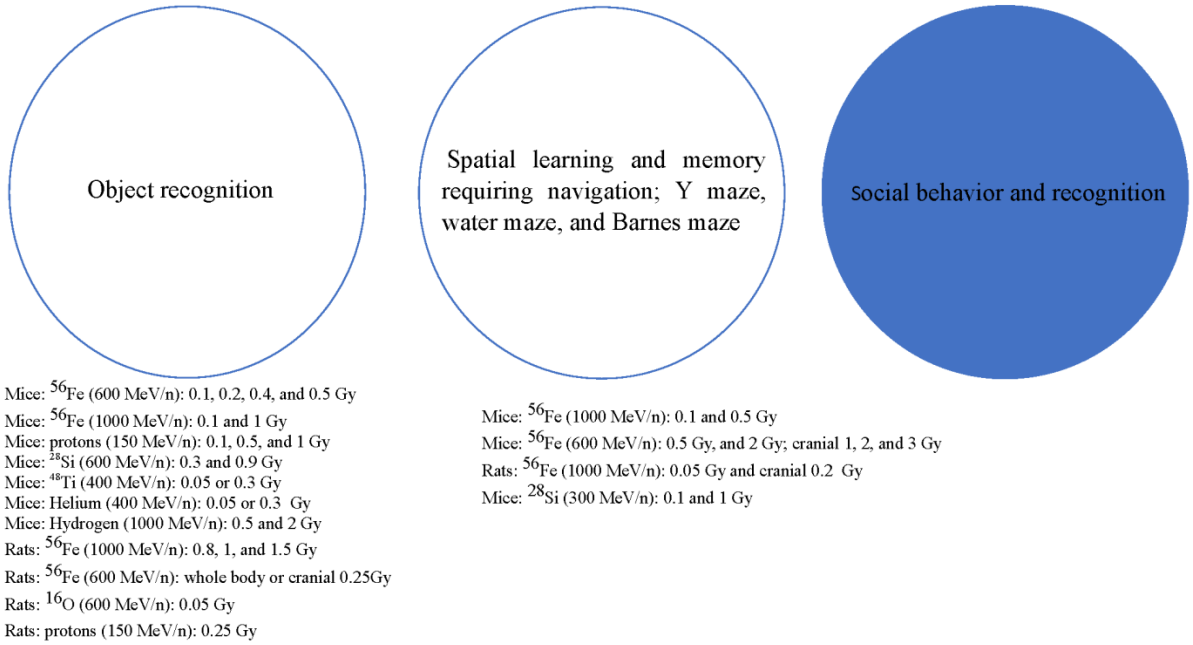

Fig. 2 (blue). Left circle. Lowest doses showing significant effects of space irradiation on object recognition. **Middle circle.** Lowest doses showing significant effects of space irradiation on spatial learning and memory requiring navigation. When cranial is mention the exposure was brain only. All other exposures were whole body.

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Two-month-old C57BL6/J male wild-type and male mice expressing human catalase in the mitochondria (MCAT) were irradiated with protons (150 MeV/n, 0.5 or 1 Gy) and tested for object recognition (both object in place (spatial component and novel object recognition) over a 3-week period started 4 weeks after exposure at Loma Linda University (Parihar, Allen et al. 2015a). Object in place and novel object recognition were impaired in wild-type, but not MCAT, mice at both radiation doses and these effects were associated with changes in neuronal architecture in the CA1 region of the hippocampus (Parihar, Allen et al. 2015a). Enhanced hippocampus-dependent memory and reduced anxiety levels of MCAT mice might have contributed to the protective effects against space irradiation (Olsen, Johnson et al. 2013). A lower dose of protons was also detrimental, but required a longer interval between radiation and testing. Novel object recognition was impaired three months after proton irradiation (150 MeV/n, 0.1 Gy) alone, or proton irradiation (150 MeV/n, 0.1 Gy) followed by ^{56}Fe ion irradiation (600 MeV/n, 0.5 Gy) 24 hours later (Raber, Allen et al. 2016b).

In male Sprague-Dawley rats, whole body irradiation at 2 months of age with protons (150 MeV/n, 0.25 Gy) impaired object recognition two months after exposure (Rabin, Joseph et al. 2008). Brain only irradiation of 2-month-old male Sprague-Dawley with ^{56}Fe ions (600 MeV/n, 0.25 Gy) or ^{16}O (600 MeV/n, 0.05 Gy) showed acute effects of irradiation, within 48 hours after exposure, on object recognition memory, but not learning (Rabin, Polose et al. 2015c).

Six-month-old B6D2 F1 female and male mice were irradiated with ^{16}O ions (250 MeV/n, 0.4, 0.8, 1.6 Gy) (Raber, Marzulla et al. 2015c). One month following exposure, there was enhanced cued fear memory seen in mice irradiated with 0.4 and 0.8 Gy (Raber, Marzulla et al. 2015c).

Six-month-old C57BL/6J mice were irradiated with ^{56}Fe (600 MeV/n, 0.1, 0.2, or 0.4 Gy) and cognitive performance, hippocampal network stability, hippocampal DNA methylation (cytosine methylation (5mC) and hydroxymethylation (5hmC), and gene expression were assessed (Impey, Jopson et al. 2016a). At the 2-week time point, novel object recognition and network stability were impaired following exposure at 0.1 and 0.4 Gy, but not following exposure at 0.2 Gy; these effects were not seen at the 20-week time point, but were associated with alterations in DNA methylation and gene expression (see Chapter 6 for a description of the pathway changes in the hippocampus following ^{56}Fe ion irradiation) (Impey, Jopson et al. 2016a). A similar study design was used to assess the short- and long-term effects of protons (150 MeV/n, 1 Gy) on hippocampal function (Impey, Jopson et al. 2017; Raber, Rosi et al. 2017b). Impairments in object recognition, spatial memory retention in the water maze, and hippocampal network stability were seen at the 2-week time point and correlated with altered gene expression and 5hmC profiles that mapped to specific gene ontology pathways (see Chapter 6) (Impey, Jopson et al. 2017, Raber, Rosi et al. 2017b). In contrast to what was seen with ^{56}Fe ion irradiation described above, novel object recognition was also impaired at the 20-week time point following proton irradiation (Impey, Jopson et al. 2017, Raber, Rosi et al. 2017). The short- and long-term effects of ^{28}Si ion irradiation (600 MeV/n, 0.3, 0.6, or 0.9 Gy) were assessed as well (Raber et al, oral communication). At the 2-week time point, novel object recognition was impaired following irradiation at 0.3 Gy, but not following irradiation at 0.6 or 0.9 Gy, and this was associated with impaired hippocampal network stability in the CA1 region of the hippocampus (Raber et al, oral communication). At 20-week time point, novel object recognition was impaired following irradiation at the 0.3 and 0.9 Gy, but not following irradiation at 0.6 Gy (Raber et al, oral communication).

Consistent with the 2-week ^{28}Si data described earlier, in a study from an independent group mice were impaired in object recognition 4 weeks following ^{28}Si ion irradiation (600 MeV/n, 0.2 Gy) (Acharya, Baddour et al. 2017).

When 6-month-old C57BL/6J male mice were irradiated with ^{48}Ti ions (600 MeV, 0.05 or 0.3 Gy), there were radiation effects on novel object recognition, object in place and temporal order at 6, 12 and 24 weeks following irradiation. Using helium ions (400 MeV/n, 0.05 or 0.3 Gy) similar deficits were observed on object in place and temporal order at 6, 15 and 52 weeks following 0.05 and 0.3 Gy doses. There was also evidence of increased anxiety (elevated plus maze) and depression-like behavior (forced swim test) 1 year after each helium exposure, and impairments on a fear extinction paradigm implicating disruption to the hippocampal-medial prefrontal cortical circuit (Limoli 2018).

As indicated earlier, recently, the effects of ^{4}He ions (250 MeV/n; 0, 0.21, 0.42 or 1.68 Gy) on behavioral and cognitive performance of B6D2F1 mice three months following irradiation at 4-6 months of age were assessed. Sham-irradiated mice and mice irradiated with 0.21 Gy or 1.68 Gy showed object recognition and spent more time exploring the novel than the familiar object but mice irradiated with 0.42 Gy did not (Raber, Torres et al. 2018).

Object recognition was assessed in 4-6 month-old female and male B6D2F1 mice two month following exposure to mixed beam space irradiation, consisting of 20 percent 250 MeV ^{16}O ions, 20 percent 240 MeV/n ^{28}Si ions, and 60 percent 1 GeV protons at 25, 50, or 200 cGy (Kronenberg, Gauny et al. 2018). While sham-irradiated female and male mice and female and male mice irradiated with 25 cGy showed object recognition and explored the novel object significantly more than the familiar one, object recognition was impaired in female and male

mice irradiated at a dose of 50 or 200 cGy. Cortical BDNF and CD68 levels were analyzed three months after irradiation.

Inhibited transcription of brain-derived neurotrophic factor (BDNF) has been implicated in whole brain clinically relevant (10 Gy) radiation-induced cognitive injury in rodents (Ji, Lu et al. 2014, Son, Yang et al. 2015). The effect of microglia on learning-dependent synapse formation, which is important for cognitive performance, involves the release of BDNF (Parkhurst, Yang et al. 2013). Activation of microglia, important in neuroinflammation, triggers the release of BDNF, which in turn induces the proliferation and prolonged activation of microglia (Gomes, Ferreira et al. 2013, Mizobuchi, Kato et al. 2014, Zhang, Zeng et al. 2014). CD68 (macrosialin), a lysosome-associated membrane glycoprotein, is a marker of activated microglia (Neuen-Jacob, Arendt et al. 1993, Tanaka, Matsuwaki et al. 2013) and increased in the brain following irradiation (Allen, Chakraborti et al. 2014, Acharya, Patel et al. 2015). For cortical BDNF and CD68 levels, there were sex x radiation interactions with a general pattern of increased levels following irradiation in females but decreased levels in males. BDNF levels in male mice irradiated with 200 cGy were lower than those in sham-irradiated male mice. In females, CD68 levels were higher in mice irradiated with 200 cGy than sham-irradiated mice. There was a significant positive correlation between BDNF and CD68 levels. These sex-dependent effects of mixed beam irradiation on cortical BDNF and CD68 levels suggest that distinct pathways might be involved in cognitive injury seen following exposure in females and males. Therefore, increased efforts are warranted to use unbiased approaches to determine altered pathways in females and males that might underlie the detrimental effects of mixed beam space irradiation in females and males.

5.2.1.8 Spatial learning and memory involving navigation; Y maze, water maze and Barnes maze. Spatial learning and memory can be assessed in dry land mazes and wet land mazes. The Barnes maze, in which the animal is searching to locate a hidden tunnel beneath an escape hole using a spatial map, is an example of a dry land maze. An advantage of the Barnes maze is that search strategies can be analyzed, but a disadvantage is that the animal need to be motivated to search for the escape tunnel (Barnes 1979). Another example of a dry land maze is the Y maze or T maze (Heyser, McDonald et al. 1999). These mazes can be used to assess spontaneous alternation (Yadin, Friedman et al. 1991, Bardgett, Taylor et al. 1994, Gerlai, Marks et al. 1994, Thompson, Levitt et al. 2005), the preference of the animal to explore a different arm than visited previously or the animal can be trained to prefer or avoid one arm. An example of a wet land maze is the water maze in which the animal is searching for an escape platform hidden beneath opaque water (Morris 1984).

In Fig. 5.2 (middle circle), the lowest doses showing significant radiation effects are indicated. When 4-month-old C57BL6/J male mice were irradiated with ^{56}Fe (1000 MeV/n, 0.1 or 0.5 Gy), those irradiated with 0.5 Gy showed less activity in the Y maze 1-2 months after exposure than sham-irradiated controls and a slight but significant increase in microhemorrhages (Liu, Liu et al. 2017). These alterations in the Y maze were not seen in similarly irradiated female wild-type mice but the female mice irradiated with 0.5 Gy did show reduced uptake of ^{18}F-GE180 using PET-imaging, suggesting either reduced glial activation or increased permeability of the blood-brain barrier (BBB) (Liu, Liu et al. 2017). Lemere et al used APPswe/PS1dE9 bigenenic male mice, an animal model of Alzheimer's disease developed by David Borchelt (Savonenko, Xu et al. 2005), in the same study. Male bigenic mice irradiated with 0.1 Gy showed reduced activity levels (entries into the closed arms) than sham-irradiated

mice, while those irradiated with 0.5 Gy showed increased activity levels in the open field and Y maze and a trend towards impaired contextual fear memory (p = 0.07) (Liu, Liu et al. 2017). In contrast to what was seen in males, female bigenic mice irradiated with 0.1 Gy showed increased activity levels in the open field and Y maze compared to sex- and genotype-matched controls (Liu, Liu et al. 2017). Consistent with the pattern seen in males, female bigenic mice irradiated with 0.5 Gy showed reduced hippocampal uptake of ^{18}F-GE180 and a trend towards reduced whole brain uptake (p = 0.064) (Liu, Liu et al. 2017). Interestingly, irradiated female bigenic mice showed reduced plaque pathology, reduced insoluble $A\beta_{x\text{-}40}$ and $A\beta_{\text{-}42}$ and increased soluble $A\beta_{\xi\text{–}40}$ and $A\beta_{\text{–}42}$ levels (Liu, Liu et al. 2017).

When 2-month-old C56BL/6J male mice expressing human apoE2, apoE3, or apoE4 under control of the mouse apoE promoter were irradiated with ^{56}Fe ion irradiation (600 MeV/n, 0.5 Gy), spatial memory retention in the water maze was impaired by irradiation in apoE2 and apoE4 mice, but enhanced in apoE3 mice (Haley, L et al. 2012). This enhanced memory performance might be related to increase in hippocampal apoE levels, which only occurred in irradiated apoE3 mice (Haley, L et al. 2012). Irradiation reduced the generation of reaction oxygen species, as measures by DHE oxidation, and levels of 3-nitrotyrosine (3-NT) and the antioxidant CuZn superoxide dismutase (SOD) in apoE2, but not in apoE3 or apoE4, mice (Haley, L et al. 2012). In another study, human apoE mice received cranial ^{56}Fe ion irradiation (600 MeV/n, 1 or 2 Gy) (Villasana, Dayger et al. 2013a). At 2 Gy, apoE4 mice were more sensitive to memory impairments in the water maze, but less sensitive to impairments in novel object recognition; at 1 Gy, only the apoE4 mice showed spatial memory retention for the second platform location after reversal training (training to locate a new platform location (Villasana, Dayger et al. 2013a). The water maze consists of a pool filled with water, and for analysis is divided into four quadrants.

During visible platform training, a platform containing a visible cue is placed in a quadrant and the mouse is trained to swim to it. Visible platform training is important to exclude potential differences in vision, motor function, or motivation. During hidden platform training, the visible cue is removed and the mouse is trained to locate the hidden platform location using spatial cues in the room. The starting location of the animal is changed each trial. If by the end of the trial the animal did not locate the platform, it is guided to it by the researcher. To assess spatial memory retention, the target platform is removed. A mouse with spatial memory retention preferentially searches the quadrant, which previously contained the platform (target quadrant) and/or swims on average closer to the platform location. In the water maze, animals can be trained to locate a single hidden platform location or multiple hidden platform locations. The latter version is used to assess cognitive flexibility and ability locate new locations (Morris 1984). In both cases, cues in the room are used by the animals to make spatial maps of the environment (Baldi, Lorenzini et al. 2003).

To determine whether space irradiation sensitizes the brain to subsequent injury, spatial learning in the water maze was assessed in C57BL/6J male mice who received traumatic brain injury two months after exposure to ^{56}Fe ion irradiation at two months of age and the mice were cognitive tested 7 weeks following irradiation (Rosi, Belarbi et al. 2012). Mice that received only trauma showed impaired spatial memory retention and reduced behaviorally-induced immediate early gene Arc expression in both hemispheres while mice that received both irradiation and traumatic brain injury did not, suggesting a protective effect of low dose irradiation in the context of a subsequent traumatic injury (Rosi, Belarbi et al. 2012).

When 3-month-old human apoE2, apoE3, and apoE4 male mice received brain-only ^{56}Fe ion irradiation (^{56}Fe (600 MeV/n, 1 or 2 Gy) and were tested three months after exposure, during

hidden platform training, sham-irradiated mice showed most robust learning, 1 Gy irradiated mice reduced learning, and 2Gy irradiated mice no improvement over the hidden platform training sessions. In the water maze probe trials, sham- irradiated apoE2, apoE3, and apoE4 mice and apoE2 and apoE4 mice irradiated with 1 Gy showed spatial memory retention, but apoE3 mice irradiated with 1 Gy, and apoE2, apoE3, and apoE4 mice irradiated with 2 Gy did not (Yeiser, Villasana et al. 2013).

Long-term effects of brain-only ^{56}Fe ion irradiation (600 MeV/n, 3Gy) 13 months following exposure of human apoE2, apoE3, and apoE4 mice at 2 months were also assessed (Villasana, Benice et al. 2011). After irradiation, spatial memory retention of apoE3 female, but not male, mice was impaired (Villasana, Benice et al. 2011). A general genotype deficit in spatial memory was observed in sham-irradiated apoE4 mice (Villasana, Benice et al. 2011). Strikingly, irradiation prevented this genotype deficit in apoE4 male mice and a similar but nonsignificant trend was observed in apoE4 female mice (Villasana, Benice et al. 2011). After irradiation, immunoreactivity of the pre-synaptic marker synaptophysin was increased in irradiated female mice, independent of genotype (Villasana, Benice et al. 2011).

A diet containing the antioxidant alpha lipoic acid (ALA) mitigated cognitive injury of 6-9 month-old C57BL/6J male mice three months following exposure (Villasana, Rosenthal et al. 2013b). ALA prevented radiation-induced impairments in spatial memory retention in the water maze probe trials following reversal learning for a new platform location (Villasana, Rosenthal et al. 2013b). In the hippocampal dentate gyrus of mice on regular diet, irradiated mice had higher levels of immunoreactivity of the dendritic marker microtubule-associated protein 2 (MAP-2) than sham-irradiated mice, suggesting that this might be a compensatory response (Villasana, Rosenthal et al. 2013b).

Impairments in spatial memory of male Wistar rats in the Barnes maze were seen in 6-11 month-old animals three months following exposure with ^{56}Fe (1 GeV/n, 0.05, 0.1, 0.15, or 0.2 Gy) (Britten, Jewell et al. 2016). Wistar male rats cranially irradiated with ^{56}Fe (1 GeV/n, 0.2 Gy) at one month of age showed impairments in spatial learning and memory in the Barnes maze (Britten, Davis et al. 2012).

In C57BL/6J wild-type male mice, irradiated (whole body) with ^{28}Si ions (300 MeV/n) at 3 months of age, enhanced spatial learning in the Barnes maze was seen 4 months following exposure at 0.1 or 1 Gy (in review). These cognitive effects were sex-dependent and not seen in female mice under the same exposure and time conditions (in review). However, in female and male C57BL6/J mice enhanced spatial learning was seen 20 months following exposure at 0.5 Gy (in review).

5.2.1.9 Social behavior and recognition. Social recognition in rodents is based on olfaction as opposed to humans that mostly use visual cues. In rodent social recognition, the olfactory investigation time will decrease with repeated or prolonged contact. Based on the preference of animals to explore novel animals, social recognition memory can be assessed. In addition, the drive to socially interact can be assessed by analyzing the animals explore (Ferguson, Young et al. 2002, Young 2002). Typically, there is one or more stimulus animal and a test animal. Aggressive behavior is typically assessed using the home intruder paradigm. For example, Co irradiation (10 Gy) decreased measures of aggressive behaviors, including the latency to attack, the frequency and duration of fighting, the frequency of bites, lunges, and chases decreased starting seven days after irradiation (Maier and Landauer 1990). Some studies have started to include assessments of social behavior in radiation studies but this has been less studied than most other behaviors so far. Social odor memory can be assessed as well. As indicated earlier,

brain-only irradiation of 6-11 month-old male Long-Evans rats with protons (1 or 2 Gy, 150 MeV/n) impaired social odor memory 24 h after training and 6 months following exposure (Davis, Mange et al. 2017).

5.2.1.10 Executive function. In the attentional set shifting test, the animals for example are trained to dig in bowls for a food reward. Following training to perform a series of discriminations to a criterion of correct choices (simple discrimination), the animals are tested in trials between pairs of food bowls that differ along two dimensions (odor, digging medium), including a reversal, an intra-dimensional shift, and an extra-dimensional shift (Barense, Fox et al. 2002, Colacicco, Welzl et al. 2002). A human or nonhuman primate test assessing this is the Wisconsin card sorting test (Grant and Berg 1948). In the extra-dimensional shift (ED shift) version of the human or nonhuman primate test, attention to compound stimuli is shifted from one perceptual dimension (*e.g.* color) to another (*e.g.* form), on the basis of changing reinforcement or feedback (Roberts, Robbins et al. 1988).

In Fig 3. (left circle), the lowest doses showing significant radiation effects are indicated. In the psychomotor vigilance test (PVT), attention and reaction times are assessed in humans and animals (Davis, Roma et al. 2016a). In tests of rodents, the front wall of an operant chamber contains a nose-poke response key and a food or liquid cup for delivery of food pellets or liquid rewards. Visual attention and impulsivity can be assessed in the 5-hole serial reaction time test, in which animals are trained to detect a light briefly presented in a pseudorandom order in one of five spatial locations over a large number of trials (Robbins 2002, Siegel, Benice et al. 2011). Depending on the version of the test used, the animal might be food restricted and/or receive food following the testing session.

Impairments in attention set shifting of male Wistar rats were seen in 6-11 month-old animals three months following whole body exposure with ^{56}Fe (1 GeV/n, 0.15, or 0.2 Gy, but not at 0.1 Gy) and in 2-month-old animals three months following whole body exposure with ^{56}Fe (1 GeV/n, 0.2 Gy) (Britten, Davis et al. 2014). Exposure to 0.2 Gy was associated with a reduced cholinergic readily releasable pool (RRP) in nerve terminals of the basal forebrain (Britten, Davis et al. 2014).

Male Wistar rats, irradiated (whole body) with 400 MeV/n He (0.01 or 0.1 Gy), 600 MeV/n ^{28}Si (0.01, 0.03, 0.05, or 0.1 Gy), 600 MeV ^{56}Fe (0.03, 0.05, or 0.1 Gy) or 600 MeV/n ^{48}Ti (0.05 Gy) at 5-6 months of age and tested three months later (90 ± 14 days), showed a significant impairment in performance on the attention set shifting test (Britten lab, unpublished observations). Male Long-Evans rats, receiving whole body irradiation of 1000 MeV/n Oxygen (0, 0.05, or 0.25 Gy), showed impairments in the PVT at one month post-exposure at both doses but at 180 days only with the 0.25 Gy dose (Davis, Mange et al. 2017). Davis et al. also assessed the effects of brain only proton irradiation (150 MeV) on PVT performance in rats. Following brain only proton irradiation (0.25, 0.5, 1, or 2 Gy) of 7-month-old Long-Evans male rats, 40% of the animals showed cognitive impairments in this dose range (Davis, DeCicco-Skinner et al. 2014). When Fisher-344 and Lewis male rats were cranially irradiated with protons (0.25 or 1 Gy) at 7 months of age, only Fisher-344 rats showed severe deficits in sustained attention that was associated with a greater loss of tyrosine hydroxylase and dopamine transporters in the prefrontal cortex (Davis, DeCicco-Skinner et al. 2015). Wistar male rats cranially irradiated with ^{56}Fe (1 GeV/n, 0.2 Gy) at one month of age showed impairments in attentional set shifting three month after exposure, with only 17% of the animals being able to complete the test as compared to 78% of the sham-irradiated rats (Lonart, Parris et al. 2012).

When the effects of ^{48}Ti (1000 MeV/n, 0.1, 0.15, or 0.2 Gy) on attention set shifting in retired Wistar male breeder rats obtained from a commercial colony was assessed 3 months after exposure, all three doses affected performance (Hadley, Davis et al. 2016).

Brain-only irradiation of 6-11 month-old male Long-Evans rats with protons (1 or 2 Gy, 150 MeV/n) impaired social odor memory 24 h after training and 6 months following exposure (Davis, Mange et al. 2017).

5.2.1.11 Operant responding and conditioned taste aversion. Learning has traditionally been defined as the acquisition of new behavior. Within an operant framework or methodology, however, the word learning implies transitional behavior that is progressing towards a steady state (Sidman M., *Tactics of Scientific Research*, 1960). These transitions are a function of the stimulus-response contingencies under which a subject is responding (e.g., positive or negative reinforcement) and the stimuli that discriminate the presence of those contingencies. Unlike learning in an operant framework, learning that is classically conditioned is a function of stimulus-stimulus contingencies or the pairing of a conditioned stimulus (CS: bell) with an unconditioned stimulus (UCS: food) to produce a conditioned response (CR: salivation). This type of learning is exemplified by conditioned taste aversion procedures where a novel taste such as saccharin (CS) is temporally paired with an injection of a substance that elicits some form of illness (UCS) to produce the avoidance of the novel taste (CR).

In Fig.5.3, the lowest doses showing significant radiation effects are indicated. Ovariectomized Sprague-Dawley female rats, cranially irradiated with ^{56}Fe (1000 MeV/n, 0.5-2 Gy) at 2 months of age, treated with capsules containing 17β-estradiol three days prior to exposure, showed reduced lever pressing following irradiation (Rabin, Carrihill-Knoll et al. 2013). Interesting enough, this pattern was not seen in animals that had received empty capsules

and even an increase in lever presses was seen in those irradiated with 1 Gy (Rabin, Carrihill-Knoll et al. 2013).

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Rats: ^{56}Fe (1000 MeV/n): 0.15 and 2 Gy and cranial 0.2 Gy
Rats: Helium (400 MeV/n): 1 or 10 cGy
Rats: ^{28}Si (600 MeV/n): 1, 3, 5, and 10 cGy
Rats: ^{56}Fe (600 MeV/n): 3, 5, and 10 cGy
Rats: ^{48}Ti (600 MeV/n): 5 cGy
Rats: ^{48}Ti (1000 MeV/n): 0.1, 0.15 and 0.2 Gy in retired breeders
Rats: ^{16}O (1000 MeV/n): 0.05 and 0.25 Gy
Rats: protons (150 MeV/n): 0.25, 1, and 2 Gy

Rats: ^{56}Fe (1000 MeV/n): 0.5 - 2 Gy, ovariectomized females
Rats: ^{56}Fe (1000 MeV/n): 0.5, 1.5, and 2 Gy
Rats: ^{28}si (380 or 600 MeV/n): 1 Gy
Rats: ^{48}Ti (500 or 1100 MeV/n): 1 Gy
Rats: ^{12}C (290 MeV/n): 1 Gy
Rats: ^{16}O (600 or 1000 MeV/n): 1 Gy

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2728 **Fig. 5.3. Left circle.** Lowest doses showing significant effects of space radiation on executive function. **Right circle.** Lowest doses showing

2729 significant effects of space radiation on operant conditioning and condition taste aversion. Note that there in two studies retired breeders

2730 and ovariectomized females were respectively used

2731

Male Fischer-344 rats, 2, 7, 12, or 16 months of age, were cranially irradiated with ^{56}Fe (1000 MeV/n, 0.25-2 Gy) and were trained to make an operant response on an ascending fixed-ratio reinforcement schedule (Rabin, Joseph et al. 2012). When performance was evaluated as a function of both age of irradiation and testing, there was an effect of age on the dose needed to produce a performance decrement; older rats exposed to lower doses of ^{56}Fe particles showed a performance decrement compared to younger rats (Rabin, Joseph et al. 2012). When performance was evaluated as a function of age of irradiation with the age of testing held constant, age of irradiation affected operant responding; older rats tested at similar ages and exposed to similar doses of ^{56}Fe particles showed similar performance decrements (Rabin, Joseph et al. 2012). Thus, the performance decrement is not a function of age per se, but is dependent upon an interaction between the age of irradiation, the age of testing, and exposure to HZE particles. Using the same experimental paradigm in terms of animal ages and radiation conditions, disruption of the acquisition of conditioned taste aversion (CTA) generated by i.p. injection of amphetamine (3 mg/kg), was limited to 2-month-old rats irradiated with ^{56}Fe ion at 0.5, 1.5, or 2 Gy (Carrihill-Knoll, Rabin et al. 2007). So, considering the rat equivalent of the typical age of astronauts, affected CTA acquisition my not be a concern.

Two-month-old male Sprague-Dawley rats were irradiated with ^{28}Si (380 or 600 MeV/n), ^{48}Ti (500 or 1100 MeV/n), ^{12}C (290 MeV/n) and ^{16}O (600 or 1000 MeV/n) ions at 0.5, 0.8, or 1 Gy and 1.5 to 2 months after exposure they were tested for operant responding to ascending higher reinforcement schedules (Rabin, Carrihill-Knoll et al. 2011). Altered responding was observed following exposure to all particles and at all energies tested with the 1 Gy dose a common threshold dose. However, the thresholds for the disruption of behavioral

responding varied as a function of both the specific particle and the particle LET. There was no main effect for dose of radiation, but the dose by reinforcement schedule interaction was significant for all HZE particles tested (Rabin, Carrihill-Knoll et al. 2011).

5.3 Magnitude of behavioral and cognitive effects as function of endpoint and dose or fluence (function of charge and velocity)

Up to this point, a modeling approach has been used for hippocampal neurogenesis following irradiation, considering neural stem cells, neural progenitor cells, immature neurons and glioblasts (Cacao and Cucinotta 2016). In addition, *in silico* mouse hippocampal granule cells were used to study effects of ^{56}Fe, ^{12}C, and ^{1}H particles and electrons (Alp, Parihar et al. 2015). Based on the hippocampal neurogenesis modeling, a dose of ^{56}Fe ions (600 MeV/n, 0.5 Gy) results in poor recovery or no recovery, which is only partially ameliorated by dose fractionation (Cacao and Cucinotta 2016). However, it became clear that effects of space irradiation on CNS function are seen at doses below those affecting hippocampal neurogenesis (see also description about neurogenesis as outcome measures in the chapter about mechanisms). Considering all the space irradiation animal CNS data current available, often unusual dose-response curves are observed for acute and long-term effects and the dose-response curves seem dependent on the CNS outcome measure assessed, definitely a challenge for CNS risk assessment of astronauts to space irradiation. To illustrate unusual dose-response curves seen following space irradiation, in Figs. 5.4 and 5.5 effects of space irradiation on object recognition, a translational test for which there is a human version available (Raber 2015a), are shown.

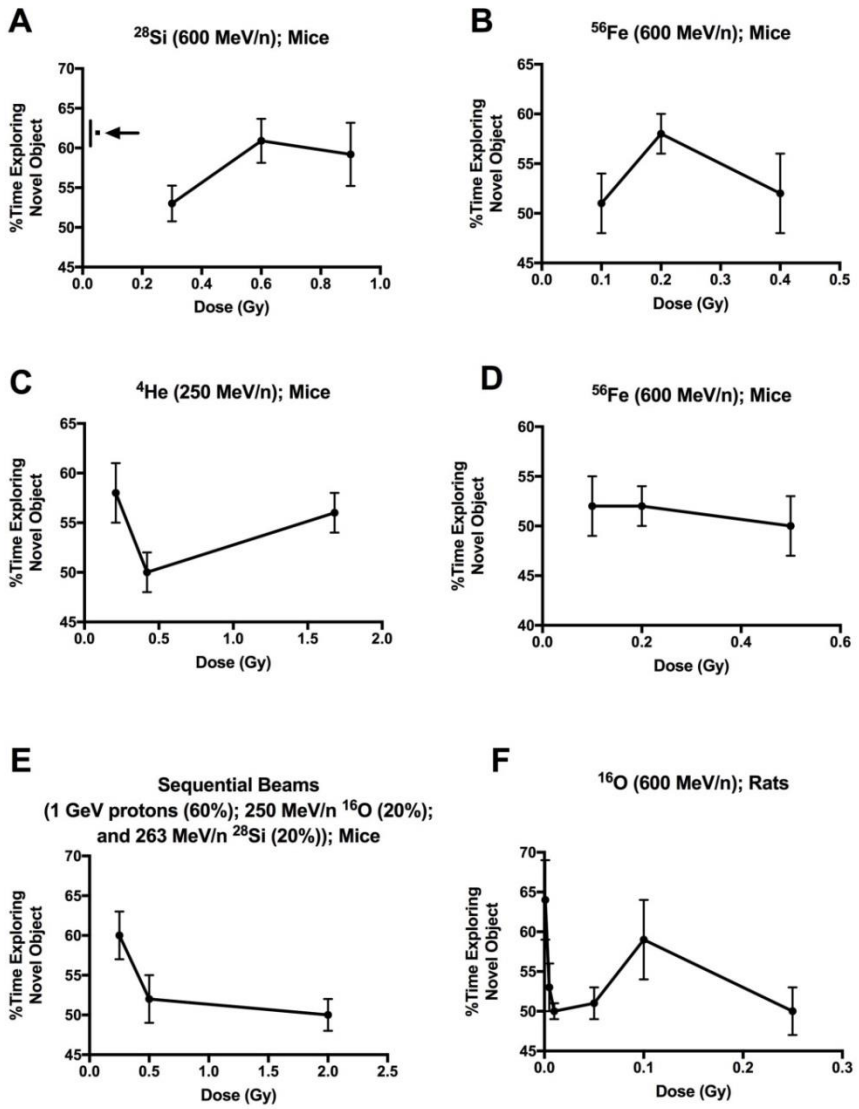
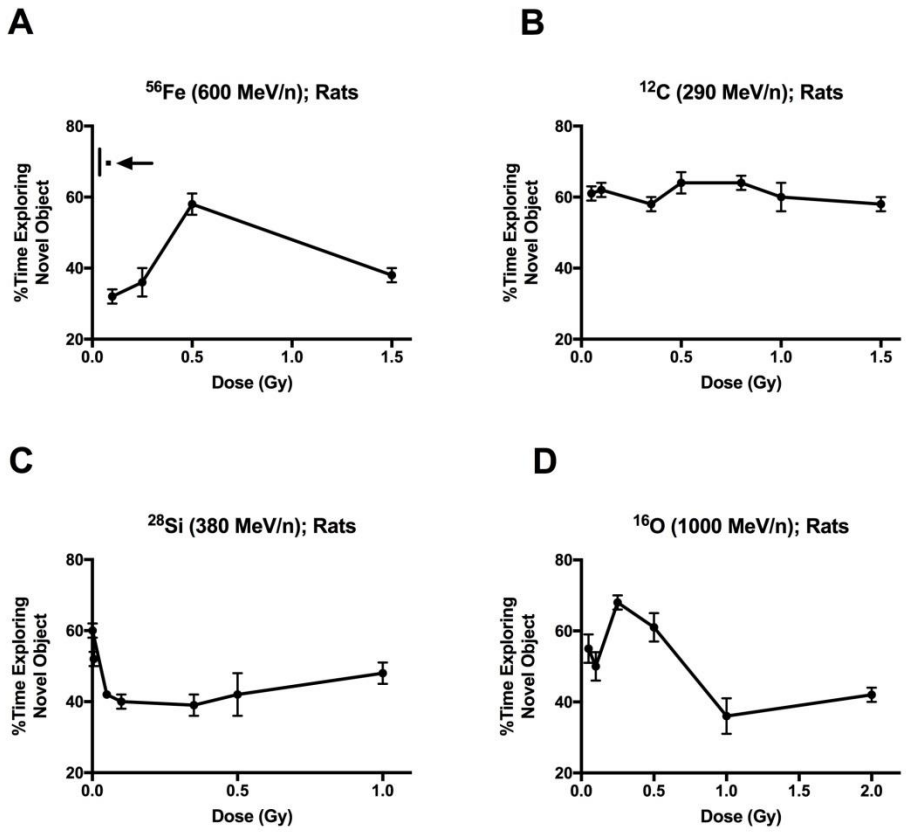


Fig. 5.4. Dose-response for acute effects of ^{28}Si (A), ^{56}Fe (B, D), ^{4}He (C), sequential beams (E), and ^{16}O (F) on object recognition. The energies and species used are indicated in the figure panels. In panel A, the error data point and vertical line indicate object recognition in sham-irradiated animals. With two objects, 50% reflects change and any value around 60% and higher reflects preferential exploration of the novel object. Time between exposure and cognitive testing: A: 1 month; B: 1 month; C: 3 months; D: 0.5 month; E: 2 months; F: 2 months. The references for the data shown are: A. (Raber, Rosi et al. 2018); B. (Impey, Jopson et al. 2017); C.

(Kronenberg, Gauny et al. 2017);D. (Haley, Yeiser et al. 2013); E. (Kronenberg, Gauny et al. 2018); F. (Rabin, Shukitt-Hale et al. 2014).

2786



2787

2788 **Fig. 5.5**. Dose-response for long-term effects of ^{56}Fe (A), ^{12}C (B), ^{28}Si (C), and ^{16}O (D) on
2789 object recognition. The energies and species used are indicated in the figure panels. In panel A,
2790 the error data point and vertical line indicate object recognition in sham-irradiated animals. Time
2791 between exposure and cognitive testing: A: 12 months; B: 11 months; C: 10 months; D: 12
2792 months. The reference for the data shown in all panels is: (Rabin, Shukitt-Hale et al. 2014).

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5.4 Development of biomarkers and bioindicators of detrimental behavioral and cognitive outcomes in animals that will also be applicable to and usable in humans/astronauts

Biomarkers, whose presence is indicative of effects of space irradiation, have to be quantifiable to detect or even predict detrimental behavioral and cognitive outcomes. In this regard, it is important to realize that the brain can compensate for damage caused by space irradiation or other environmental challenges during space missions. Thus, it is conceivable that not only a biomarker that relates to impaired CNS function but also a biomarker that relates to a neurotrophic marker and typically is associated with beneficial effects on brain function might be increased following space irradiation. As biomarkers, one could consider outcome measures in individual tests, particularly in those tests for which there either is a humanized version or a clear human counterpart outcome measure. In addition, based on the various functions affected by space irradiation, one could consider an integrated score based on multiple tests using Z scores. The outcome of unbiased pathway analyses following space irradiation described in Chapter 6 could also be used to develop biomarkers or bioindicators that could be relatively noninvasively measured in astronauts prior, during, and following missions.

5.5 Future directions and recommendations

From a historical perspective, effects of space radiation on neurobiological responses in animals have evolved over the years. In addition, while the earlier studies often involved brain only irradiation, most recent studies have involved whole body irradiation. As for brain-only irradiation anesthesia is typically used, it is conceivable that the anesthesia might protect against the effects of space irradiation. Further, there has been a change in the behavioral and cognitive tests used in the various space irradiation studies. Finally, there has been a change in the age of

the animals at irradiation used in most studies. Although earlier studies mostly involved young adult animals, current studies typically involve animals that are at least 6 months of age at irradiation to better correspond to the age window of astronauts during space missions. As a result, there are gaps in our understanding of the effects of space irradiation on the CNS. Based on the current body of knowledge, the following recommendations are suggested:

1. Use chronic exposures to complement the acute exposures and fractionated doses used so far. Technically this is not trivial but from a biological perspective the effects of an acute versus a chronic exposure might be very different.

2. Increase use of mixed beam exposures as the interaction of effects of different particles is largely unknown. One study so far involved mixed proton-^{56}Fe ion irradiations with a 24 h interval between both exposures (Raber, Allen et al. 2016b) but there are many combinations not assessed yet.

3. Consider the effects of space irradiation in the context of other environmental challenges pertinent to astronauts during space missions. Examples include effects of stress and circadian challenges, as well as effects of space irradiation on the stress response and circadian activity levels.

4. Consider the effects of space irradiation on emotional behaviors, including depressive-like and social behaviors.

5. Consider more studies comparing performance of females and males in space radiation studies. Although some recent studies involve females, the majority of studies have been performed in males only.

6. Consider assessing effects of mitigating strategies to treat or even prevent detrimental effects of space irradiation on the brain.

7. Consider assessing effects of multiple exposures over time to mimic multiple space missions astronauts are exposed to.

8. Consider studies combining behavioral and cognitive assessments with unbiased strategies to determine pathways altered following space irradiation to get a better understanding of mechanisms underlying the space radiation effects (see also next chapter about potential mechanisms).

9. Consider focused studies in nonhuman primates once results from recommendations 1-7 are conclusive.

6. Observations Relevant to Potential Mechanisms

6.1 Cellular Mechanisms

The CNS is a complex tissue comprised by multiple cell types that each play critical roles in normal function. This inter-dependence of different cell types means that injury to one type can manifest as dysfunction of the entire system. For example, immune-mediated injury to oligodendrocytes, as seen in multiple sclerosis, results in a wide range of sensory, motor, behavioral and cognitive deficits that vary from individual to individual and depend on the location and duration of injury. Major cell types in the CNS include neurons, astrocytes, oligodendrocytes, microglia, and cells associated with the vasculature, including endothelial cells and pericytes. Neurons comprise an incredibly diverse variety of cells in the mammalian CNS of different sizes, connection patterns and function; they work together as ensembles of specialized function to gather and process information, integrate it, and generate behavioral and cognitive responses. Astrocytes and oligodendrocytes, collectively called macroglia, arise from the same embryonic origin (neuroectoderm) as neurons, but do not maintain the unique electrically excitable properties shared by neurons. Instead, they each function in critical ways to help preserve the specialized environment of the CNS that allows neurons to carry out neural transmission. In particular, the abundant astrocytes help to maintain water and ion balance in the CNS (*e.g.*, by taking up excess K^+), and have specialized functions at excitatory synapses where they take up excess glutamate and recycle it back to neurons via production of glutamine. Astrocytes are also intimately associated with the vasculature through specialized processes called endfeet, which encircle capillaries and small arterioles throughout the CNS. There is accumulating evidence that astrocytes may assist with ensuring proper nutrient perfusion of local

active neurons by sensing neural activity and controlling local blood flow to the region, a process called neurovascular coupling (Petzold and Murthy 2011, Nuriya and Hirase 2016). Oligodendrocytes contribute to neural transmission by myelination, a process that greatly enhances the speed and efficiency of electrical signal conduction through the axon. Interestingly, some recent data suggests that oligodendrocytes may also provide substrate (lactate) for axonal metabolic demands (Morrison, Lee et al. 2013).

Microglia are brain-specific tissue macrophages that arise during early embryonic development from the yolk sac (Ginhoux and Prinz 2015). Microglial transcriptomes are distinct from peripheral monocytes and other macrophage populations (Bennett, Bennett et al. 2016). Nevertheless, microglia function, as other macrophages, to survey the local environment and quickly respond to injury (Nayak, Roth et al. 2014, Tay, Savage et al. 2017). Indeed, within the last decade, *in vivo* multi-photon imaging of genetically labeled microglia clearly shows a continuous extension and retraction of cell processes that presumably carry out local tissue surveillance. In response to injury, microglia rapidly adapt to challenges by taking on unique phenotypes that for instance, secrete chemokines to recruit additional immune cells, phagocytize cell debris, or signal astrocytes to secrete extracellular matrix and form "glial scars". This local reaction of microglial cells and ensuing signaling to other cells is a process called *neuroinflammation.* While brain injury or disease processes can lead to recruitment of other myeloid lineage cells from the bone marrow, and such recruitment is observed after relatively high doses of low LET radiation (Moravan, Olschowka et al. 2011, Morganti, Jopson et al. 2014, Moravan, Olschowka et al. 2016), microglia represent a long-lived pool of cells with clear capacity for self-replication (Elmore, Najafi et al. 2014). Finally, in addition to their ability to respond to injury, microglia also play important roles in normal brain development, particular

with respect to synaptic plasticity and the removal of synaptic structures (Hong and Stevens 2016, Tay, Savage et al. 2017).

In addition to the cell types mentioned above, the CNS vasculature plays a critical role in maintaining normal function. In the brain and spinal cord (as well as in peripheral nerves), blood vessels are characterized by endothelial tight junctions, which provide the unique blood brain (or nerve) barrier (BBB) (Zhao, Nelson et al. 2015). This specialized adaptation maintains the proper ionic and water balance required for effective neural transmission within the CNS. While detailed descriptions of BBB functions and constituents of the neurovascular unit are beyond the scope of this introduction, alterations to the BBB are commonly described in many disease states and contribute to dysfunction and degeneration (Kisler, Nelson et al. 2017). Potential targets for such alterations include the endothelial cells themselves, as well as surrounding pericytes, which play a critical function in maintaining BBB integrity (Sweeney, Ayyadurai et al. 2016).

6.1.1 Loss of Progenitor Cell Populations

Although the adult CNS is largely considered post-mitotic and therefore relatively insensitive to the DNA damaging effects (*e.g.*, cell death and carcinogenesis) of ionizing radiation exhibited by highly proliferative tissues (e.g. bone marrow, intestinal endothelium), the adult CNS contains several progenitor cells populations or potential progenitor populations that show classic radio-sensitivity. In particular, these include neurogenic regions in the dentate gyrus of the hippocampus and subventricular zone and oligodendrocyte precursor cells (OPCs), which are distributed throughout the CNS. Evidence for potential space radiation effects for each of these cell types follows.

6.1.1.1 Adult Neurogenesis. The hippocampus is one of the best-studied CNS structures, in part because of its unique anatomical organization, its clear role in fundamental processes associated with learning and memory, and its involvement in multiple neurological diseases including AD, global hypoperfusion, and epilepsy. A major source of hippocampal input is the perforant path, which synapses on dendrites of the dentate gyrus granule cells, through which signals are passed on to neurons of the hippocampus proper for further processing and integration. Within the subgranular zone (SGZ) of the dentate gyrus is a population of neural precursor cells (NPCs) that have the capacity to divide, differentiate, and migrate through a series of well-studied steps, from neuroblasts to mature dentate gyrus granule cells that are fully integrated into the circuitry of the dentate gyrus (Kempermann, Song et al. 2015). This process, known as hippocampal neurogenesis, was recognized more than 50 years ago and clearly occurs in the adult human brain (Monje, Vogel et al. 2007, Spalding, Bergmann et al. 2013). Substantial research, mostly performed in rodent models, reveals that hippocampal neurogenesis is a dynamic process that can be modified by behavior (*e.g.*, voluntary running is strongly associated with increased hippocampal neurogenesis (van Praag, Christie et al. 1999)), drugs (*e.g.*, fluoxetine causes increased neurogenesis (David, Samuels et al. 2009)), and disease (Eisch, Cameron et al. 2008, Winner and Winkler 2015). Aged rodents show reduced neurogenesis relative to young mice (Kempermann, Song et al. 2015, Goncalves, Schafer et al. 2016) and there are dramatic differences in the amount of neurogenesis across inbred mouse strains (Kempermann, Chesler et al. 2006). Although the precise role(s) of hippocampal neurogenesis are still debated, these newly differentiated neurons are involved in behavioral and cognitive changes associated with stress (Ben Menachem-Zidon, Goshen et al. 2008, Koo and Duman 2008, Lagace, Donovan et al. 2010), depression (Sahay and Hen 2007, David, Samuels et al. 2009), and learning and memory

(van Praag, Christie et al. 1999, Shors, Miesegaes et al. 2001, Cao, Jiao et al. 2004, Leuner, Mendolia-Loffredo et al. 2004, Clark, Brzezinska et al. 2008, Deng, Saxe et al. 2009).

In animal models, ionizing radiation has been used as a tool to investigate the effects of hippocampal neurogenesis ablation. Notwithstanding other mechanisms evoked by radiation exposure, there is growing consensus in clinical radiooncology practice that relative avoidance of the hippocampus in irradiated fields reduces cognitive deficits, particularly in young individuals receiving whole brain radiotherapy (Gondi, Pugh et al. 2014, Tsai, Yang et al. 2015). Thus, the effect of HZE particle irradiation on hippocampal neurogenesis and hippocampal function in general has received a significant amount of attention in the field.

Adult neurogenesis can be quantified by counting cell nuclei that have taken up bromodeoxyuridine (BrdU) *in vivo*, the use of immunohistochemical (IHC) staining for markers of cell proliferation (*e.g.*, Ki67), or IHC staining for specific cell markers expressed by neural precursor cells and neuroblasts (*e.g.*, Doublecortin, DCX). Rola *et al* (2004b). first demonstrated decreased hippocampal neurogenesis in young female C57BL/6 mice exposed to whole body ^{56}Fe (1000 MeV/n) using staining for BrdU uptake, Ki67 and DCX. There was a dose-dependent decrease in all of these measures with 1, 2 or 3 Gy observed 3 months post-irradiation (Rola, Otsuka et al. 2004a). The same group found similar reductions in neurogenesis using 1-3 Gy ^{56}Fe (1000 MeV/n) or ^{12}C (290 MeV/n) in male C57BL/6 mice nine months post-irradiation, indicating that effects were sustained (Rola, Sarkissian et al. 2005). Interestingly, a radiation-associated increase in dentate gyrus granule cell expression of monocyte chemokine receptor CCR2 was reported in this same study (Rola, Sarkissian et al. 2005). Young male C57BL/6 mice receiving cranial radiation under isoflurane anesthesia with a collimated ^{56}Fe (600 MeV/n) beam showed reduced numbers of BrdU labeled mature hippocampal neurons co-labeled with

Neu-N , 2 months post-irradiation, with dose dependent effects seen at 1, 2, and 4 Gy, but no effect at 0.5 Gy (Rola, Fishman et al. 2008). These reductions in new neurons were correlated with a dose-dependent increase in BrdU labeled hippocampal microglia, highlighting a relationship between reduced hippocampal neurogenesis and markers of neuroinflammation previously established with low-LET radiation (Monje, Toda et al. 2003). Reduced hippocampal neurogenesis following whole-body ^{56}Fe (1000 MeV/n) exposure (2.5 Gy) has also been reported in young male Sprague-Dawley rats and likened to changes seen with aging (Casadesus, Shukitt-Hale et al. 2005). In a study designed to determine a threshold for radiation effects on hippocampal neurogenesis, Sweet *et al*. reported a significant decrease in acutely labeled BrdU positive SGZ cells 48 hours after radiation with 0.3 Gy of whole body ^{56}Fe (1000 MeV/n), but not lower doses (*e.g.*, 0.01-0.1 Gy) (Sweet, Hurley et al. 2016). A persistent effect on neurogenesis, quantified by DCX cell counts at 1 month post-irradiation, showed significance with 1 Gy, but not lower ^{56}Fe doses. Parallel studies comparing effects of low-LET (^{137}Cs) irradiation showed significant decrements at 1 Gy for both acute cell division (48 h BrdU) and DCX measures of neurogenesis (Sweet, Hurley et al. 2016). The apparent similarity of dose thresholds seen for low and high-LET radiation effects on hippocampal neurogenesis may reflect limits in direct DNA damage due to reduced fluence of HZE particles at lower doses.

Although most space radiation-related studies of hippocampal neurogenesis have been carried out with ^{56}Fe HZE particles, there are a few reports with protons. Using a design similar to that described for comparisons of ^{56}Fe and gamma exposure, Sweet *et al*. reported decreased numbers of SGZ cells acutely labeled with BrdU after 0.5 Gy proton (1000 MeV) whole body exposure, that was not observed with 0.1 or 0.2 Gy, in young male C57BL/6 mice, 48 hours after irradiation (Sweet, Panda et al. 2014). DCX staining one month post-irradiation was similarly

decreased by 0.5 Gy protons. Interestingly, female mice showed reduced acute BrdU uptake following 0.1 Gy proton exposure, but additional studies would need to be completed to verify this apparent sex difference. Raber and colleagues investigated the effects of whole body proton or proton combined with ^{56}Fe irradiation in young male C57BL/6 mice and did not detect reduced hippocampal neurogenesis with doses up to 1 Gy protons (150 MeV) or with protons (0.1 Gy; 150 MeV) plus ^{56}Fe (0.5 Gy; 600 MeV/n) (Raber, Allen et al. 2016b). However, rather than immediate labeling with BrdU as in previous studies, neurogenesis was quantified by counting BrdU positive mature granule cells (Neu-N^{+}) three months after radiation exposure (BrdU was administered one month before sacrifice), which may account for differences between the studies mentioned here.

In a recent report using ^{28}Si (300 MeV/n) exposure in 10-week-old C57BL/6J mice of both sexes, Whoolery *et al.* reported decreased granule cell proliferation, detected by BrdU incorporation and Ki67 staining, as well as reduced number of DCX positive cells 24 h after 1 Gy exposure (Whoolery, Walker et al. 2017). Reduced numbers of Ki67 and DCX stained cells were also observed with 0.2 Gy at this early time point. At three months post-irradiation, cells co-labeled with BrdU and Neu-N and DCX positive cells numbers were reduced in 1 Gy treated mice, but not in those exposed to 0.2 Gy (Whoolery, Walker et al. 2017). There were some sex differences noted; for example, Ki67 levels were still decreased in males, but not females exposed to 1 Gy, suggesting some recovery of granule cell proliferation (Whoolery, Walker et al. 2017).

Some investigators have explored the effect of HZE radiation on specific stages of NPC cell proliferation and survival using transgenic mouse models that genetically label neural precursors. Using a transgenic nestin-CFPnuc reporter mouse that labels nuclei of neural

precursor cells, Encinas *et al*. found evidence for reduction not only in BrdU-positive cells 24 hours following head-only 1 Gy ^{56}Fe (1000 MeV/n), but also in a quiescent adult neural stem cell population based on morphological and staining criteria (Encinas, Vazquez et al. 2008). However, using a different mouse model with tamoxifen-induced labeling of nestin-expressing cells and their progeny, the Eisch group reported preservation of the quiescent neural precursor cell population, defined as radial glial cells, at time points ranging from 24 hours to 3 months following 1 Gy whole body ^{56}Fe (300 MeV/n) particle exposure (Rivera, Shih et al. 2013, DeCarolis, Rivera et al. 2014). Additional findings from this group suggest that precursor cell proliferation may recover from 1 Gy ^{56}Fe exposure based on no significant decrease in DCX labeled cells 2 months after irradiation (DeCarolis, Rivera et al. 2014). While this last finding contrasts with data suggesting that reduction in DCX labeling persist for 9 months (*e.g.*, (Rola, Sarkissian et al. 2005)), as well as data at 3 months (Raber et al., 2016b), there are differences in radiation parameters and mice used that hamper direct comparisons.

A major issue with interpretation of ground-based studies of space radiation is that nearly all experiments conducted to date depend on single acute doses rather than the chronic low dose rates encountered in space. One approach that starts to address this issue has been to compare fractionated to single doses of HZE particles. Along these lines, 5 daily fractions of 0.2 Gy ^{56}Fe (300 MeV/n) was no different, with regard to measures of hippocampal neurogenesis, than a single 1 Gy dose using 10-12 week old, male and female nestin reporter mice on a C57BL/6 background (Rivera, Shih et al. 2013). As better models of chronic exposure become available, including the recently started chronic neutron exposure rodent studies and upgrades in the NASA Space Radiation Laboratory (NSRL) facilities at Brookhaven National Laboratory (BNL), it will

be important to establish whether clear effects of particle radiation, such as reductions in hippocampal neurogenesis, are dose-rate dependent.

Figure 6.1 compiles results from multiple studies investigating persistent deficits in hippocampal neurogenesis, defined as reductions in BrdU incorporation or DCX stained neurons, 30 or more days post-irradiation. The range of experiments shown include both mice and rats, but all are with young animals, typically 2 months of age at the time of irradiation. To compare across different measures, values are reported as proportion of control and shown with regard to ion used, but not energy. Given the relatively limited dose range for most ions tested, linear plots have been included as the simplest way to consider data trends. From these plotted data one can start to consider some upper and lower limits for RBEs of the particles, within the range of energies tested, at least for substantially reduced 'proportions remaining' (e.g., 60% or less, indicating RBEs up to about 3). For higher survival levels, the curves are less well defined and the data suggest that the RBEs may be a larger, but quite a bit more data in the low dose regions, which is most relevant to space missions, would be required to pin this down. Along these lines, the model presented by Cacao et al. provides some predictions regarding low dose effects (Cacao and Cucinotta 2016). However, as already described above, the majority of single ion studies show little or no effects on persistent neurogenesis with doses below 0.5 Gy. If there are small reductions in persistent neurogenesis at these lower doses, the consequences of such effects are currently not known. By way of comparison, current clinical protocols for sparing of hippocampus in whole-brain radiotherapy treatment plans for adult metastatic cancer (e.g., NRG-CC001) call for limits to the maximum dose and dose to 100% of the hippocampus of 16 Gy and 9 Gy, respectively (Pokhrel, Sood et al. 2016).

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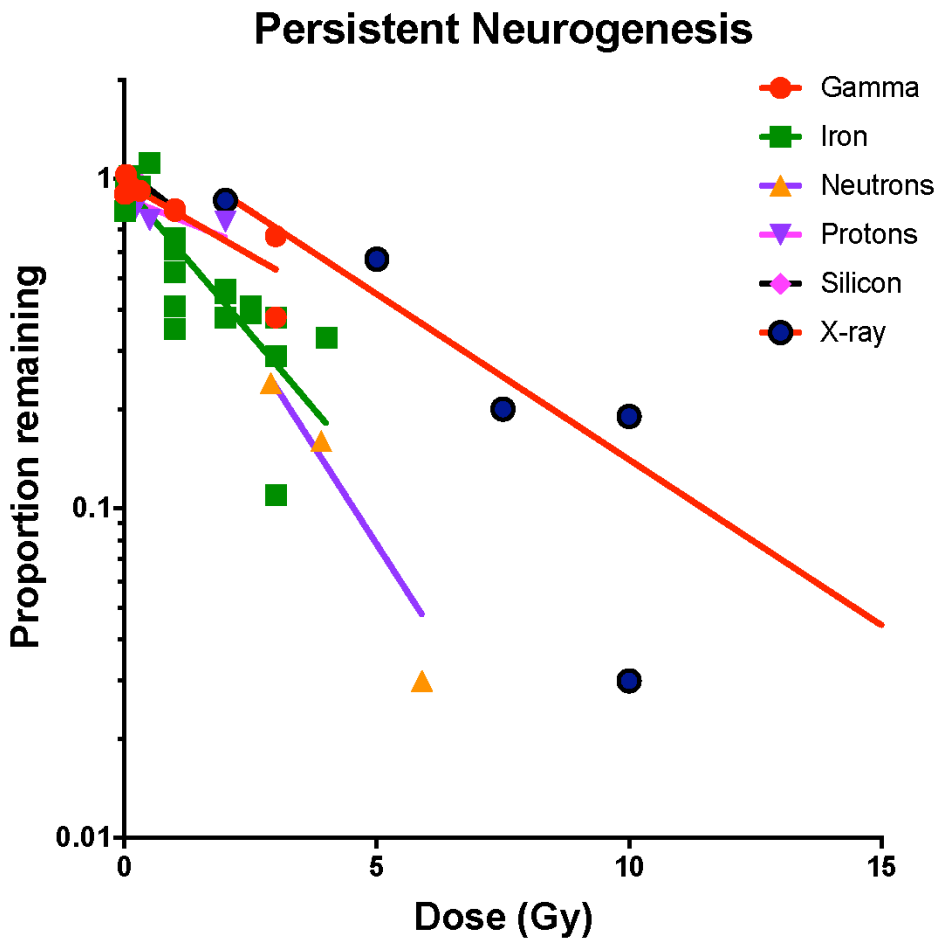

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3057

3058 **Fig. 6. 1** Dose-response for persistent effects of multiple ions on neurogenesis. Data are plotted
3059 from studies using both rats and mice, all irradiated at relatively young ages (e.g., 2 months).
3060 Proportions of neurogenesis remaining are based on measures of DCX or BrdU staining, 30 days
3061 or longer after exposure. In some cases (e.g., iron), experiments with multiple energies are
3062 grouped. When specific values were not provided, data points were estimated from figures. Data
3063 are drawn from the following references: (Mizumatsu, Monje et al. 2003, Rola, Otsuka et al.
3064 2004a, Casadesus, Shukitt-Hale et al. 2005, Rola, Sarkissian et al. 2005, Otsuka, Coderre et al.
3065 2006, Encinas, Vazquez et al. 2008, Rola, Fishman et al. 2008, Rivera, Shih et al. 2013, Sweet,
3066 Panda et al. 2014, Sweet, Hurley et al. 2016, Whoolery, Walker et al. 2017)

6.1.1.2 Oligodendrogenesis. In the adult brain, oligodendrocyte precursor cells (OPCs) play an important role in normal myelin turnover and in the production of new myelin in response to physiologic or injurious stimuli. Under normal circumstances, OPCs are quiescent or have a long cell cycle time, but can be recruited to rapidly proliferate in the setting of injury (Hughes, Kang et al. 2013). Although there is much evidence that high therapeutic doses of low-LET radiation can lead to white matter damage (*e.g.*, radiation necrosis) and lower doses may impact white matter more subtly (Begolly, Shrager et al. 2016), there is a relative paucity of studies examining the effects of HZE radiation on this critical cell type. Rola *et al*. reported a dramatic, dose-dependent decrease in OPCs, identified as NG2 immunoreactive cells, 2 months after brain-only exposure with 1 through 4, but not 0.5 Gy, ^{56}Fe (600 MeV/n) particles (Rola, Fishman et al. 2008). Reduced NG2-immunoreactive cell staining was also reported in 10-12 month-old male Wistar rat spinal cord, 3 months after exposure to 1 Gy protons (250 MeV) (Suresh Kumar, Peluso et al. 2015). These limited data suggest that particle radiation can impact OPC populations, which may lead to late white matter deficits, but that such effects are apparent with acute doses of 1 Gy and higher.

6.1.1.3 Other Cell Populations. There are other CNS cell types with the capacity to replicate, including astrocytes and microglia, as well as pericytes and endothelial cells, which contribute to BBB integrity. In general, replication in these cell types, at least in the adult, is a response to injury. For example, microglia proliferation has been observed in the hippocampus following ^{56}Fe particle irradiation (Rola, Fishman et al. 2008), presumably as part of a response to apoptosis and/or loss of proliferating neural progenitor cells (Encinas, Vazquez et al. 2008). Radiation exposure, whether acute or chronic, has the potential of causing CNS injury, which

may lead to proliferative responses that are themselves sensitive to radiation. This is likely an issue with higher dose exposures seen, for example, with fractionated radiotherapy (Begolly, Olschowka et al. 2017), but whether such effects would be expected in the deep space setting of chronic, low dose exposure is unclear.

6.1.2 Neuronal Structural Effects

Unlike most other cell types, neurons maintain highly complex and extensive cell processes that are critical for their function. Collectively called neurites, these processes include dendrites, which greatly increase the receptive area of neurons for incoming signals, and axons, which represent the route for transmission of signals to other neurons or effector tissues such as muscle. These processes greatly increase the cross-sectional areas of neuronal cell membranes, and represent a unique target for radiation, particularly traversal of HZE particles, separate from the nucleus. Neuronal architecture of select cell types (*e.g.*, dentate gyrus granule cells) has been modeled to better understand the potential interactions of particulate radiation with the cell (Alp, Parihar et al. 2015, Alp and Cucinotta 2017); however, the diversity of neuronal types, each with unique dendritic fields of greater or lesser extent means that some neurons may be relatively more sensitive to radiation effects on processes.

Accumulating evidence indicates that neural processes are sensitive to HZE radiation. Indeed, decreased CA1 hippocampal synaptic density assessed by electron microscopy was reported in mice exposed to argon particles over 30 years ago (Philpott, Sapp et al. 1985). More recent studies have included impacts of radiation on overall dendritic complexity as well as the specific synaptic contacts between neurons. Each of these structural effects will be considered in turn. Importantly, changes in neural processes and synapses would be expected to manifest as

alterations in electrophysiological properties and/or changes in neural connectivity, and ultimately affect behavioral and cognitive performance. Conversely, behavioral and cognitive performance affect dendritic complexity and spine density and these effects are modulated by radiation (Allen, Raber et al. 2015). These functional endpoints of HZE and proton exposure are described in Section 6.2.4.

6.1.2.1 Effects on Neurites. Limoli and colleagues have examined the effects of HZE radiation on dendritic arbors using digitally reconstructed confocal images obtained with transgenic mice that express enhanced green fluorescent protein from a Thy1 promoter, which is active in specific subsets of cortical neurons (Parihar, Allen et al. 2015, Parihar, Allen et al. 2016). Detailed neuronal morphometric analyses of such tissues provide multiple indices related to dendritic arbors as well as information about spine densities and spine types. Using this approach with mice 6-months-old at the time of whole body irradiation, Parihar *et al.* reported significant decreases in dendritic branches, branch points and total dendritic length in the prelimbic area of medial prefrontal cortex 8 weeks after exposure to 0.3 Gy ^{16}O (600 MeV/n) or 0.05 and 0.3 Gy ^{48}Ti (600 MeV/n) (Parihar, Allen et al. 2015). Notably, each of these doses was associated with deficits in performance in the novel object recognition and novel object in place tests performed at 6 weeks post-irradiation. The authors elected to investigate dendritic changes in the medial prefrontal cortex because of its known role in working memory (executive function) and performance in object recognition tests when the interval between training/learning and testing are within the same session (Parihar, Allen et al. 2015). In a second study of mice examined 15 weeks post-exposure, similar changes in dendrite measures were seen for all doses tested (0.05 and 0.3 Gy of both ^{16}O and ^{48}Ti) (Parihar, Allen et al. 2016), indicating persistence

of such changes. Using an identical approach, decreases in dendritic complexity were observed in the dendritic fields of dentate gyrus granule neurons, 30 days following exposure to 1 Gy protons (250 MeV) (Parihar, Pasha et al. 2015c). Similar findings were reported by the same group following gamma irradiation using doses of 1 and 10 Gy (Parihar and Limoli 2013), indicating a common response of neuronal dendrites and spines to ionizing radiation of all types tested.

Using Golgi staining, a classic method for visualizing neuronal processes that depends on silver impregnation of individual neurons, Allen *et al.* carried out morphometric analyses of the dentate gyrus molecular layer, representing dendrites of dentate gyrus granule cells, as well as apical and basal dendrites of hippocampal CA1 and CA3 subfield pyramidal neurons after ^{56}Fe (600 MeV/n) irradiation (Allen, Raber et al. 2015). Male C57BL/6 mice received 0.5 Gy whole body irradiation and tissues taken 3 months later were analyzed using Sholl analysis to quantify dendritic lengths. ^{56}Fe exposure led to modest, but significant decreases in granule cell dendrite length and more complex responses in CA1 neurons with decreased lengths of apical and increased lengths of basal dendrites (Allen, Raber et al. 2015). No effects on dendritic length were observed in CA3 pyramidal neurons.

6.1.2.2 Effects on Synapses and Dendritic Spines. The studies showing changes in dendritic complexity described above also included measures related to synaptic structures, including density and types of dendritic spines, which represent specialized post-synaptic structures as well as levels of both pre- and post-synaptic associated proteins. Allen *et al.* described significant reductions in spine density (*i.e.*, number of spines per unit length of dendrite) in dentate gyrus granule cells, CA1 basal, and CA3 apical dendrites 3 months following 0.5 Gy ^{56}Fe (600 MeV/n)

irradiation, as well as some changes in spine morphology (Allen, Raber et al. 2015). Parihar *et al.* found significant decreases in medial prefrontal cortex spine number and density with 0.05 and 0.3 Gy ^{16}O (600 MeV/n) and ^{48}Ti (600 MeV/n) exposures at 6 and 15 weeks post-irradiation (Parihar, Allen et al. 2015, Parihar, Allen et al. 2016). Morphological, these decreases included spines characterized as filopodia, long, and mushroom-like, but not stubby spines. Interestingly, increased numbers of post-synaptic density protein-95 (PSD-95) immunoreactive puncta were found 6, 15, and 27 weeks post-irradiation with both doses (0.05 and 0.3 Gy) of the two ions (Parihar, Allen et al. 2015, Parihar, Allen et al. 2016). According to the authors, this increase in PSD-95 puncta might represent a radiation-induced response that inhibits dendritogenesis. An alternate hypothesis is that increased PSD-95 expression might represent an attempt to form new connections that were lost. Regardless of direction, changes following space irradiation in markers regarded as important for brain function might be detrimental. Consistent with this notion, increased PSD-95 expression as well as decreased spine density were both correlated, for nearly all radiation conditions, with individual mouse performance on an object in place task administered 2 weeks before sacrifice (Parihar, Allen et al. 2016). These data provide an important link between structural and cognitive measures after HZE radiation and raise concerns regarding potential CNS dysfunction in humans given the relatively modest doses at which this correlation was observed (0.05 Gy) (Limoli 2017).

Dose- and ion-response curves for measures of spine density at time points 30 days or more after exposure are shown in Figure 6.2. To compare across multiple studies, spine densities in irradiated animals are plotted relative to control densities, and data is plotted for multiple brain regions as indicated in the figure legend. Notably, as indicated, some data was quantified from Golgi-impregnated tissues, which labels a subset of neurons in the field of interest, while other

data was generated from computer-aided image analysis of neurons genetically labeled with eGFP. In both cases, the original data is reported as spines per unit length of dendrite; however, in comparing control values, the Golgi method typically records about twice as many spines per unit length as the eGFP method. More over, the reduction from control is not as profound with data derived from the Golgi stain, possibly reflecting sensitivity of the two methods to specific spine types. Unlike the data shown in Figure 6.1 for neurogenesis, the available data for spine density are not sufficient to draw conclusions about dose-response other than reductions are seen at very low doses for some HZE ions. As already mentioned, spine density and dendritic complexity have been correlated with behavioral performance, raising specific concerns about the observed effects at low doses.

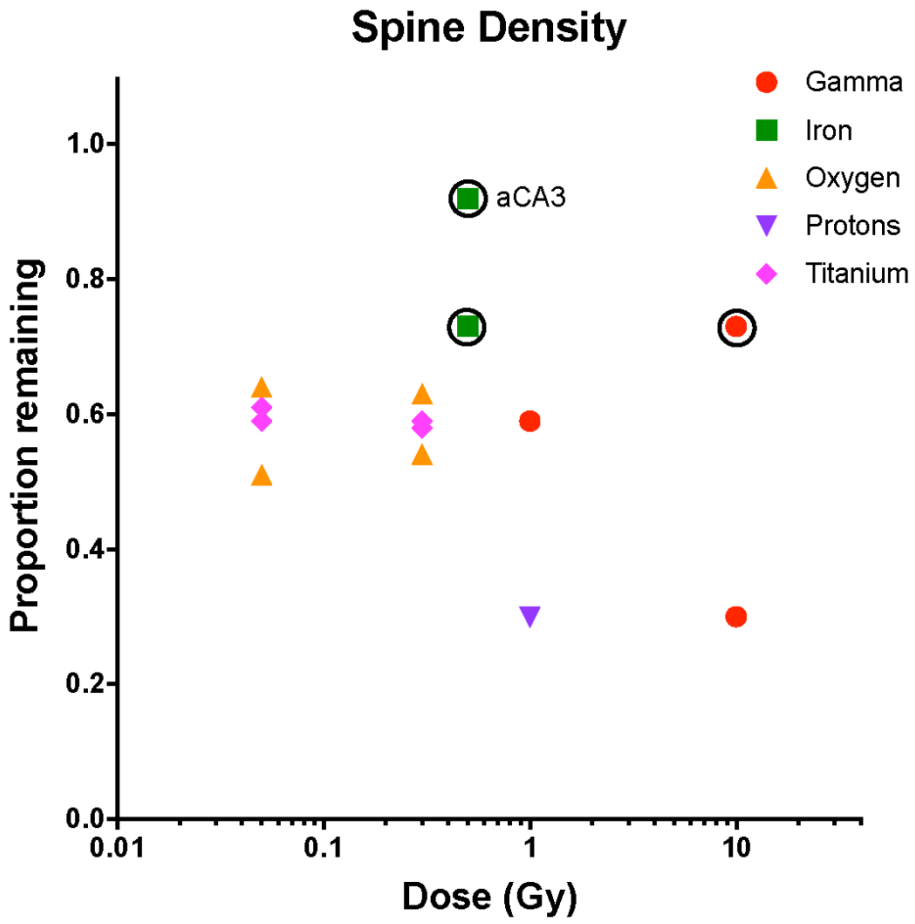

Fig. 6. 2 Dose-response for persistent effects (30 days or more) of multiple ions on dendritic spine density. With the exception of one measure for apical CA3 dendrites (indicated on graph), data for gamma, protons and ^{56}Fe represent analysis of dendrites in the dentate gyrus molecular layer, and data for ^{16}O and ^{48}Ti are from the mPFC. All data are from studies using mice irradiated at relatively young ages (e.g., 2 months) with the exception of the ^{16}O and ^{48}Ti data, which were derived from mice irradiated at 6 months of age and examined 8 and 15 weeks post-exposure. Data points derived from Golgi stained tissue are indicated with a black circle. When specific values were not provided, data points were estimated from figures. Data are drawn from the following references: (Chakraborti, Allen et al. 2012, Parihar and Limoli 2013,

Allen, Raber et al. 2015, Parihar, Allen et al. 2015a, Parihar, Pasha et al. 2015c, Parihar, Allen et al. 2016, Raber, Allen et al. 2016a).

One important aspect related to the studies presented above showing synaptic changes following irradiation is the fact that synapses are highly plastic structures that are readily modified by behavioral and cognitive input, and all mice studied underwent relatively extensive behavioral and cognitive testing. Indeed, Raber *et al.* reported that whole body 0.5 Gy ^{56}Fe (600 MeV/n) irradiation inhibited acute behavior-associated increases in dendritic spine density across several hippocampal regions when assessed 3 months after exposure using the Golgi method (Raber, Allen et al. 2016a). Therefore, unless behaviorally naïve animals are included in a particular study, it is hard to distinguish whether the observed CNS effects are due to only HZE exposure per se or an interaction of effects of radiation and behaviorally testing/environmental enrichment.

6.1.3 Vascular Effects

Because of high energy demands to maintain ionic balance and excitability, blood flow and continuous tissue oxygenation are critical for proper brain function. Indeed, despite its relatively small mass, the richly vascularized human brain consumes 20% of the body's oxygen intake. In addition, as already described, proper neural function depends on an intact BBB. There is ample evidence that the brain's vasculature is damaged by high-dose, low LET radiation: vascular lesions are prominent in areas of radiation necrosis in irradiated humans and non-human primates (Hanbury, Robbins et al. 2015, Andrews, Metheny-Barlow et al. 2017) and there is experimental evidence that radiation effects on endothelial cells may underlie such white matter damage (Calvo, Hopewell et al. 1988, Hodges, Katzung et al. 1998, Lyubimova and Hopewell 2004). Experiments in rodents also show clear effects of low LET radiation. For example, a cranial X-ray dose of 9 Gy led to reduced hippocampal microvessel volumes at 2

days that persisted until 1 month after exposure in 8-month old C57BL/6 male mice (Craver, Acharya et al. 2016). Similar effects were also reported in rats one month after 10 Gy cranial irradiation (Craver, Acharya et al. 2016). Despite this evidence, there is a relative paucity of information about brain vascular effects of lower dose or high-LET radiation.

Using stereological techniques to quantify endothelial cell number and microvessel measures, Mao *et al*. reported dose-related changes 12 months following cranial ^{56}Fe (600 MeV/n) irradiation of 10-week old C57BL/6 male mice. Interestingly, radiation reduced endothelial cell density at 0.5 Gy and 2 Gy, and microvessel length density at 0.5 Gy, but not at the highest dose tested (4 Gy) in the hippocampal CA1 region (Mao, Favre et al. 2010); such changes were not seen in the dentate gyrus and no changes were detected at 6 months post-irradiation. Decreases in microvessel diameter were also limited to the CA1 region, but these were only observed with the 4 Gy dose at the 12-month time point (Mao, Favre et al. 2010). The authors suggest that the effects on endothelial cell number at lower doses might reflect a differential dose-response to presumed acute loss (and repair) of endothelial cells at the higher dose tested; however, no data on acute cell loss were reported. Interestingly, using a 3-D human brain microvascular endothelial cell culture model, Grabham and colleagues demonstrated unique effects of 1 Gy ^{56}Fe and protons (both at 1000 MeV/n) on vasculogenesis, suggesting that regeneration of damaged vessels might be hindered after radiation injury (Grabham, Sharma et al. 2013). Further studies of particle radiation effects on brain vasculature are clearly warranted, particularly given accumulating evidence of radiation promoting atherosclerotic disease (Yu, Parks et al. 2011) and other cardiovascular changes (Sasi, Yan et al. 2017). These studies should include experiments using lower doses and a range of time points.

6.2 Molecular Mechanisms

6.2.1 DNA Damage

HZE causes DNA damage in the CNS as evidenced by increased numbers of 53BP1-immunoreactive foci in the dentate gyrus 7 days and 2 months after 1 Gy ^{56}Fe (1000 MeV/n) exposure (DeCarolis, Rivera et al. 2014). As already mentioned, the rapid apoptotic loss of hippocampal neural precursor cells is certainly related to such damage (Encinas, Vazquez et al. 2008). In post-mitotic or slowly dividing cells, DNA damage can provoke cellular senescence associated with chronic redox imbalance to a more oxidized state and inflammation (Kang, Xu et al. 2015, Choubey and Panchanathan 2016). DNA damage in neurons and resulting chronic oxidative stress have also been proposed as important contributors to the development of neurodegenerative disorders (Wang, Dharmalingam et al. 2017). Studies exploring increased neural oxidative stress following heavy ion exposure are described in Section 6.2.3.

6.2.2 Neuroinflammation and Peripheral Immune Responses

Originally described in the context of Alzheimer's disease (Akiyama, Barger et al. 2000, Heneka and O'Banion 2007), the local expression of cytokines, chemokines, and other inflammation-associated mediators by microglia and other CNS cell types, a process known as neuroinflammation, has been recognized as an important component of most brain disorders whether resulting from injury, infection, degeneration or developmental processes. Neuroinflammation, evidenced by phenotypic alterations in microglia and astrocytes (glial activation) and increased mRNA expression of pro-inflammatory cytokines such as TNF-α and IL-1β, is readily observed following relatively high doses of low LET radiation (Chiang,

McBride et al. 1993, Chiang, Hong et al. 1997, Moravan, Olschowka et al. 2011). Moreover, therapeutic agents or genetic manipulations targeting inflammatory signaling can reduce brain radiation effects (Moore, Olschowka et al. 2004, Belarbi, Jopson et al. 2013). Therefore, there is much interest in the possible involvement of neuroinflammation following HZE exposure.

Although not a typical measure, qualitative observation of increased CCR2 expression in the dentate gyrus of male C57BL/6 mice suggested a possible neuroinflammatory response 9 months after exposure to 1 or 3 Gy ^{56}Fe (1 GeV/n) or ^{12}C (290 MeV/n) ions (Rola, Sarkissian et al. 2005). Rola *et al.* followed up these initial findings using stereology to count newly born activated microglia (cells co-labeled with BrdU and CD68) 2 months after ^{56}Fe particle (600 MeV/n) irradiation and observed a clear dose-responsive increase in such cells within the dentate subgranular zone with doses of 1 to 4 Gy (Rola, Fishman et al. 2008). Increases in such newly born activated microglia were inversely correlated with decreased measures of newly born neurons (BrdU-Neu co-labeled) and newly born oligodendrocyte precursor (BrdU-NG2 co-labeled) cells. In a separate set of studies with 2-month-old male C57BL/6 mice, numbers of BrdU-CD68 co-labeled cells in the dentate gyrus did not increase following exposure to protons (0.1 to 1 Gy, 150 MeV) or ^{56}Fe particles (0.5 Gy, 600 MeV/n), but did negatively correlate with performance on a novel object recognition task 3 months after exposure (Raber, Allen et al. 2016b). Changes in protein levels of several cytokines and chemokines were observed at 1 and 3 months after combined proton (0.1 Gy) and iron (0.5 Gy) exposure using a mouse cytokine array. These changes included decreased IL-4 and IFN-γ levels, as well as increased IL-12p70 and TNF-α levels at 1 month and decreased CCL11 and CCL22 levels plus increased IL-6 levels at 3 months (Raber, Allen et al. 2016b). Although specific roles for these observed responses in brain function following space irradiation are not understood, they indicate that

neuroinflammation is seen at space relevant radiation doses and are consistent with previous evidence from low-LET exposures showing complex patterns of chemokine and cytokine expression over time (Chiang, Hong et al. 1997, Moravan, Olschowka et al. 2011).

In addition to inducing chemokine expression, radiation can "activate" brain endothelial cells to express proteins involved in immune cell adhesion and trafficking; together these signals can influence the entry of peripheral immune cells into the CNS, augmenting or modifying the ongoing neuroinflammatory response. Such effects are clearly evident following higher doses of low LET radiation. For example, Moravan *et al.* reported persistent increases in endothelial intracellular adhesion molecule-1 (ICAM-1) expression and delayed CCR2 dependent influx of peripheral monocytes that persisted in the brain parenchyma as MHC-II and CD11c+ cells following cranial irradiation with 15 Gy and higher doses of gamma irradiation in young male C57BL/6 mice (Moravan, Olschowka et al. 2011, Moravan, Olschowka et al. 2016). Other reports indicate that such influx occurs with 10 Gy low LET exposure (Morganti, Jopson et al. 2014), and more importantly, that blocking such influx using CCR2 deficient mice resulted in less radiation-induced decrements in Morris water maze performance and CA1 and CA3 expression of Arc, a plasticity-related immediate early gene product induced after spatial exploration (Belarbi, Jopson et al. 2013). Based on this finding and earlier evidence that CCR2 is increased in the iron-irradiated hippocampus, Raber and colleagues explored the effects of ^{56}Fe particle (600 MeV/n) irradiation in C57BL/6 mice lacking CCR2 (Raber, Allen et al. 2013a). However, no effect of radiation was apparent for behavioral, cognitive, and histologic endpoints with the chosen 0.25 Gy dose of radiation at a single 3-month time point. Additional experiments using behavioral and cognitive endpoints and histological indices (*e.g.*, novel object place and synaptic density) that are sensitive to lower doses (Parihar, Allen et al. 2016) would

help address the importance of CCR2 signaling to HZE effects. Notably, Cherry *et al.* reported increased ICAM-1 expression in male APPswe/PS1dE9 bigenic mice 6.5 months after whole-body exposure to 1 Gy (1 GeV/n) ^{56}Fe particles (Cherry, Liu et al. 2012). In contrast, whole-body proton irradiation (1 GeV) *reduced* ICAM expression in male C57BL/6 mice 3 months after exposure (Sweet, Panda et al. 2014).

Peripheral immune responses and inflammation can have dramatic effects on brain function. This is clearly evident in animal models of "sickness behavior" and in anyone experiencing the flu. In these cases, circulating inflammatory factors signal directly, or indirectly, to modify hypothalamic and other brain responses. CNS radiation effects can be exacerbated by peripheral inflammation. For example, thermal injury was shown to exacerbate low-LET radiation effects on cognitive performance and neuroinflammation (Cherry, Williams et al. 2013). Thus, it is important to consider possible synergies between particle radiation and injury or infection experienced by astronauts during space flight. Conversely, recent evidence suggests that alterations in peripheral immune responses, including T cell and myeloid cell populations residing in the brain's meninges, can have beneficial effects on CNS function (Kipnis 2016, Herz, Filiano et al. 2017). Although the effect of radiation on such populations has not been explored, the opportunity to manipulate peripheral immunity to improve brain function or resistance to radiation injury offers novel approaches for mitigation of space radiation CNS effects.

6.2.3 Oxidative sSress

A hallmark of radiation injury is oxidative stress invoked by the immediate and persistent effects of ionizing radiation. In addition to direct oxidative damage to nucleic acids, proteins and

lipids, alterations in mitochondrial metabolism and activation of inflammatory pathways that produce reactive nitrogen and oxygen species (RNS and ROS, respectively) can persist for many months. Indeed, oxidative stress has been posited as a major contributor to late radiation effects and multiple strategies to prevent or mitigate oxidative injury have been tested to prevent such effects (Zhao and Robbins 2009, Hladik and Tapio 2016).

Exposure of cultured rat hippocampal precursor cells to ^{56}Fe (1000 MeV/n) particles across a range of doses, led to increased oxidative stress measured by increased fluorescence using an RNS/ROS sensitive dye (Limoli, Giedzinski et al. 2007). Changes at 6 h were particularly prominent with 1 Gy, but also occurred at 0.25 and 0.5 Gy; increased ROS persisted for at least 48 h. Signals were reduced when irradiated cells were treated with the antioxidant α-lipoic acid (Limoli, Giedzinski et al. 2007). In similar experiments using human derived hippocampal precursor cells, 250 MeV protons at doses as low as 0.1 Gy increased oxidative stress using the same fluorogenic dye; such changes were especially prominent at the latest time point examined (48 h) and occurred with both high and low dose rates (0.25-0.50 Gy/min vs. 0.20-0.25 Gy/h) (Tseng, Lan et al. 2013). Studies with other fluorogenic dyes suggested that both nitric oxide and superoxide ions contributed to overall increases in oxidative stress. Interestingly, hippocampal neural precursor cells derived from mouse brain did not show consistent RNS/ROS increases following proton irradiation (Azzam, Jay-Gerin et al. 2011; Tseng, Lan et al. 2013). Using primary neural stem and precursor cells isolated from mice and grown as neurospheres, Tseng *et al*. examined the effects of low dose ^{56}Fe (600 MeV/n) particle irradiation. These studies showed dose-dependent elevation of RNS/ROS signals with doses as low as 0.02 Gy (range 0-0.15 Gy), 12 and 24 h post-irradiation, with evidence of effects persisting for as long as 8 weeks with somewhat higher doses (Tseng, Giedzinski et al. 2014).

These findings, along with other experiments using low fluence beams, demonstrate that neurospheres are remarkably sensitive to heavy ion irradiation (Tseng, Giedzinski et al. 2014). Mechanisms underlying observed effects of particle irradiation, particularly at low fluences, has been previously reviewed (Li, Gonon et al. 2014).

Indications that oxidative stress might contribute to HZE radiation effects include studies using dietary supplements with antioxidant properties. For instance, male Sprague-Dawley rats fed diets containing fruit extracts for 8 weeks prior and after irradiation with 1.5 Gy ^{56}Fe particles (1000 MeV/n) performed better in the Morris water maze task than rats fed control diets (Shukitt-Hale, Carey et al. 2007). Interestingly, KCl-induced release of dopamine from striatal slices of the fruit-fed rats did not show such a radiation-induced decrease (Shukitt-Hale, Carey et al. 2007). One caveat to such studies is that fruit extracts also contain anti-inflammatory activity and may involve other possible mechanisms of action, raising challenges about attribution of benefits to antioxidant properties.

Using 10-week old male C57BL/6 mice pretreated with the antioxidant α-lipoic acid 30 min prior to irradiation with 1.5 Gy ^{56}Fe ions (500 MeV/n), Manda *et al.*, showed improved retention of Morris water maze training 30 days post-irradiation (Manda, Ueno et al. 2008). At this time point harvested cerebellum showed evidence of DNA damage, oxidized lipids, and oxidized proteins that were each attenuated in tissue from mice that received α-lipoic acid (Manda, Ueno et al. 2008). In contrast to these findings, Haley *et al.*, failed to demonstrate evidence of oxidative damage (3-nitrotyrosine or lipid peroxidation) in hippocampal or cortical tissue of C57BL/6 mice of both sexes whole-body irradiated with 0.1, 0.2 or 0.5 Gy ^{56}Fe (600 MeV/n), about 4 weeks after exposure (Haley, Yeiser et al. 2013). Moreover, mice fed a diet containing α-lipoic acid showed no benefits in performance on a novel object recognition task

(Haley, Yeiser et al. 2013). Whether the lack of evidence for oxidative changes at these lower doses reflect a true difference in tissue responses and/or the requirement of ROS for normal cognitive performance is difficult to conclude. Consistent with a role for oxidative stress in radiation-induced cognitive injury, EUK-207, a superoxide dismutase and catalase mimetic designed to scavenge reactive oxygen species (ROS) (Doctrow, Huffman et al. 2002), reduced ^{137}Cs-induced cognitive injury; mice undergoing head-only irradiation (^{137}Cs, 15 Gy) or sham-exposure were treated with EUK-207 one day after exposure and underwent cognitive testing 3 months later using a water maze test in order to assess hippocampus-dependent spatial learning and memory (Raber, Davis et al. 2017). There were significant detrimental effects of irradiation on cognitive performance which were prevented in animals treated with EUK-207. In these studies, EUK-207 reduced hippocampal 3-nitrotyrosine (3-NT) staining, a marker of peroxynitrite-mediated oxidative modification of proteins. Importantly, EUK-207 had no detrimental effects in sham-irradiated mice, which is important since ROS are, as indicated above, required for normal synaptic plasticity and learning and memory.

The complex role of ROS in cognitive performance in the context of irradiation is illustrated in studies in which the generation of ROS was assessed in hippocampal slices of cognitively tested and irradiated mice using dihydroethidium, a fluorescent dye that is oxidized by superoxide into the stable dihydroxy ethidium (DHE). In one study, apolipopotein E2 and E4 female mice received sham-irradiation or cranial irradiation (^{137}Cs,10 Gy) at 8 weeks of age and a standard mouse chow or a diet supplemented with α-lipoic acid starting at 6 weeks of age; behavioral and cognitive performance of the mice were assessed 12 weeks later, and subsequently, the generation of ROS in hippocampal slices was analyzed (Villasana, Akinyeke et al. 2017). Compared to sham-irradiated E4 mice, irradiated E4 mice showed enhanced spatial

memory in the water maze. This was associated with increased hippocampal phorbol-12-myristate-13-acetate (PMA)-induction of ROS. Similar effects were not seen in E2 mice. Irradiation increased hippocampal ROS levels in E2 mice while decreasing those in E4 mice. In addition to the levels of ROS and oxidative damage, it is also important to assess the enzymes involved in generating ROS and the antioxidant response as they may be potential therapeutic targets to mitigate the radiation response. For example, NADPH activity and MnSOD levels were higher in sham-irradiated E2 than E4 mice. Irradiation increased NADPH activity and MnSOD levels in hemi brains of E4 mice but not in those of E2 mice. In another study from the same group, the generation of ROS in hippocampal slices of mice irradiated at BNL with head-only ^{56}Fe ion irradiation (600 MeV/n, 3 Gy) was assessed (Villasana, Weber et al. 2016). Independent of the radiation condition, E4 mice had greater anxiety-like and conditioned fear behaviors than WT mice, and these genotype differences were associated with greater levels of ROS in E4 than WT mice.

In E4, but not WT, mice, hippocampal slices treated with PMA showed more DHE oxidation in sham-irradiated than in irradiated mice and hippocampal heme oxygenase-1 (HO-1) levels were higher in irradiated than sham-irradiated E4 mice. HO-1 catalyzes the degradation of heme to generate carbon monoxide, biliverdin and free iron (Dwyer, Nishimura et al. 1992, Vincent, Das et al. 1994). Increased HO-1 levels are seen in many neurological conditions associated with increased oxidative stress and inflammation, as well as following X-Ray irradiation (Rugo and Schiestl 2004). While elevated HO-1 levels can restore redox homeostasis and reduce inflammation (Ndisang and Jadhav 2009), excessive heme degradation may result in toxic levels of CO, bilirubin, and iron (2Cuadrado and Rojo 2008). Thus, alterations in DHE oxidation might be related to changes in HO-1 levels. With regard to the role of ROS in the CNS

radiation response, it is important to distinguish the generation of ROS, which is required for normal brain function, from oxidative damage. Additional studies exploring the role of oxidative stress, the generation of ROS and oxidative damage, on endpoints relevant to low dose space irradiation are clearly warranted.

Two-month old male Sprague-Dawley rats exposed to doses of ^{16}O particles (1000 MeV/n) ranging from 0.05 to 1 Gy showed dose-dependent decreases in hippocampal protein kinase C (PKC)-α and increased expression of NF-κB and GFAP levels at 36 h and 75 days post-irradiation; the authors interpret these findings as evidence of both oxidative and inflammatory responses (Poulose, Bielinski et al. 2011). Notably, changes were also observed in levels of several proteins associated with autophagy, especially at the earlier time point, suggesting radiation-induced inhibition of this important process (Poulose, Bielinski et al. 2011).

6.2.4 Synaptic sSgnaling and Neurotransmitter Systems

Electrophysiological studies, which provide detailed quantitative data about neuronal properties and connections related to synaptic plasticity, have also been used to investigate CNS effects of HZE exposure. Because of its unique and relatively simple, well-characterized architecture, as well as its important roles in normal CNS function and disease, the hippocampus is often the subject of electrophysiological investigation. Such studies include examination of local field potentials generated following stimuli as well as individual neuron recordings using patch clamp techniques. Both approaches have been applied to hippocampal slice preparations from mice irradiation with HZE particles or protons.

One of the most common investigated hippocampal pathways is the connection between CA3 and CA1 pyramidal neurons known as the Schaffer collaterals. Tetanic stimulation of this

pathway can result in a phenomenon termed long-term potentiation (LTP) in which the magnitude of a local field response to a specific stimulus increases above the pre-tetanic (baseline) level and remains elevated for hours. LTP arises because of increased synaptic efficacy and is often considered a physiological correlate of learning and memory since signaling between neurons is reinforced. Vlkolinsky *et al.* investigated LTP in hippocampal slices from young (10-week-old) male C57BL/6 mice one month after exposure to 1, 2, or 4 Gy cranial ^{56}Fe (600 MeV/n). Half of the mice in this experiment were treated with a high dose of lipopolysaccharide (LPS; 1 µg/g, given *i.p.*) as an immune stressor 4 h before killing the mice. A dose-dependent increase in synaptic excitability, measured as the magnitude of postsynaptic depolarization (slope of the field excitatory post-synaptic potential), was significant in hippocampi irradiated with 4 Gy, but not observed in hippocampi of LPS treated mice (Vlkolinsky, Krucker et al. 2007). Interestingly, 2 Gy, but not 1 or 4 Gy ^{56}Fe irradiation reduced LTP. A similar effect was seen with LPS treatment alone; however, the combination of 2 Gy and LPS led to a paradoxical increase in LTP (Vlkolinsky, Krucker et al. 2007). In a follow-up study, hippocampal slices were analyzed from ^{56}Fe-exposed mice, all with LPS treatment, at 1, 3, 6, or 12 months post-irradiation. 2 Gy-exposed mice showed reversal of the LPS-associated decrease in LTP at 1 and 3 months that disappeared at 6 and 12 months (Vlkolinsky, Krucker et al. 2008). Moreover, there was a significant decrease in the maximal dendritic excitatory response of CA1 cells from mice treated with LPS at 6 and 12 months post 4 Gy exposure (Vlkolinsky, Krucker et al. 2008). These and other analyses suggest radiation-induced damage to CA1 dendrites at these later time points. This point is further supported by observations that rat hippocampal synaptosomes showed reduced hyperosmotic sucrose-evoked glutamate release and modest changes in NMDA receptor subunits 90 and 180 days after 0.6 Gy ^{56}Fe particles

(1000 MeV/n) (Machida, Lonart et al. 2010). Although only examined using *in vitro* cultures, glutamate uptake by neurons and astrocytes might also be modulated by HZE irradiation, resulting in other effects on synaptic transmission (Sanchez, Nelson et al. 2010).

Electrophysiological assessments following HZE radiation have been made in APP23 transgenic mice, which overexpress the Swedish double amyloid precursor protein (APP) mutation and accumulate amyloid plaques starting at about 6 months of age. In addition to behavioral alterations and cognitive abnormalities, these mice show age-dependent reduction in synaptic excitability. Anesthetized, young (7-week-old) male APP23 mice were cranially irradiated with 1, 2, or 4 Gy ^{56}Fe (600 MeV/n) and killed for hippocampal slice preparation and electrophysiology at multiple time points ranging from 1 week post-irradiation to 24 months of age (Vlkolinsky, Titova et al. 2010). The major effect of radiation was the appearance of reduced synaptic efficiency for CA1 neurons stimulated via the Shaffer collaterals, as a result of reduced postsynaptic excitability and neuronal output rather than presynaptic excitability. Such changes were most prominent at 9 months of age and occurred for all radiation doses tested; in contrast, age-associated decreases in synaptic efficiency did not become apparent until mice were 14 months of age in non-irradiated controls (Vlkolinsky, Titova et al. 2010). The investigators limited their analyses to electrophysiology; histological studies of plaque deposition or other pathological changes might have corroborated with the early appearance of reduced synaptic efficiency. Nevertheless, these studies highlight possible synergies between space radiation and ongoing pathological processes that may be underway long before clinical disease is noted (Braak, Thal et al. 2011).

In contrast to electrophysiological studies of iron particle irradiation that showed decrements in excitability and LTP, a study using silicon particles at lower doses showed a very

different result. Male C57BL/6 mice were whole-body irradiated with 0.25 or 1 Gy ^{28}Si (600 MeV/n) at 80 days of age and dorsal hippocampal slices were tested about 3 months after irradiation (Raber, Rudobeck et al. 2014). Interestingly, both doses increased the LTP response above control levels. Moreover, for a subset of mice that had been cognitively tested using contextual fear conditioning, the magnitude of LTP was even greater for the 0.25 Gy exposed mice, who also showed greater percent freezing in the cognitive test (Raber, Rudobeck et al. 2014b). In a parallel study of CA1 pyramidal cells in the ventral hippocampus using slices isolated from the same mice, LTP was not significantly affected by silicon exposure, however, mice irradiated with 1 Gy showed reduced CA1 output and impaired E-S coupling without an effect on dendritic excitability (Rudobeck, Nelson et al. 2014). These findings demonstrate regional differences in dose-dependent radiation effects that may reflect unique circuitry of these two hippocampal areas.

Electrophysiological properties of neurons exposed to protons have been explored in some depth. For example, patch-clamp was used to examine intrinsic membrane properties of CA1 neurons in male C57BL/6 mice, 3 months following 1 Gy proton (150 MeV) exposure (Sokolova, Schneider et al. 2015). Investigators observed reduced neuronal membrane resistance and reduced resting membrane potential (hyperpolarization). These changes in passive membrane properties that reduce neural excitability were counterbalanced by changes in persistent sodium currents and an increased rate of miniature excitatory postsynaptic currents (Sokolova, Schneider et al. 2015). In a similar set of experiments, dentate gyrus granule cells showed increased excitability 3 months after 1 Gy proton exposure associated with decreased inhibitory GABAergic synaptic transmission (Marty, Vlkolinsky et al. 2014). These effects were not observed with similar doses of ^{28}Si or ^{56}Fe (both ~ 600 MeV/n). Interestingly, detailed

examination of GABAergic interneurons in the hippocampus that control pyramidal neuron excitability, revealed a distinct result. In particular, the cannabinoid type 1 receptor expressing basket cells (CB_1BCs) showed increased GABA release that resulted from decreased 2-arahidonoylglycerol (a CB_1 ligand) five to nine weeks after 0.5 Gy 150 MeV proton exposure in male C57BL/6 mice at 2-3 months of age (Lee, Dudok et al. 2017). The specificity of this effect was reinforced by an absence of radiation effect on GABA release from parvalbumin-expressing, fast spiking interneurons (PVINs), the other major inhibitory interneuron providing input to hippocampal pyramidal cells. Interestingly, proton irradiation modified hippocampal circuitry with evidence of increased connections between CA1 pyramidal cells and PVINs, but not CB_1BCs (Lee, Dudok et al. 2017). Taken all together, these studies exploring effects of protons show region specific effects, highlighting the complex responses of brain to radiation.

Wild type and APPswe/PS1dE9 double transgenic mice harboring two mutant transgenes associated with familial Alzheimer's disease on a mixed strain background were exposed to 142 MeV protons at 3 months of age using a dose range up to 1 Gy. Behaviorally, transgenic mice performed worse on a water maze than wild type mice at 3 and 6 months, but effects of radiation were not observed except in a reversal task at 6 months in wild type mice (Rudobeck, Bellone et al. 2017). Electrophysiological analysis of hippocampal preparations at 9 months showed distinct radiation effects: whereas wild-type mice showed increased synaptic efficiency with radiation (0.5 Gy), APPswe/PS1dE9 mice showed decreased PS amplitudes at 0.1 and 1 Gy. In addition, presynaptic glutamate release probability, measured by paired-pulse facilitation, was reduced in APPswe/PS1dE9 mice with 1 Gy proton exposure (Rudobeck, Bellone et al. 2017). Radiation did not affect LTP in either transgenic or wild type mice. Notably, 1 Gy protons increased amyloid deposition in dorsal cortex, 9 months post-irradiation; consistent with results

using iron irradiation in this same transgenic line (Cherry, Liu et al. 2012); but did not influence cytokine levels at this time point (Rudobeck, Bellone et al. 2017).

6.3 Brain Imaging

Neuroimaging is a mainstay diagnostic procedure for neural injury and disease, and reveals clear evidence of radiation damage such as white matter necrosis in patients receiving high-dose radiotherapy. Several imaging approaches, including micro-positron emission tomography (PET) and magnetic resonance imaging (MRI), have been used to evaluate rodent brains following HZE exposure. In one study, male Sprague-Dawley rats were head-only irradiated with 1.2 or 2.4 Gy of 600 MeV/n ^{56}Fe particles and analyzed for [^{18}F]2-deoxy-2-fluro-D-glucose (FDG) accumulation (Rice, Saintvictor et al. 2006). Radiation failed to modify brain FDG uptake in any region analyzed (striatum, thalamus or hippocampus) measured *in vivo* at 6 or 9 months post-irradiation with microPET or *ex vivo* at 9 months using autoradiography (Rice, Saintvictor et al. 2006). The authors concluded that this approach was not sensitive to detect changes associated with HZE exposure.

In a series of studies, Obenaus and colleagues carried out dose and time-dependent MRI analyses of male Sprague-Dawley rats exposed to iron particles. For all studies, rats were irradiated at approximately 2 months of age using head collimated beams of 600 MeV/n ^{56}Fe and doses of 1, 2 and 4 Gy. In the first report, MRI analyses conducted 1 month post-irradiation showed no overt anatomical changes, but quantitative regional analysis of the hippocampus showed a modest rise in T2 signal only for the 2 Gy dose, and a clear increase of the diffusion weighted image (DWI) signal known as apparent diffusion coefficient (ADC), which is related to tissue water mobility, at all tested doses, with ADC at 1 Gy being the highest (Obenaus, Huang

et al. 2008). Proton magnetic resonance spectroscopy revealed an increase in the N-acetylaspartate/choline ratio and a lactate peak at 4 Gy, suggesting possible compensatory changes to radiation exposure (Obenaus, Huang et al. 2008). Histological analyses of brain tissues revealed no overt pathology, but some evidence of microglia morphology changes at all doses tested (Obenaus, Huang et al. 2008). In a follow-up study examining MRI signals at one-week post-irradiation, these investigators reported similar increases in T2 relaxation time for all doses tested in 3 different brain regions (hippocampus, entorhinal cortex and thalamus), but decreased ADC values, which contrast with their observations at 1 month (Huang, Smith et al. 2009). Interestingly, ADC values for rats exposed to 1 and 2 Gy fell below control levels at 3 months post irradiation, but MRI analyses of rats at 6, 9, 12, and 18 months did not show clear divergence of T2 and ADC values from controls (Huang, Smith et al. 2010). Based on histological analyses of iba-1-immunoreactive microglia and GFAP-immunoreactive astrocytes, the authors described an inverse relationship between ADC and glial activation across the time points analyzed (Huang, Smith et al. 2010); however, additional studies would be required to establish such a relationship. Given the doses used in these studies and the dynamic changes observed across time (including those observed in control rats), it is not clear how useful current imaging approaches will be for analyses at more space-relevant doses.

6.4 Use of Unbiased Approaches and Large Data Sets, Including 'Omics, to Assess Pathways Involved in Effects of Radiation on the Brain.

Most analyses to assess pathway changes following environmental challenges such as radiation exposure involve biased analyses of changes in a particular pathway or alterations in the level or activation state of small subsets of proteins or mRNAs. A limitation of these biased

approaches is that there might be alterations as a result of radiation exposure that are missed. So, in case significant changes are seen, the pattern might not be complete and there might be other changes that are important to appreciate for risk assessment and mitigation purposes. In case no changes are seen, it is conceivable that they might exist and are simply missed. This would be a particular concern in assessing risk to the brain following space irradiation. We recognize that it would not be realistic to analyze pathway changes in every single brain area or under all conditions, but use of a non-reductionist and unbiased approach in at least one brain area pertinent to behavioral and/or cognitive performance of astronauts during missions is critical to determine CNS risk. The power and potential of a systems biology approach for understanding pathway changes under physiological and pathological conditions and following environmental challenges, including the biological response to radiation exposure (Feinendegen, Hahnfeldt et al. 2008), has been appreciated, although the analytical opportunities can be associated with methodological challenges as this is a relative young area of science (Barcellos-Hoff and Costes 2006, Arnold, van Nas et al. 2009, Dinov 2016). For example, a systems radiobiology approach has been used to generate personalized data-driven risk profiles for radiotherapy outcomes following prostate radiotherapy (Coates, Souhami et al. 2016) and computational oncology involving genotyping and modeling approaches (El Naqa, Kerns et al. 2017). Similarly, 'omics approaches are being used for increased understanding of mechanisms underlying various neurodegenerative conditions, including AD and PD, as reviewed and discussed during the 2018 Alzheimer's Disease Research Summit organized by the NIA (https://www.nia.nih.gov/research/nih-ad-summit-2018-program-agenda).

A proteome analysis using an ion mobility-enhanced data-independent acquisition (MS(E))-based label-free quantitative proteomic analysis of hippocampal tissue was carried out

with low-dose whole body ionizing radiation (X-ray, 1 Gy) of 5.5-month-old male C57BL/6J mice after contextual fear conditioning training (Huang, Wickramasekara et al. 2016). Deregulated proteins indicated adverse effects of irradiation on myelination and perturbation of energy metabolism pathways involving a shift from the TCA cycle to glutamate oxidation. In addition, proteins associated with synaptic activity, including vesicle recycling and neurotransmission, were altered in the irradiated mice (Huang, Wickramasekara et al. 2016). A follow up study used the same hippocampal tissues for an unbiased lipidomics analysis based on a workflow that combined untargeted ion mobility-enhanced data independent mass spectrometry coupled with liquid chromatography (UPLC-HDMSE). Following irradiation, there was a significant accumulation in the levels of diacylglycerols, phosphatidic acids, glycosylated ceramides (HexCer), sulfatides (ST), and phosphatidylethanolamine plasmalogens (PlsEtn) (Zandkarimi, Morré et al. 2016). In contrast, ceramides and phosphatidylethanolamines levels were significantly decreased following irradiation (Zandkarimi, Morré et al. 2016). Due to their important roles in hippocampal cognitive function, increased levels of ST and PlsEtn are especially interesting. A proteomics approach also revealed that canonical pathways for Huntington's, Parkinson's, and Alzheimer's disease were acutely affected by radiation within 72 h of 20 Gy whole brain X-Ray irradiation (Shukla, Shankavaram et al. 2015).

Proteomics are also being used in the context of space irradiation in rats that showed intact and impaired spatial memory three months following exposure to ^{56}Fe (1 GeV/n, 0.2 Gy) (Britten, Jewell et al. 2017). Proteins upregulated in the irradiated rats, irrespective of their spatial memory performance status, included proteins involved in oxidative damage response, calcium transport and signaling. In addition, expression of various proteins correlated with impaired spatial memory performance and are implicated in poor spatial memory performance,

neurodegeneration, neuronal loss or neuronal susceptibility to apoptosis, or neuronal synaptic or structural plasticity (Britten, Jewell et al. 2017).

C57BL/6-lacZ mice (male and female animals between 6 – 12 wks of age) were exposed to a range of low dose-rate simulated solar particle event (sSPE) radiation at the NSRL. The hippocampus was surveyed for differential transcriptional regulation of genes known to be associated with neurogenesis. Results showed differential expression of neurotrophin and their associated receptor genes within 2 weeks after a single 0.5 or 1 Gy of sSPE exposure (Chang, Doppalapudi et al. 2010). Progressive changes in the profile of expressed genes known to be involved in neurogenic signaling pathways were dependent on the sSPE dose. On the other hand, alteration in the profile of genes known to be involved in neurotrophic functions in the hippocampal tissue appears to persist for up to 8 weeks after radiation exposure (Chang, Doppalapudi et al. 2010). Such temporal changes confirm that, although cytogenetic changes measured from peripheral blood samples taken from the same animals after a single dose of sSPE protons appear to be transient, the impact of this exposure is sufficient to lead to persistent dynamic changes in neuronal tissues long after the initial radiation exposure.

A number of neurotrophic/neuropeptide and synaptic transmission pathway associated genes were altered by > 2-fold within 2 week after 0.5 Gy radiation exposure. Such changes were normalized against sham-treated animals from the same study. Although the impact of the altered expression of these pathway specific genes are unknown at this time, nevertheless, such changes suggest that a single acute sSPE exposure of 0.5 or 1 Gy was sufficient to perturb the gene-expression profiles of these tightly regulated neuronal pathways.

Radiation quality and sequence of mixed beam exposures impacts differential gene expression associated with neurotrophin and receptors in the hippocampus. In a study where

C57BL/6 mice were exposed to a single dose of 1 Gy 1 GeV/n Iron ions, or a single dose of 1 Gy 250 MeV protons, or 50 cGy of iron ions followed by 50 cGy of protons, or 50 cGy of protons followed by 50 cGy of iron ions separated by 2.5 h between doses, the gene expression profile from hippocampus isolated from these animals 1 week post irradiation showed that most of the neuotrophin and their receptor genes were down regulated after a single dose of iron, or protons or iron followed by protons. In contrast, tissue harvested from animals that were exposed to protons first followed by iron ions showed that most neurotrophin and their receptors, neuropeptide and their receptors, neurogenesis, immune responsive, cell growth and differentiation genes were upregulated (presented at Port Jefferson, NY, NASA investigators' workshop, 2010).

Unbiased approaches are also being used to assess epigenetic mechanisms involved in the response to HZE exposure. For example, hippocampal changes in cytosine methylation, a major epigenetic modification involving the addition of a methyl group to cytosine (5mC), play a key role in regulating expression of genes required for spatial learning and memory (Miller and Sweatt 2007, Lubin, Roth et al. 2008). A second form of DNA methylation, hydroxymethylcytosine (5hmC), is derived from 5mC by the action of three ten-eleven translocation enzymes (TET 1-3) (Chen, Dzitoyeva et al. 2012). The levels and clear relationship between changed gene expression and one form of DNA methylation (5hmC) after exposure suggest altered gene ontology and KEGG pathways that account for altered cognitive function. 5mC and 5hmC levels are high and exceptionally dynamic in the brain; their high and dynamic levels in neurons during development and aging (Jin, Wu et al. 2011, Szulwach, Li et al. 2011) suggest strongly that they play critical roles in CNS function.

There is a fundamental epigenetic remodeling for a subset of genes with critical neuronal functions and other cellular functions as a result of HZE exposure. Hippocampal DNA hydroxymethylation, which is correlated with gene expression, is affected 4 (Impey, Jopson et al. 2017) and 22 (Impey, Pelz et al. 2016b) weeks following proton irradiation (150 MeV/n, 1 Gy). Significant overlap was observed between DNA methylation changes at the two time points, demonstrating specificity and retention of changes in response to radiation. The radiation-induced changes were mapped to genes encoding neuronal functions including post-synaptic gene ontology categories. Thus, the brain's response to proton irradiation is both specific and prolonged and involves novel remodeling of non-random regions of the epigenome. Analysis of hippocampal DNA methylation data also revealed epigenetic remodeling of a subset of genes with critical neuronal functions within four weeks of exposure. Genes that are differentially-regulated in the synapse category at the early time point included the GABA receptor. Interestingly, 5-9 weeks after proton irradiation (150 MeV, 0.5 Gy) of 2-3 month-old C57BL/6J mice, there was increased GABA release from the cannabinoid type 1 receptor (CB1)-expressing basket cells (CB1 BCs) onto pyramidal cells (Lee, Dudok et al. 2017).

In addition to proton irradiation, ^{56}Fe ion irradiation also affects DNA methylation in the hippocampus (Impey, Jopson et al. 2016a). Comparison of changes in DNA methylation following irradiation with protons and ^{56}Fe showed an overlap in changes in distinct pathways, demonstrating a shared radiation signature. Gene ontology analyses revealed categories linked to cell adhesion, cell junctions, neuronal growth, and synapse function. The comparison of protons and ^{56}Fe ions also showed changes in distinct pathways, demonstrating a radiation quality effect.

As described earlier and reported (Impey, Jopson et al. 2016a), while 0.1 Gy of ^{56}Fe (600 MeV/n) impaired object recognition in mice at the early time point, 0.2 Gy did not. For increased

RNA transcripts, there was more enrichment in pathways for the 0.2 than the 0.1 Gy dose. This observation is consistent with the dose-dependent pattern in cognitive injury and suggests a compensatory mechanism by which the greater response to radiation damage at 0.2 Gy prevents the cognitive impairment observed at 0.1 Gy. For decreased RNA transcripts, there was a highly significant enrichment for categories linked to neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease (PD), and Huntington's disease, and the decrease was greater at the 0.1 Gy dose; again, this is consistent with increased cognitive impairment at this dose. This effect was transient. Indeed, for the late time point there was no significant enrichment for categories linked to neurodegenerative disease, nor were cognitive impairments observed (Impey, Jopson et al. 2017).

The proton and ^{56}Fe data highlight the potential risk of developing transient and chronic mission-critical cognitive impairments during space missions. While the detrimental effects on cognitive function might be the same, the pathways responsible for these changes after exposure to different particles might be similar or very different. This is especially a concern in the context of planned long-term space missions, such as those to Mars, because astronauts will be exposed to different radiation qualities during a space mission. Additive or even synergistic effects on pathway changes that result in more profound and longer-term effects on the brain might occur. The long-term detrimental effects of proton irradiation on hippocampal function are remarkable, considering that the effects of ^{56}Fe ion irradiation were more pronounced at 2 rather than 20 weeks following exposure. Post-synaptic synapse remodeling might be particularly important in these effects. In the HZE studies, a novel class of DNA methylation change was observed characterized by both increased and decreased 5hmC levels along the entire gene body (the region spanning introns and exons together). These changes were mapped to genes encoding

neuronal functions including postsynaptic gene ontology categories. At this moment, it is unclear whether this kind of DNA methylation change is unique for the response to HZE exposure.

To determine effects of ^{28}Si ion (600 MeV/n, 0.3 Gy) on hippocampal DNA methylation, cytosine methylation (5mC) and/or cytosine hydroxymethylation (5hmC)-DIP-Seq libraries were generated from the hippocampi of mice 4 weeks after exposure to ^{28}Si ion irradiation or sham-irradiation; 1060 differentially methylated regions (DMRs; $p < 0.01$) and 1531 differentially hydroxymethylated regions (DHRs; $p < 0.01$) were identified (Raber, Rosi et al. 2018). Similar to the observations 4 weeks following proton irradiation, only genes that were associated with significantly-increased 5hmC were enriched for gene ontology categories and KEGG pathways linked to neuronal function (FDR-adjusted $p < 0.001$). When ^{28}Si ion irradiated gene-associated DHRs were compared with previously published proton and ^{56}Fe ion data by selecting the top 1500 DHRs from all data sets (ranked by p value), a highly significant overlap was revealed between ^{28}Si ion DHR-associated genes and the analogous ^{56}Fe and proton DHR-associated genes (Raber 2018, Raber, Rosi et al. 2018). Remarkably, the overlap between the ^{56}Fe and ^{28}Si sets was more significant (Fisher exact, $p < 1x10^{-99}$) than the overlap with the proton data (Fisher exact $p < 2x10^{—55}$). The significant overlapping set of DHRs was used to analyze the subset of DHR-associated genes that were in common to all three forms of radiation exposure;176 identified genes that were associated with significantly increased hydroxymethylation in response to all three forms of space irradiation (Permutation test, $p < 1x10^{-12}$) and these genes showed significant enrichment for overlapping gene ontology pathways linked to presynaptic vesicle fusion and Rab/Rho GTPase activity (Raber, Rosi et al. 2018). Related to assessment of CNS risk, these genes are linked to severe neurological disorders, including AD, Amyotrophic lateral sclerosis (ALS) and Zellweger syndrome, also called cerebrohepatorenal syndrome.

Zellweger syndrome, one of a family of disorders called leukodystrophies, is a congenital disorder characterized by the reduction or absence of functional peroxisomes in the cells of an individual. Importantly, considering the timing between radiation exposure and hippocampal tissue analysis, the pathways are related to conditions we would anticipate to be occurring as late effects (following the mission) rather than acute effects, highlighting that heavy ion exposure likely has long-term consequences.

One set of proteins identified in the ^{56}Fe-^{28}Si-proton signature are Tre-2/Bub2/Cdc16 (TBC) 1D proteins. Strikingly, TBC1D1 has been identified as an ionizing radiation-induced gene in multiple studies (Yin, Nelson et al. 2003, Paul and Amundson 2008, Niu, Qin et al. 2010) and TBC1D4, TBC1D5, and TBC1D9 have also been identified as radiation-induced genes in single studies (Xu, Gao et al. 2008, Godoy, Mello et al. 2013). TBC1D9 was reported to be upregulated in radiation-associated tumors that developed many years after exposure, suggesting a long-term epigenetic mechanism (Godoy, Mello et al. 2013). Humans have about 40 different TBC proteins. TBCD1 and D4 are best known and both linked to disease. TBC often functions as a specific Rab/Rho GTPase activity and are essential for intracellular and membrane transport and signal transduction. However, TBC proteins likely have additional functions that are not known yet.

This shared radiation signature is also of interest in the context of ongoing mixed ion beam exposure studies. The profound phenotype seen with those genes confirms the potential of this approach to identify genes and pathways involved in the CNS space irradiation response that are critical for CNS function. The synapse category was also highly significant.

Consistent with a role for DNA methylation in effects of space irradiation on cognitive performance, mice impaired in object recognition 4 weeks following ^{28}Si ion irradiation (600

MeV/n, 0.2 Gy), showed an increase in 5-methylcytosine and 5-hydroxymethylcytosine levels in the hippocampus that coincided with increased levels of the DNA methylating enzymes DNA methyltransferase DNMT3a, TET1 and TET3 (Acharya, Baddour et al. 2017).

Besides using a single 'omics approach to identify pathway changes, data from two 'omics approaches can be integrated for obtaining converging evidence. For example, DNA methylation and metabolism are tightly linked, with crosstalk at the molecular level (Chiacchiera, Piunti et al. 2013). Data integration of these two approaches was used to determine the overlap in pathways identified by the 5hmC and metabolomics analyses, using a platform that maps changes against established KEGG pathways (Johnson, Torres et al. 2017), in a study using a high-fat diet as environmental challenge (Johnson, Torres et al. 2017). Similar data integration based on more than one 'omics approach could be used to assess pathways changes in the brain following space irradiation.

The identified pathways, particularly those identified as part of a shared HZE radiation signature, are critical for understanding the mechanisms underlying detrimental CNS effects of space irradiation that are associated with and likely contribute to cognitive injury. The shared pathways are also important with regard to current interest in assessing effects of mixed beam space irradiation. Based on the data described above, one would hypothesize additive and potentially synergistic effects involving these pathways. Further, this radiation signature is of great help in selecting potential mitigators against HZE radiation-induced cognitive injury out of either the pool of FDA-approved drugs or compounds that are not yet approved, but are anticipated to be beneficial based on their ability to affect the shared pathways involved in the HZE radiation response of the CNS.

Interestingly, based on RNAseq data a shared signature was recently reported in the cerebral cortex of patients with autism, bipolar disorder, and schizophrenia (Gandal, Haney et al. 2018). Four sets of genes were less active in neurons, one set of genes was more active in astrocytes, and one set of genes was hyperactive in microglia. So, one could envision how a shared HZE radiation signature could be compared with signatures, not only of individual conditions, but also shared signature of various neurological conditions that could be of further help in assessing CNS risk to space radiation and identifying optimal mitigators astronauts could use during space missions.

Finally, significant technological advances provide new ways for carrying out unbiased approaches. For example, it is now possible to establish transcriptomic profiles for individual cells, which creates a quantitative profile response diversity across a population of cells. For example, single cell RNA sequencing of microglia led to identification of a unique microglial type associated with Alzheimer's disease (Keren-Shaul, Spinrad et al. 2017), and single cell sequencing of the neurogenic niche has revealed unique niche-associated signatures for neural precursors and non-neuronal cells (Artegiani, Lyubimova et al. 2017). In addition, recent advances in single cell whole-genome sequencing provide a means to follow accumulating somatic mutations in neural cells associated with disease and development (Bae, Tomasini et al. 2018, Lodato, Rodin et al. 2018). Application of these new technologies to space irradiation is likely to provide new insights regarding the complexity of brain cell responses.

The gut microbiome can communicate with the CNS via the gut-brain axis, and affect stress-related behaviors, anxiety, and depression (Foster and McVey Neufeld 2013, Kelly, Kennedy et al. 2015, Allen, Dinan et al. 2017). The gut microbiome also affects cognitive performance in the water maze (Jorgensen, Hansen et al. 2014) and sensorimotor and fear

learning following exposure of mice to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Raber et al., oral communication). In 4-6 month-old female and male B6D2F1 mice two month following exposure to mixed beam space irradiation, consisting of 20 percent 250 MeV ^{16}O ions, 20 percent 240 MeV/n ^{28}Si ions, and 60 percent 1 GeV protons at 25, 50, or 200 cGy, the gut microbiome is affected (Raber et al., oral communication=). As the gut microbiome could be noninvasively monitored in astronauts during missions, increased understanding of relationships between the gut microbiome and CNS function might be particularly valuable.

6.5 Future Directions and Recommendations

Multiple mechanisms involving different cell types and responses have been investigated in the context of HZE particle irradiation. Many of the mechanisms explored are well established responses to higher dose, low LET radiation, and current evidence suggests that high LET radiation can elicit such responses, including inhibition of neurogenesis, oxidative stress and neuroinflammation. As experimental doses have decreased and studies have moved from using head-only brain irradiation to whole body radiation studies, there is evidence that some mechanisms (*e.g.*, inhibition of neurogenesis) may not occur below certain thresholds and therefore may be less relevant for the low dose and low dose rate radiation environment in space. Nevertheless, it is difficult to dismiss the possibility that subthreshold cumulative doses may impact these or related processes and contribute to deleterious effects on behavioral and cognitive tasks. At this point, little is known about effects of mixed beam space irradiation and effects of chronic exposures. One of the biggest challenges has been linking radiation-induced cellular and/or molecular responses to radiation-induced behavioral alterations and deficiencies in cognitive performance. Emerging evidence that HZE radiation can reduce connections

between neurons through effects on dendritic arbors and dendritic spine density is consistent, and indeed correlated, with reduced cognitive performance, and is therefore a promising area for further investigation. However, the underlying mechanisms for such changes are currently not known and may well involve converging tissue responses rather than any single process. To assess these mechanisms, unbiased large data 'omics studies are critical. The current state of knowledge provides both challenges and opportunities for preventing or mitigating the negative impacts of particle radiation exposure; at one extreme, there may be redundant processes and pathways that each have negative impacts on performance, at the other extreme, intervention in any one of several involved pathways may be sufficient to provide benefit. In consideration of our current knowledge, the following recommendations for mechanistic studies are offered. Note that some of these overlap with recommendations made for the behavioral and cognitive studies because of their importance for work with animal models.

1. Use chronic exposures to complement the acute exposures and fractionated doses used so far. Technically this is not trivial but from a biological perspective the effects of an acute versus a chronic exposure might be very different.

2. Increase use of mixed beam exposures as this more closely mimics the space radiation environment and the interactive effects of different particles is largely unknown.

3. Consider CNS effects in the context of HZE radiation interactions with physiological systems, such as the cardiovascular and immune systems, which are intimately linked to proper brain function.

4. Encourage studies that attempt to correlate mechanistic and behavioral and cognitive endpoints.

5. Encourage comparisons between females and males in space radiation-related studies.

6. Test specific mitigating or preventative strategies to move the field from description to hypothesis testing.

7. Encourage application of new tools in neuroscience such as high density *in vivo* recordings, optical imaging, optogenetics and pathway tracing to probe particle radiation effects beyond local circuits.

8. Encourage studies combining behavioral and cognitive assessments with unbiased "omic" strategies to determine pathways altered following HZE irradiation and gain insight into mechanisms underlying space radiation effects and to guide the selection of mitigators for space radiation-induced CNS injury.

7. Applications of Mechanistic Models

7.1 Applicability of Adverse Outcome Pathway Approach

7.1.1 Definition/Framework of AOP

The adverse outcome pathway (AOP) framework represents a conceptual linkage of biological pathway(s), which when sufficiently disturbed can lead to an adverse outcome that can be used for regulatory purposes (Ankley *et al.*, 2010; Garcia-Reyero, 2014; Villeneuve *et al.*, 2014a; Vinken, 2013). An AOP consists of a series of key events (KEs; measurable biological changes), which are linked together by key event relationships (KERs). The most recent version of the AOP framework introduces event components, serving as structured representations of KEs (Ives *et al*., 2017). There can be one or more event components per KE, and the event components will be described using a set of pre-defined biological ontologies. The interaction of a stressor (chemical or non-chemical factor) with a biological system at the molecular level is known as the molecular initiating event (MIE), and represents the first KE of the system (Allen *et al.*, 2014, 2016). The MIE, when of sufficient magnitude and duration, results in changes (KERs) if the next quantifiable step (KE) in a series of biological changes. A KER can range in detail from a simple qualitative indication that an increase or decrease in the upstream KE could result in an increase or decrease in the downstream KE, to a more quantitative mathematical relationship between the events that may account for critical adaptive/compensatory biology. Given sufficient perturbation (e.g., exposure to an adequate dose and duration of a contaminant, radiation, etc.), the biological pathway results in an adverse outcome (AO), which serves as the final step in the AOP. The number of KEs that are included in the AOP is a function of the

current state of physiological knowledge of the plausible connections that are involved in producing the AO. The number of KEs and KERs that are required for acceptance of the AOP will depend on the use of the AOP (legal regulations vs. defining knowledge gaps) (Maxwell *et al.*, 2014; Patlewicz *et al.*, 2015; Wittwehr *et al.*, 2016) and may change over time as knowledge of biological relationships increases. Finally, the use of AOPs and structure-activity relationships, have been discussed in the context of avoiding adverse outcomes in the pharmaceutical field (Patlewicz and Fitzpatrick, 2016). As stated in Allen *et al*., (2014) "In the field of human toxicology, the focus is on adverse outcomes, but we do not wish to exclude the use of MIEs in a therapeutic sense, as these may become toxicologically relevant for other chemicals or applications. For example, a beneficial MIE fits well into pharmacological understanding, as does an adverse MIE in overstimulation pharmacology. The distinction between adverse and therapeutic outcomes is less important than the applicability of the term MIE, as we expect the outcomes to be dependent on the dose." Therefore, there is no inherent reason that an AOP could not also be constructed for beneficial outcomes, such as in pharmaceutical treatment of a disease state to improve the health status of the individual (Carusi *et al*., 2018). Finally, although an AOP is often depicted as a linear process, it is understood that for most biological processes, an AOP network is needed for actual accurate predictions of outcome effects (Villeneuve *et al*., 2014a). The importance of factors such as event modifiers, compensatory mechanisms, multiple hit events, effect duration, biological thresholds, etc. has been emphasized (Leist *et al*., 2017). It is through linkages of KEs in multiple AOPs that networks may be constructed.

Development of AOPs is an international effort that is captured in the AOP Wiki located at: https://aopwiki.org/, with guidance provided for how to construct AOPs at: http://www.oecd-

ilibrary.org/environment/users-handbook-supplement-to-the-guidance-document-for-developing-and-assessing-adverse-outcome-pathways_5jlv1m9d1g32-en; OECD, 2013). It is known that interaction of an external stressor with a biological system will result in changes in multiple processes, and that a given KE may be involved in several biological pathways. Best practices for constructing an AOP, which may involve interactions of KEs in multiple pathways, have been published (Villeneuve *et al.*, 2014b). Several principles for development of an AOP are commonly accepted. These are depicted in Figure 7.1. First, an AOP is not chemical specific. An AOP describes the biological response to a perturbation of the MIE. Any stressor that alters the MIE in the same manner should set the same sequence of KEs in action. Second, AOPs are modular in nature. They consist of KEs which are connected by KERs. The number and biological detail in a KE and KER will reflect the current state of biological knowledge. Third, an individual AOP is a pragmatic unit of development and evaluation. Fourth, it is acknowledged that interactions between AOPs, at the level of KE and KERs, form the basis for biological responses. This implies that networks of AOPs will be necessary for accurate prediction of biological outcomes. Finally, AOPs will change over time, as knowledge of biological pathways increases. Additional KE and KERs may be added, or removed, to better reflect the current state of scientific knowledge. Eventually, the steps of a proposed AOP are evaluated using modified Bradford-Hill criteria (Becker *et al.*, 2015; Hill, 1965). These include factors such as: 1) dose-response concordance – are KEs altered at doses less than those that produce the AO? 2) temporal association – do the KEs occur in the expected order? 3) consistency/specificity – is the incidence of the AO less than the KEs? If exposure is stopped, or a KE blocked, is the sequence of events reversible? ; and 4) biological plausibility – are the effects observed over species, strains, and biological systems consistent with the proposed AOP, and does the AOP make sense

with current biological knowledge? Evaluation of the proposed AOP with these criteria can reveal specific portions that may need additional data to strengthen the weight of evidence, leading to a strategy to focus resources for maximal impact on the development of the AOP.

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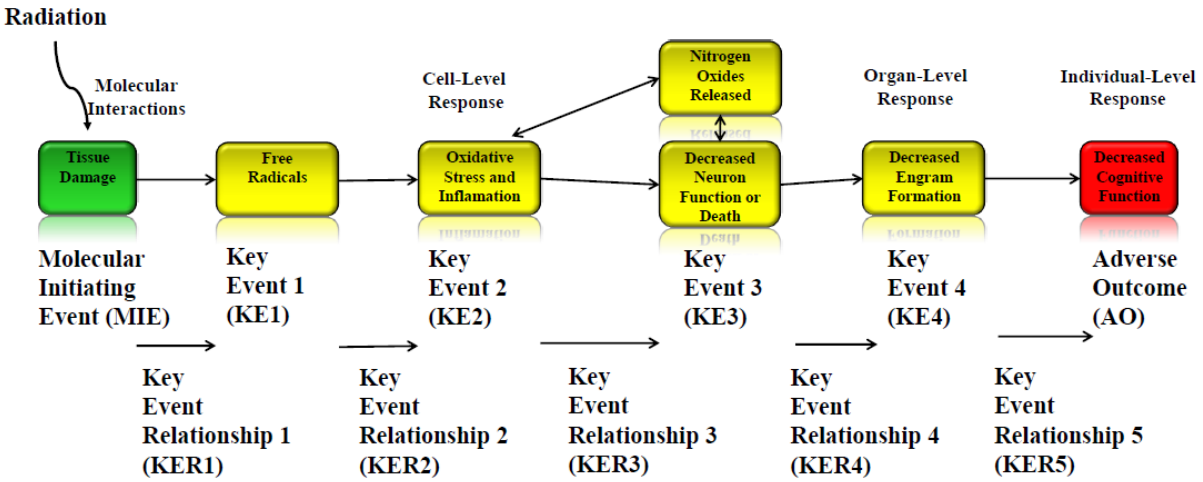

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3961 **Fig. 7.1** Schematic of a hypothetical, simplified, Adverse Outcome Pathway (AOP) showing the
3962 major components of its relationships. The first event of the pathway is the MIE, representing the
3963 initial interaction of the insult with the body at the molecular level. This initial interaction leads
3964 to a series of quantifiable KEs which are related to each other by KERs. Changes will result in
3965 the AO of concern. In this case the nitrogen oxide feedback may be beneficial and actually
3966 reduce the magnitude of the AO as well. Additional key events, feedback loops, and networks of
3967 interacting AOPs are involved in most real-world scenarios.
3968 .

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Development of an AOP can proceed in several directions. If sufficient biological knowledge exists for a specific AO, the AOP can be proposed working through KEs toward the MIE (Kimber *et al.*, 2014). This will allow testing the proposed KEs using the criteria described in the preceding paragraph. A second strategy could include developing weight of evidence for KEs that are believed to be involved in the expression of an AO (Groh *et al*., 2015). An example could include cellular processes that are known to be involved in development of the nervous system that if sufficiently altered, could result in developmental neurotoxicity (Bal-Price *et al.*, 2015, 2017). This approach is initially dependent on existing knowledge of proposed KEs, with experiments designed to support/refute the role of these KEs in the AOP. While this may initially involve a "biased" approach toward which KEs to include/test, the weight of evidence assessment will eventually provide support for the inclusion/exclusion of the proposed KEs. A third strategy can involve the use of data generated by data-rich technologies such as transcriptomics, proteomics, metabolomics, lipidomics, and unbiased epigenetic analyses such as DNA methylation analyses. This approach can be useful when a test species can be exposed to a biological perturbation (toxicant), and a large number of potential KEs can be assessed simultaneously. Computer algorithms can be used to determine enriched nodes (possibly representing KEs) and networks. Mapping the measured changes into biological pathways can lead to the development of putative AOPs (Perkins *et al.*, 2011). This third strategy may lead to pathways and interactions that were not previously recognized as important in an AO. However, replication and the weight of evidence assessment for the KEs and KERs will be necessary to biologically "validate" the AOP. This may lead to a more focused, hypothesis-driven approach, as described in the second strategy above.

Identification of KEs, KERs, and biological pathways that are conserved across species has the potential to allow AOPs to assist with extrapolations between test species and humans (Celander *et al*., 2011; Perkins *et al*., 2013). For example, if a protein is known/proposed to be altered as a MIE for a substance, the primary amino acid sequence of the target protein has been used to assess similarities in the MIE target across species (LaLone *et al*., 2013). This approach has been expanded to a multi-step assessment of conserved functional domains and amino acid sequences in the MIE target (LaLone *et al*., 2016). Identification of critical KEs that are required for the expression of an AO may allow the use of screening tests to prioritize chemicals for further testing (Burden *et al*., 2015). Thus, the knowledge of conserved genes, proteins, and biological pathways may allow the AOP framework to serve as the basis for extrapolation between species and other model systems to humans.

Although the AOP framework was developed around chemically-induced AOs, there is no reason that radiation-induced biological changes cannot be described by properly developed AOPs. By definition, an AOP is agnostic to the agent producing the MIE. The AOP maps out the biological changes produced by KEs which lead to the AO. Application of the AOP concept to radiation-induced neurological changes will require identifying the MIE(s), the KEs involved in the biological pathway, and the AO(s) of concern. Recently, the AOP process has been applied to radiation-induced cancer risk (Brooks *et al.*, 2016). This work focused on genomic changes produced by radiation, but also listed inflammation and reactive oxygen species (ROS) as risk factors for cancer. These later changes can also be involved in neurological damage, and could be included as KEs for other AOs. Recent work has specifically addressed the use of inflammation as a KE in the AOP framework, through the use of three “hub KEs”, in a manner that may allow the process of inflammation to be generalized across may different tissues

(Villeneuve *et al.*, 2018). We recognize that it is often hard to distinguish between beneficial compensatory responses/pathway changes and adverse injury responses in the central nervous system (CNS) following radiation. The issues of non-linearities, such as feedback loops, event modifiers, compensatory mechanisms, multiple "hits" by initiating an initiating event, effect duration for various KEs, and thresholds have recently been reviewed for AOPs (Leist *et al.*, 2017). Therefore, for using the AOP framework in the context of space irradiation, it may be important to consider these additional issues in a framework for radiation-induced AOPs.

Various research, including both biased and unbiased approaches, into radiation-induced neurobehavioral changes and neurocognitive injury has resulted in many proposed MIEs or KEs, as well as AOs. Many such MIEs and KEs have been noted in Sections 5 and 6 of this document. As described in these sections, animal research has indicated that radiation exposure can result in long-lasting decreases in behavioral measures of cognitive function, and endpoints that are believed to be associated with neuronal correlates of learning, memory, and cognitive function. For example, the three figures (Figs. 5.1-5.3) in this document illustrate many radiation-induced alterations in behavioral and cognitive function in animal models. As shown in these figures, much of this animal research has been performed using different exposure protocols (and often by different laboratories). The AOP framework may serve as a means to consolidate the findings and evaluate the strength of evidence for various pathways. For example, constructing an AOP for altered behavioral performance and cognitive changes after radiation exposure may involve KEs that have been used in existing AOPs. A searchable list of KE and KERs has been included in the AOP Wiki, to facilitate creating such linkages (https://aopwiki.org/events; https://aopwiki.org/relationships). Such network linkages may help identify other proposed pathways for common or different biological outcomes. For example, a KE involving

neuroinflammation (https://aopwiki.org/events/188) has been linked to four AOPs that may involve neurodegeneration and impairments in learning and memory, including impairments in motor learning and memory (last checked 11/06/2017). When coupled with a weight of evidence evaluation, the AOP framework can help evaluate the strength of data in the literature for different pathways. This in turn can focus research efforts to fill in critical data gaps, as well as possible insights into additional research directions.

7.1.2 Comparison with Chemical Toxicity

Initiation of an AOP by a chemical requires sufficient concentration of the chemical (or its metabolite(s)) at the active biological site to cause a sufficient level of change in the MIE. The AOP framework begins with the altered function of the MIE and does not formally include pharmacokinetic parameters (absorption, distribution, metabolism, or elimination) (Teeguarden *et al.*, 2016). While distribution, metabolism, and elimination may be less important for radiation exposures, the anatomical site of exposure (whole body vs. head), rate of exposure, and type of radiation will influence the rate, amount, and duration of perturbations in a MIE. Thus, consideration of the differences in effect(s) produced by high charge and energy nuclei (HZE) and galactic cosmic rays (GCR, protons and He nuclei) may be important in determining which MIE are altered. However, once an MIE is altered to a sufficient level, for a sufficient duration, the predicted changes in the next stage KE (biological pathway) should occur, leading down the pathway to an AO. This aspect should not differ between chemical and radiation exposure. However, we recognize that based on the unusual dose-response relationship identified for effects of radiation on the CNS, prediction modelling of effects of space radiation on AOPs may be more complicated than that for chemical exposures.

Chemicals have been shown to alter psychological constructs of interest, such as learning and memory. While the literature is too large to be summarized in this exercise, several brief cases can be provided. As examples, treatment of adult (150 day-old) rats with 0.375 mg/kg/day of mercury chloride ($HgCl_2$) for 45 days resulted in reduced horizontal and vertical movements in an open field arena, increased time to acquire inhibitory avoidance, decreased long-term (24 h) memory, and decreased short term memory (30 s retest) in the elevated T-maze, as well as decreased latency to fall in the rotarod test at 16, 20, 25, and 28 rpm (Teixeira *et al.*, 2014). Treatment with lead (Pb) in feed over postnatal days 1-21 or 1-55 impaired recall of trace fear conditioning in female Long Evans rats (150 ppm Pb), while exposure of males (via dams) to 150 or 750 ppm Pb from 10 days prior to breeding through weaning impaired recall in the fear conditioning paradigm (Anderson *et al.*, 2016). Treatment of male Sprague Dawley rats with 100 ppm Pb in drinking water from 24 – 56 days of age impaired conditioned fear memory and increased anxiety-like behavior. Long-term potentiation in the hippocampus was impaired in 8-week-old animals, and the density of dendritic spines was reduced in the CA1 region of the hippocampus (Wang *et al.*, 2016). These examples illustrate that chemicals can alter regions of the brain associated with cognitive function, and result in behavioral and cognitive changes indicative of memory dysfunction.

Because changes in cognitive function and possible predisposition to developing diseases such as Parkinson's and Alzheimer's following to radiation are of concern during space missions, concordance of changes in KEs between chemicals and radiation would allow use of the AOP paradigm and the chemical literature to predict pathways that are altered in neurological function produced radiation. Unfortunately, in spite of years of research evaluating the effects of chemicals on cognitive processes, there have been very few pathways proposed that detail

biological changes from the molecular level (e.g. MIE) to changes in constructs such as learning and memory (AOs). The AOP Wiki is a work in progress, and contains only a few such pathways. Example AOPs related to NMDA receptor interactions have been proposed to be related to cognitive dysfunction: https://aopwiki.org/aops/48, https://aopwiki.org/aops/13, and https://aopwiki.org/aops/12 (last verified on: 11/20/2017). These AOPs include KEs such as mitochondrial dysfunction, changes in intracellular Ca^{+2} levels, decreased neural network function, neuroinflamation, altered levels of brain-derived neurotrophic factor, altered dendritic morphology, decreased synaptogenesis, and cell injury and/or death. Alterations in KEs involving oxidative stress status have been proposed to be involved in neurotoxicity produced by a variety of chemicals reported to alter cognitive function, such as manganese (Martinez-Finley *et al*., 2013), methylmercury (Aschner *et al*., 2007), and toluene (Kodavanti *et al*., 2015). Such effects may differ between young, middle-age, and senescent animal models (Kodavanti *et al*., 2011; Royland *et al*., 2012). These examples show that proposed KEs in alterations of neurological function produced by chemicals are similar to some of those proposed to be altered by radiation.

Maps of biological processes that are altered in disease states usually involve networks of changed biological pathways. These networks do not necessarily involve the AOP framework, but include a series of pathways that include KEs involved in chemical-related AOPs. Inhibition of mitochondrial complex 1 in nigro-striatal neurons has been proposed to result in Parkinsonian motor deficits https://aopwiki.org/aops/3 (verified 11/20/2017). A proposed map of the pathways involved in Parkinson's Disease has been proposed (http://minerva.uni.lu/MapViewer/; Fujita *et al*., 2014) which includes KEs including calcium homeostasis, mitochondrial dysfunction, synaptic pathology, neuroinflamation, and apoptosis. Other researchers have also emphasized the

role of inflammatory processes involved in mitochondrial dysfunction and oxidative stress (Tansey *et al*., 2007). Another example of disease pathways involves the role of amyloid pathology, including amyloid plaque formation in Alzheimer's Disease signaling; many different KEs such as phosphorylation of tau protein, formation of reactive oxygen species, formation of amyloid plaques, microglial activation, lipid peroxidation, and many other KEs that are linked into a biological network (https://www.cellsignal.com/contents/science-cst-pathways-neuroscience/amyloid-plaque-formation-in-alzheimer-s-disease-signaling-interactive-pathway/pathways-alz). The role of KEs, such as oxidative stress, has been emphasized in multiple neurodegenerative disorders (Reynolds *et al*., 2007) and neurotoxicity from environmental agents (Kodavanti, 1999; Viviani *et al*., 2014). These examples make it clear that there are common KEs that are proposed to be altered in disease states, or involved in cognitive function, that may also be affected by chemicals, and have been proposed to be involved in changes in neurological function produced by radiation.

Given the AOP paradigm, the question then becomes what common KEs leading to AOs of concern are common between chemical exposures and radiation exposures. Adverse outcomes of interest include learning, memory, including motor learning and memory, emotion recognition, processing speed and reaction time, vigilance, fatigue, and neuropsychological changes (McPhee and Charles, 2009; Parihar *et al*., 2015b; Slack *et al*., 2009; Strangman *et al*., 2014). The current state of neurobiological knowledge precludes these highly apical human psychological constructs from being described as an outcome from a singular cellular process. However, changes in various cellular processes in brain regions such as the hippocampus and cortex have been correlated with changes in such psychological constructs. Cucinotta and coworkers have presented a summary table of rodent studies relating exposure to HZE particles

to behavioral and cognitive alterations (Cucinotta *et al*., 2014). The importance of neuroinflamation and mitochondrial function in mediating the effects of radiation has been emphasized (Betlazar *et al*., 2016). The use of broad-based genomic responses may yield information regarding important KEs resulting from radiation exposure. For example, 22 weeks after exposing six month-old C57BL/6J mice to 1 Gy of protons, hippocampal DNA methylation was altered in pathways related to neuron differentiation, axon outgrowth, neuron/synapse development, and neurogenesis (Impey *et al*., 2016a). However, when 0.1, 0.2, or 0.4 Gy of 600 MeV ^{56}Fe irradiation was used, cognitive impairments in object recognition and epigenetic changes in hippocampal neuronal pathways associated with axon guidance, axogenesis, and neuronal development 2 weeks after treatment, but not 20 weeks after treatment (Impey *et al*., 2016b). There was some concordance between the changes resulting from proton exposure with ^{56}Fe for pathways related to cell adhesion, cell junctions, neuronal growth, and synapse formation. However, there were also differences in the responses resulting from the two different types of radiation, indicating different pathways may be involved (Impey *et al*., 2017). Thus, identification of appropriate KEs to include in an AOP may require systematic studies involving unbiased approaches that examine a wide range of possible pathways.

It is clear that both chemicals and radiation can alter KEs that have been proposed to be involved in behavioral performance and cognitive function and several disease states. A need for the development of theoretical models, and independent review of research has been emphasized for space radiation research (Dicello, 2002). This process is included in the formalized AOP Wiki. A synopsis of the radiation-induced changes in nervous system function with a focus on AOP pathways/networks may highlight areas of research needed to fill data gaps, and for which pathways currently there is the strongest biological support based on the available data.

4154

4155 **7.1.3** Summary

4156 1. The AOP framework serves as a means to organize knowledge about relationships
4157 between the initial biological perturbation and an apical outcome.
4158 2. The AOP framework describes biological pathways and is agnostic to the specific
4159 physical/chemical stressor that produces the initial biological alteration.
4160 3. The AOP framework is can be used to describe the weight of evidence for the
4161 different portions of the biological pathway, to identify knowledge gaps, and to
4162 indicate which areas may be a priority for future research.
4163 4. A well-defined, quantitative AOP may assist with the reduction of default uncertainty
4164 factors and enable the use of targeted assays for Key Events instead of apical Adverse
4165 Outcomes in risk assessment.
4166 5. Although developed for chemical-induced biological changes, the AOP framework
4167 could also be used to describe radiation-induced biological changes. This approach
4168 may assist with the organization of the literature base on radiation-induced effects,
4169 and indicate areas for future research emphasis.

4170 **7.1.4** Recommendations

4171 1. Conduct a systematic review of existing literature and data, organized in an AOP
4172 framework, to identify the strength of converging evidence for radiation-induced changes
4173 in various biological pathways. Although limited to what is published in the literature,
4174 this process may lead to identification of knowledge gaps for research prioritization and
4175 targets for mitigation of detrimental radiation-induced effects on the brain.

4176

2. Clearly define the adverse outcome(s) of concern. This will assist in determining what is a biologically-significant change (such as 10% increase in reaction time). This process will focus work on the biological pathways (AOPs) which may be the most important.

3. Fund research into the similarity of biological pathways between multiple species for radiation-induced changes in neural function. This will assist in constructing AOPs to organize data which may be collected in other species (mice, NHPs), and help determine the relevance of the data to describe biological changes which produce the adverse outcome(s) of concern

7.2. Risk Models and Methods for Characterizing Magnitude of Exposure in Order to Predict CNS Effects

7.2.1 Background

In order to predict the consequences of a radiation exposure some model of the dose response relationship is essential. Traditional radiation protection policies are based largely on the assumption that cancer induction is the most sensitive endpoint of radiation exposure, and therefore limiting it to an acceptable level will also limit the damage due to other biological processes to acceptable levels. Furthermore, a linear no threshold model is used to predict cancer induction by low LET radiation and weighting factors are used to predict the effect of higher LET radiations. It is recognized that for many specific biological endpoints, the linear model is not directly supported by data, but it is believed that the linear model is unlikely to

under estimate the consequences of exposure for any cancer endpoint and its overestimates of risk are acceptable in that they provide a desirable safety margin.

In order to provide useful recommendations for exposure limits to prevent CNS damage, it is necessary to determine the maximum acceptable level of such damage and sufficient of the dose response relationship that can be used to predict when that level of damage will occur. It is possible that the exposure limits set to prevent excessive levels of cancer induction will be sufficient to prevent significant CNS damage, but suitable models will be needed to determine if that is true or not.

Most forms of CNS damage that have been observed clinically or which have been measured in experimental systems appear to be non-stochastic in nature. That is, the larger the radiation exposure, the larger the effect, or the shorter the time before a significant effect occurs. This has traditionally been referred to as a deterministic effect, but it is now generally assumed that observed damage is the result of the accumulation of stochastic damage to a number of cells in the tissue (Hall and Giacci 2012). Generally there is a threshold dose, below which there is not enough damage to the function of the tissue to be detected by the available assay. As new, more sensitive assays are developed the threshold dose for an observable effect is likely to decrease. Similarly for human performance, there is likely to be a level of exposure below which effects cannot be observed. However, the fact that a statistically significant effect is observed in a specific test does not necessarily indicate that the effect is significant with respect to the completion of a mission or the long term health of the crew member. Furthermore, in the case of CNS effects there appear to be a variety of adverse effects which may result from different forms of stochastic damage (different MIEs initiating the same, or different, AOPs) or different spatial or temporal distribution of the same type of initial damage (MIE). In order to recommend

radiation protection procedures and limits, models of the magnitude of each CNS effect as a function of an appropriate description of radiation exposure are needed and a determination of the level of effect that is likely to have an adverse effect on mission performance needs to be determined.

7.2.2 General Requirements for a Quantitative Risk Model

Formulation of a general model for quantitative projection of risk for defined behavioral, cognitive or other health effects requires basic information of the following types:

- Specification of radiation exposure in terms of relevant physical parameters that may affect the quantitative health outcomes.
- Quantitative associations between the significant health effect(s) of concern and radiation exposure for defined exposure parameter values.
- Principles for extrapolation of the risk relationship to other relevant radiation parameter values that describe the variety of exposure scenarios of interest (including mixed radiation fields),
- Generalization of the model in suitable parametric form to encompass the range of exposure scenarios foreseen for application of the risk projection model.

In principle these quantitative associations could be based on human epidemiological data, human data for surrogate biological events (key events?) that can be quantitatively linked to the final behavioral, cognitive or other health effects of concern and/or experimental data that can be quantitatively linked to the relevant human effects. Alternatively, they could in principle be based on a sufficiently detailed quantitative understanding of the molecular basis of the relevant human CNS reactions to radiation exposure and of the systems biology that leads to the

significant human health effects of concern, that is, a quantitative expression of the relevant AOP. At present, for both short-term and late effects on the CNS, there is a lack of relevant epidemiological data and major gaps in the understanding of relevant biological processes. Many experimental results have been accumulated on behavioral, cognitive effects and tissue changes in experimental animal (primarily rodent) models, and these results currently serve as the primary source of information from which guidance on extrapolations to human risk may be sought; however, the relevance for this purpose of much of the existing data and the endpoints studied remains uncertain. With any approach, the health effects may be quantified, for example, as dose-response relationships for level of effect, or for probability of occurrence or as threshold doses.

7.2.2.1 Specification of Radiation Exposure. Results of biological studies utilizing different irradiation procedures suggest that most biological systems include a variety of mechanisms for dealing with radiation induced damage and consequently a single parameter describing radiation exposure is unlikely to be successful. An adequate specification of exposure for quantitative relationship to biological effects is likely to require specification of the radiation quality, temporal distribution, and spatial distribution in addition to the quantity of radiation.

7.2.2.1.1 Quantity of radiation. This is usually expressed either as absorbed dose or as particle fluence. Since there is a direct (1/LET) relationship between average fluence and average absorbed dose for heavy charged particles, the preferred choice may depend on which of these two parameters most closely predicts the biological response across particles of differing energy (E) and charge (Z) in the volumes of interest. Another consideration may be the role that photon

radiation plays in the risk model, since a fluence-based description for the secondary electrons is somewhat problematic because of the multitude of additional electrons set in motion in the slowing-down spectrum.

7.2.2.1.2 Quality of radiation. The commonly used parameter LET (L) provides only a partial indication of the track structure or spatial distribution of ionization-density of the radiation, so additional or alternative specifications may be required. For prospective radiation protection on Earth the ICRP (2007) has specified radiation-weighting factors (w_R) and quality factors (Q(L)) on the basis of its judgement of risks for stochastic effects (particularly cancer) from terrestrial radiations. For space radiation, the NASA risk projection model for risk of cancer induced death (REID) applies quality factors (QF_{nasa}) as a function of E and Z of heavy charged particles, parameterized to relate more closely to the track structure properties of the particles (Cucinotta et al 2013). In the case of both the above ICRP and the NASA approaches, the quality factors are expressed relative to high-energy photons because the main data base for risk of cancer induction in humans comes from epidemiological studies of the survivors of the A-bombs in Japan, exposed predominantly to high energy gamma rays. These quality factors are not suitable for application to non-stochastic (tissue) effects. New radiation-quality descriptions will be required for CNS effects, irrespective of whether they are regarded as tissue or stochastic. The term gray-equivalent (Gy-equiv) has sometimes been used for tissue effects when comparing different radiations, particularly when the reference radiation is high-energy photons, as in conventional radiotherapy. If some CNS effects are the result of the high spatial and/or temporal concentration of initial damage produced by HZE particles there will be no comparable effects produced by low LET radiation and use of a reference radiation with known risk may not be possible.

7.2.2.1.3 Temporal pattern of delivery of the radiation. On the macroscopic scale HZE exposures in the space environment are generally at very low dose rates (very low fluence rates, random timing between particles), but most laboratory studies are at high dose rates, due to practical constraints of the radiation sources and experimental conditions. Exposure to protons can be at somewhat higher dose rates during solar particle events. However, on the microscopic scale the dose rate within a HZE track is very high.

7.2.2.1.4 Spatial distribution of exposure. Space radiation exposures are generally to the whole body. Transport codes are used to estimate finer detail of variations due to particle interactions in the spacecraft and the body. For the NASA risk projection model for REID due to cancer, the fluence spectrum of particles (E, Z) is evaluated at the level of individual organs within the body. On the microscopic scale the spatial distribution of energy deposition and radiation chemistry products depends strongly on E and Z and decreases rapidly with distance normal to the charged particle's trajectory.

7.2.2.2 Data Available for Risk Model for CNS Effects of Space Radiation

7.2.2.2.1 Epidemiology. There are essentially no human epidemiological data on CNS effects from exposure to space radiation (see Section 4). In the case of cancer risks, there is a rich epidemiological database from human exposures to photons and this has provided a means to link to risk projections for space radiation via experimental and theoretical studies. It remains unclear whether such a future link is feasible for specific CNS effects, particularly if the nature of the effect, or its pathway, caused by HZE is fundamentally different and beyond the scope of photon radiation.

7.2.2.2.2 Experimental data**.** There is a wide range of experimental data on CNS effects in animals, notably rodents, from exposure to accelerated charged particles corresponding to components of space radiation; these data are summarized in Section 5. These studies have had a diversity of objectives and overall they have included a variety of mouse and rat models, a wide range of biological assays, a selection of charged particles from protons up to ^{56}Fe, specific energies up to 1000 MeV/n and acute doses from as low as 0.01 Gy up to a few gray. However, these studies leave many gaps, which make it problematic to formulate a radiation response model for any CNS effect.

- It is currently unclear which effect(s) in the animal models are most relevant to the 'significant risk(s)' in humans for which a risk model is required or whether any of them are suitable for translation of risk to humans?
- It is currently unclear also how the effect(s) in animal models can be related quantitatively to significant effect(s) in humans.
- From the particular perspective of constructing a space radiation risk model relatively few of the studies provide quantitative information across a sufficient range of radiation parameters to guide generalization of biological effectiveness in relation to radiation quality parameters.
- Dose response information for specific assays of effect is usually quite limited and not readily comparable between studies because of differences in systems used and other possible variables. In several instances dose responses appear quite irregular, further compounding the difficulty of comparisons with other radiation qualities or studies and determination of a specific dose level that equates to a given risk.

- For much of the animal data summarized in Section 5 for the CNS effects from exposure to heavy charged particles there are no directly corresponding data from exposure to photons (X-rays or gamma-rays). In such cases, the lack of corresponding data will preclude the use of photons as a link between human and animal data, which link has been crucial for development of the NASA cancer risk projection model (Cucinotta et al. 2013). (Section 5 does not generally include photon data even in those cases where corresponding photon and heavy particle experiments were carried out under somewhat similar conditions. Instead the reader is referred in Section 5.2.1 to a published general review of behavioral and neurophysiological changes of exposure to X-rays and gamma irradiation (Obenaus et al. 2012). For the mechanistic studies reviewed in Section 6, results for photons have been included in selected cases.)
- Responses to high-energy protons (and to ^{4}He ions) might well be informative of expectations for photons because of the strong similarities in their patterns of energy deposition, which in both cases are overwhelmingly via secondary electrons. When significant biological effects are observed for high-energy protons, the first-order expectation should be for broadly similar effects from similar absorbed doses of photons (especially X-rays of energies that produce first-collision electrons with an energy spectrum similar to that of the proton delta rays). Spatial correlations of delta rays along the path of the high-energy protons are unlikely to alter this expectation materially because of the large ionization mean free paths of the protons. Where substantial differences are observed between the proton and photon effectiveness, the explanation may itself be informative and worthwhile to seek (for example, beam

contamination, surprisingly large consequences of relatively rare proton-nuclear interactions or surprising importance of some subtle difference between the spatial or temporal patterns of energy deposition by photons and high-energy protons).

- There is one series of published experiments that may be indicative of the type of comprehensive set of data that is required across a broad range of radiation parameters for a specific effect. This particular series is for changes to executive function assessed by operant responding tests in irradiated rats (Rabin et al. 2007b, 2011, 2015a). The series covers 7 different heavy particles (from ^{4}He to ^{56}Fe), with from 1 to 3 different energies for each particle, giving 10 different radiation qualities in all and with LET values ranging from 0.89 to 150 keV/μm. Furthermore the series includes some results for ^{137}Cs gamma-ray exposures. Provided that the experimental methods were sufficiently constant across the experiments and that inter-experiment variability were not too large (Rabin et al. 2015b), then a data set of this type might provide useful guidance for selection of the more appropriate radiation quantity parameter (absorbed dose or fluence) on which to base a risk model for a defined effect (for example in this case, a threshold dose to disrupt cognitive (operant) performance in male Sprague-Dawley rats), as well as providing meaningful rules for interpolation and extrapolation for radiation-quality across space-relevant particles over a wide range of charge, energy and LET. Additionally, comparisons with the gamma-ray data might provide some potential for developing a link to information on humans after exposure to terrestrial radiation.

7.2.2.2.3 Track structure modeling of initial biological damage. In the case of CNS effects that show deterministic characteristics and for which a full AOP is not known, it may be useful to consider two or more steps independently along the pathway. The first step would be the initial production by the radiation of microscopic damage to biomolecules or subcellular structures, the MIE, and the second and subsequent steps would be the changes in performance of the tissue or system in response to that damage. The production of microscopic damage of a specific type is likely to be a stochastic process and can best be modeled utilizing the stochastic properties of radiation track structure.

Analysis of the tracks of charged particles in relation to geometric models of structures of particular biological target volumes and their spatial associations enables evaluation of a variety of physical quantities of potential interest. These include hit probabilities (by the direct passage of heavy charges particles and also by their delta-ray electrons), frequency distributions of the magnitudes of energy deposition within the targets, spatial correlations between hit targets, relative contributions from multiple tracks (inter-track) compared to single tracks (intra-track) (with potential implications for shapes of dose response and potential dose-rate dependence), etc. Where results from radiobiological experiments enable these physical data to be associated with particular biological consequences, or early processes leading to those consequences, the physical data can then provide guidance for extrapolation of expected effects across heavy particles of differing E, Z and LET, as well as comparisons with photons. In such ways, models based on track structure analyses can contribute to development of risk models, as well as contributing to mechanistic understanding of critical events and pathways that lead to the biological effects and to potential mechanistic components of a risk model.

As an example of such an approach, Alp et al. (2015) developed a stochastic computational model of microscopic energy deposition events of radiation tracks to study the initial damage to irradiated neuronal cells of the mouse hippocampus, including by construction of in silico mouse hippocampal granule cells with spine and filopodia segments stochastically distributed along the dendritic branches (see also Section 6.1.2 above). The model was evaluated with track structure simulations of large numbers of 20 μm-length track histories of ^{56}Fe (600 MeV/n), ^{12}C (300 MeV/n) and ^{1}H (250 MeV) particles, of mean LET 172.4, 12.9 and 0.4 keV/μm, respectively. This approach can support the development of biophysical models of the modifications of spine and dendritic morphology observed after low dose charged particle irradiation by providing accurate descriptions of the underlying physical insults to complex neuron structures at the nanometer and larger scales. The model has been applied subsequently to heavy particles of lower energy, of relevance to hadron therapy, namely ^{12}C (59 MeV/n), ^{3}He (38 MeV/n) and ^{1}H (32 MeV) (Alp et al 2017). In further advances of this approach, morphological data for mouse dentate granular cell layer neurons, together with the stochastic model of particle track structure and microscopic energy deposition, have been used to develop a predictive model of HZE-particle-induced morphological changes to the complex structures of dendritic arbors (Alp and Cucinotta 2018). Initial evaluation of the model was for a comparison of the changes induced by ^{16}O and ^{48}Ti particles of equal specific energy (600 MeV/n) but differing LET (16.3 and 129 keV/μm, respectively); this led to the suggestion that delta-rays play a major role in neuron morphological changes and hence that the dependence on LET is quite small (Alp and Cucinotta 2018). Future comparisons with experimental measurements of neuron morphology should enable testing of the model, and specific assumptions within it, as well as its progressive development and application to other heavy particles and also to photons. It remains

to be demonstrated what role such changes in neuron morphology may or may not have in relation to early or late risks to humans from space radiation. Apart from direct radiation damage to dendrites and spines, other possible mechanism for degradation of neuron morphology by radiation include damage to mitochondria, damage to early response genes, activation of microglial cells and non-targeted effects via chronic reactive oxygen species and inflammatory responses (Cucinotta *et al*. 2018). At present there are insufficient experimental data to characterize the radiation-quality dependence of effects on these targets and to guide model development.

The complex dose response relationships observed in many of the rodent experiments (section 5) suggests that the relationship between initial biochemical damage and altered CNS function is often highly non-linear (although experimental variability and diversity of endpoints studied may impede generalizations). Different key event relationships may dominate depending on the concentration or distribution of MIE, or different MIE may initiate different outcome pathways which interact to modify the observed CNS effect. One form of interaction that has been suggested as a cause for non-monotonic dose response is radiation induced activation of "repair" systems. In this context "repair" does not necessarily indicate reversal of damage or any particular target molecules or structures, but would include any change that resulted in decreased probability of observing the endpoint being studied.

7.2.3 Approaching Modeling From the Adverse Outcome

In order to meet the requirements for predicting risk in different radiation fields a model that relates the physical characteristics of the charged particle tracks which initiate biological changes is required. However, it may not be necessary to develop such a model by starting with

the characteristics of different charged particle tracks, as is described above. It may be possible to utilize the Adverse Outcome Pathway approach (section 4.1) to identify the molecular initiating event, MIE, which is responsible for a specific AO. Then from the MIE and knowledge of radiation chemistry it may be possible to deduce the probability of producing the MIE and the AO as a function of the energy deposition patterns of the different particle charges and velocities in the space radiation spectrum. This approach is expected to be exceptionally difficult considering the wide range of key events and key event relationships involved in the wide range of acute and delayed adverse outcomes that can be produced, but adverse outcomes in the CNS is a very active research area. Much of this research has no relationship to radiation exposure, but it is possible that key events produced by other environmental factors or even genetically based metabolic differences may also be in the pathway initiated by ionizing radiation.

Using dose-response functions to assess effects of radiation on multiple endpoints (different human behaviors down to cellular changes in the hippocampus of animal models) is theoretically similar to the chemical risk assessment process. The classical method of risk assessment for chemicals is described in the National Academies of Science “Red Book” (NRC, 1983). The basic steps include: Hazard Identification, Dose-Response Assessment, Exposure Assessment, and Risk Characterization. For example, identification of endpoints of concern to NASA and whether they are altered by radiation would be involved in the hazard identification process. An assessment of the nature and evidence for causation are also involved (including an assessment of the scientific literature). This could involve assessing the impact of radiation on a behavioral change such as reaction speed. Many times, an evaluation of which biological change is produced at the lowest dose, or is of greatest concern, is performed. The next stage involves characterizing the response to the doses of the agent and the incidence or severity of the adverse

effect. This will include different doses, variability in amount or pattern of exposure, and other factors that may alter the biological response. An extrapolation between high to low doses, between animals and humans, acute to chronic exposure, and vulnerable populations may be included in this dose response process. Determination of Lowest Observed Effect Levels, No Observed Effect Levels, or modeling of a continuous dose-response function may be involved. Additionally, this process may involve the application of uncertainty factors to compensate for data/knowledge gaps, which should be clearly explained (see Dorne and Renwick, 2005 for examples). Similar approaches could be used with radiation-induced changes in function. The exposure assessment process involves estimating frequency, amount, and duration of human exposures. Estimates of uncertainty, possible vulnerable populations, and potential interventions are identified. Again, these processes would be applicable to radiation exposures. Finally, the risk characterization process estimates the incidence of predicted health effects under the conditions of exposure that was detailed in the exposure and dose-response assessments. Again, this process would be applicable to radiation as well as chemical exposures.

More recently, the limitations of the traditional approach to risk assessment have been highlighted by another NAS report describing a vision and strategy for future approaches to assessing chemical risk (NRC, 2007). This approach consists of the following components: chemical characterization, toxicity testing, dose-response modeling, and extrapolation modeling. The toxicity testing component revolves around the concept of toxicity (biological) pathways that are altered after chemical exposure. These pathways may be described by a series events, which may range from biochemical alterations to changes in organ function. Alterations in the biological pathway may eventually overcome homeostatic processes, leading to adverse health effects. It may be difficult to relate changes in a molecular endpoint to an apical outcome

(behavioral change). A wide range of screening tests may be used to assess changes in multiple steps along the biological pathway (see section on Adverse Outcome Pathways (AOPs)). If a sufficient number of biological steps are understood and assessed, it may be possible to replace animal testing with a battery of *in vitro* assays. Alternatively, if there is a sufficient quantitative understanding of the key event relationships to allow for reasonable extrapolation from an early key events to a predicted severity or probability of an adverse outcome, this can also be achieved through assessment of a relatively limited number of biological steps (Judson *et al*., 2015). Development of such biological pathways may allow integration of cellular effects of radiation to be linked to behavioral deficits of concern (see section on AOPs).

7.2.4 Integration of Data Across All Biological Scales

As described in Section 3.4 above, the Phase I report recommends exploring approaches for integration of data across all biological scales (organizational, spatial and temporal), from the initial subcellular damage initiated by the interactions of charged particle tracks through cell responses and tissue changes to cognition and behavioral outcomes. It states further that the approaches need to integrate data from cells, experimental animals and humans, including any epidemiology data available and any relevant astronaut data in particular, into a coherent model that will facilitate development of risk projections and accordingly, risk management.

These recommendations serve as useful longer-term goals, but progress towards them requires accrual of robust, reproducible and systematic data on quantitative relationships between specific radiation exposures and relevant outcomes at each, or most, of these scales in experimental systems that can be effectively translated to outcomes in humans for “significant impairment”. At present there are almost no epidemiological data for populations exposed to

charged particles or for CNS effects in astronauts (with the exception of cataracts), so every opportunity should be taken to collect such data and also to seek other means to link observations in experimental systems to outcomes in humans, including for example by comparison with effects from exposures to photons.

As discussed in other sections of this report, the AOP approach can serve as a useful framework for organizing existing knowledge, identifying the many gaps that currently exist and prioritizing research. In these ways it can facilitate integration of data across scales. Multiscale modeling (both in space and time) can be developed most efficiently in situations where many of the key events and pathways have been sufficiently well characterized, including with empirical or mechanistic quantitative relationships between them, such that models at various scales are available for simultaneous application. In some cases, however, sufficiently accurate predictions cannot be obtained with the assumption of scale separation and the predictive model must be extended to account for causation across multiple scales. In other cases, empirical correlations across scales are invoked to avoid specific modelling of intermediate stages. There are many examples of multiscale modelling of radiation effects of relevance to radiation therapy (cell inactivation) and to radiation carcinogenesis, including for charged particle exposures in both cases. In these fields, understanding of induction by radiation of the initiating events and development of subsequent key events are reasonably well advanced, but at present the knowledge gaps for CNS effects are very much larger. The gaps could be narrowed through a more unified translational research program that combines rodent, larger animal, mechanistic and biophysical studies with available human data to provide the most robust datasets to feed an AOP type framework for the human space radiation response.

7.2.5 Conclusion

Currently available data are insufficient in many respects for the development of formal quantitative risk projection models for either in-mission or long-term effects of exposure of the brain to space radiation. Current deficiencies and obstacles include lack of direct data on effects of exposure of humans to space radiation, inability to translate observed radiation effects in rodents to humans (qualitatively or quantitatively), clear definition of what effects in humans are "significant" for in-mission operations and what rodent analogues might correspond, variable patterns of dose-response for behavioural and cognitive effects in rodents, insufficient systematic data on radiation-quality dependence, and insufficient identification of key events and pathways linking initial radiation properties and damage to short-term or late effects.

7.2.6 Recommendations

Develop methods for translation to humans of results from studies in rodents and other biological models, including by identification of key molecular and tissue changes and pathways linking the early radiation damage to relevant short-term behavioural or cognitive changes or to late effects. The Adverse Outcome Pathway approach (see Section 7.1) may provide a helpful formalism to guide research, with emphasis being put on conservation between rodents and humans and on quantitative as well as qualitative relationships.

Concentrate experimental research efforts on obtaining reproducible dose response relationships for protons and two specified heavy ions (possibly Si and Fe) at 3 specified velocities, as well as corresponding results for gamma- or X-ray photons which will provide a potential linkage to human data. This will facilitate the development of quantitative risk projection models of the type discussed in Section 7.2,1 and will be necessary in order to estimate the dependence of relevant

behavioural, cognitive and long-term health changes on radiation parameters (for specification of radiation exposure in terms of quantity and quality).

Conduct tissue equivalent proportional counter (microdosimetry) measurements of proton beams used for CNS effects research. Some experiments have shown proton beam exposure to be significantly more effective than photon exposure. Since TEPC measurements will detect any high lineal energy component in the proton beam they will be able determine if beam contamination or a unique property of proton tracks which is responsible for the unexpected effectiveness.

Use low dose rate neutron exposures, comparing the results to high dose rate exposures with nearly identical neutron spectrum, to estimate the effects of dose rate on CNS effects. Although the secondary radiation produced by neutron exposure can not produce the long tracks of cells affected by a single GCR particle, they are a reasonable simulation in other respects.

8. Extrapolation Between Species

Many small animal behavioral studies, as well as cellular and molecular studies (see sections 5 and 6) show statistically significant effects of simulated space radiation at mission relevant doses for many CNS-related effects. These studies provide fundamental information relevant to the mechanisms leading from energy deposition by space radiation to the biological consequences. However, they do not necessarily provide sufficient information to determine the level of radiation exposure that would lead to operationally significant detriment. Consequently, estimated risk of during-mission or post-mission effects based on only rodent data have relatively large uncertainties. Ideally, human data would be used to determine the level of exposure that will produce specific detrimental mission-critical effects in human populations. The available human clinical data has been reviewed in sections 4.1 and 4.2, and clearly show that significant damage occurs at relatively high doses. However, there are very little data at mission relevant doses and energies for exposure of relevant populations (healthy adults not on medication) to relevant radiations (high velocity charged particles, not x or gamma rays). There is a very limited amount of data that appears to be relevant, including healthy workers exposed to polonium (section 4.2.3) and AVM patients treated with accelerator-generated charged particle beams (section 4.2.1). Unfortunately, there are insufficient data, from these studies to determine the risk resulting from exposure in space and polonium exposure of a significant population is not likely to occur in the future. High energy charged particle beams are again being used for cancer therapy and in the future it may be possible to identify patient populations who, having received high doses to a very limited region of the brain, have received a low dose of well-defined radiation outside of the treatment volume, and have not received chemotherapy or other drugs which would impact CNS function. However, this population is likely to grow slowly and

may not be available in time to support radiation safety decisions required for early deep space missions.

Data from well-designed non-human primate experiments can potentially reduce the uncertainty in risk estimates derived from rodent data. The degree to which the uncertainty can be reduced depends on the extent to which mechanisms mediating CNS effects in rodents are relevant to effects on NHP and humans and on the precision with which NHP behavioral and cognitive assays can predict human behavioral and cognitive performance. Compared to rodents, NHPs share many genetic and physiological similarities with humans. For example, female Rhesus macaques, like humans, show about 28-day menstrual cycles and undergo menopause, whereas mice go acyclic with age. NHPs are considered a good animal model for age-related cognitive decline and age-related changes in circadian rhythms (Haley, Landauer et al. 2009, Haley, Kohama et al. 2010, Haley, McGuire et al. 2011a, Messaoudi, Urbanski et al. 2011). They are outbred and relatively long-lived. As described in the following, NHP experiments designed to reduce the uncertainty in risk estimates will be both time consuming and expensive and cannot produce useful results relatively quickly.

8.1 Non-Human Primate Considerations

When planning non-human primate (NHP) experiments, it is essential that careful consideration is given to: (1) objectives of the experiments; (2) endpoints to be evaluated; (3) sub-species; (4) age; (5) sex(es) of the animal; (6) number of animals required for obtaining conclusive data based on the statistical design; and (7) radiation source: considerations such as relevant radiation quality, dose, exposure geometry, dose rate and exposure time.

The rhesus macaque (*Macaca mulatta*) has been the predominant nonhuman primate model used in neuroscience studies. The information that has been generated will help reducing the uncertainty in the design of experiments using macaques relative to other NHP. However, alternative NHP species must be considered. To determine suitability of alternatives, past use for the study of CNS effects or CNS plus radiation should be evaluated.

The number of animals needed for experiments to reduce the uncertainty in risk to human health will be study design-dependent. Major factors influencing the design include statistical design relative to endpoints, individual variability in assay results, variance in dose delivery, and expected difference in critical endpoints at prospective radiation doses, and age and sex of NHP relative to human subjects. Many additional factors will impact the total duration of the project and the timing of the need for specific numbers of qualified NHP. Critical variables are: (1) The total number of NHPs; (2) Exposure time relative to radiation quality, dose and dose rate (in context of a space environment); (3) Radiation physics relative to radiation quality and ability to mimic the space radiation environment; (4) Availability of radiation source(s) for exposure and research sites with holding capacity and experimental setup for adequate throughput; and (5). Time-course of research relative to selected CNS endpoints for acute and late-effects (effect on throughput, holding capacity and availability and supply of the animals, especially if they have to be bred from birth for this effort). These factors and the time to reach the required NHP age, 15 years for macaques to simulate a human age of 48 years, will determine the breeding schedule and colony housing requirements. This will be a significant confounding variable in the experimental design. A research program will most likely require a vendor-managed consortium and potentially a consideration of multiple research sites.

If it is necessary, as indicated by small animal data, to assess a significant sex difference for endpoints using NHPs, there will be a significant increase in the total number of animals required. The research to reduce uncertainty in risk estimates will ideally require animals that are research naïve; sero-negative for STLV, SIV and malaria, negative for Herpes B virus and tuberculosis. The study design should include a preferred body weight (bw) range and age to minimize known variables. If the effects of different radiation mitigators were to be tested in a NHP model, additional animals would be needed. If these mitigators are not currently approved by the FDA for other conditions, additional animals for pharmakinetic studies in the NHP model would be required.

8.2 Radiation Effects and CNS Biology Endpoints/Outcome Measures

8.2.1 Model Development. An animal model is described as "…. a specific combination of animal species, challenge agent (radiation), and route of exposure (external) that produces a disease, process or pathological condition (CNS effect) that in multiple important aspects corresponds to the human disease or condition of interest." (FDA 2015) If proposed NHP experiments will include pharmaceuticals intended for use as medical counter measures, the FDA guidance criteria for a "well characterized" animal model will be applicable (FDA 2015).

Even if testing MCM is not planned, the animal model must be sufficiently "well characterized" for an accurate prediction of the human response to the radiation in the context of the space environment. The effort to characterize an animal model should include a "natural history" study to gain an understanding of the development and progression of the key radiation-induced CNS effects (FDA 2015). The observations along the time course of expected radiation effects should define the primary, secondary, and tertiary endpoints for CNS pathology and

possible treatment-triggers for medical management and administration of MCM. The experimental protocol should define the latency, time to onset of manifestations of injury, incidence, severity, progression and resolution of selected acute and late CNS pathology. Prior knowledge of the dose response relationships and selected outcome measures will aid in determining the frequency and timing of critical observations, secondary endpoints, or outcome measures and biomarker analysis.

The animal model should also be validated relative to three aspects: (1) Phenomenological validity; is there a confluence of signs, symptoms and biological processes that demonstrate that the model is similar to the human condition; (2) Predictive validity; this is paramount and indicates that performance of the animal model in a test or response to treatment is predictive of performance of the human response to radiation and treatment; (3) Construct validity; which implies that the model has a sound theoretical rationale relative to the network of associations that support our procedures and assumptions relating to the human condition.

8.2.2 Biomarker Analysis. The identification and analysis of biomarkers can be critical diagnostic tools and predictive of outcome relative to any number of clinical endpoints. The FDA in a guidance document for Alzheimer's disease defined the use of biomarkers as single primary and supportive secondary outcome measures (FDA 2013). It is emphasized that there should be "…widespread evidence-based agreement in the research community that the chosen biomarker reflects a pathologic entity that is fundamental to the underlying disease process."

Relevant outcomes to be assessed include acute effects (during the mission), such as development of the transient somnolence syndrome, altered cognitive function, including short-term memory, reduced motor function, and behavioral changes that affect performance and

human health, and late effects such as accelerated ageing and age-related cognitive decline, and neurological disorders, including Parkinson's disease, Alzheimer's disease, and other dementias.

The NHP chosen must be compatible, primarily in terms of size, with available source of simulated space radiation. This will depend on choice of TBI vs head-only models, omni-directional vs bilateral exposure and uniform, homogeneous exposure to the prescribed tissue point to simulate the omnidirectional space environment.

Relative biological effectiveness for selected radiations and endpoints must be estimated in order to design exposures. Uncertainty in the RBE will require bracketing doses, increasing the number of animals required.

8.3 Statistical Considerations.

The stochastic nature of biological responses makes it difficult to determine dose response relationships when the dose is low and the probability of seeing responders is small. In order to illustrate the difficulty in obtaining significant risk estimates from low probability data in isolation the effects at low dose sample calculations follow. They indicate that experimental studies that could detect differences in the 1-10% range would require populations exceeding 100 NHPs per dose point. The introduction of higher dose, higher probability data, would significantly enhance the evaluation if it were established that mechanisms are independent of dose.

Some recently accessed human data will be used to illustrate this issue. Since the data are presently unpublished, the adverse neurological complication is expressed in terms of its incidence and the dose is given in arbitrary units. The results from patients associated with eight different dose levels were available from this study, the group size varying from 19-82 cases. The lowest 4 dose groups were associated with a low incidence of complications that varied

between 3.9 and 11.1% (p = 0.039-0.111) for a small increase in dose, ~ 13%. With the higher dose groups, the incidence rose progressively to 42% (p = 0.42). For the purposes of the present illustration, the results of a standard probit fit to the crude incidence data for the whole data set (8 data points) were compared with those using only the 4 lowest doses where, the incidence of the specified event was low. The data imputed for the analysis are given below.

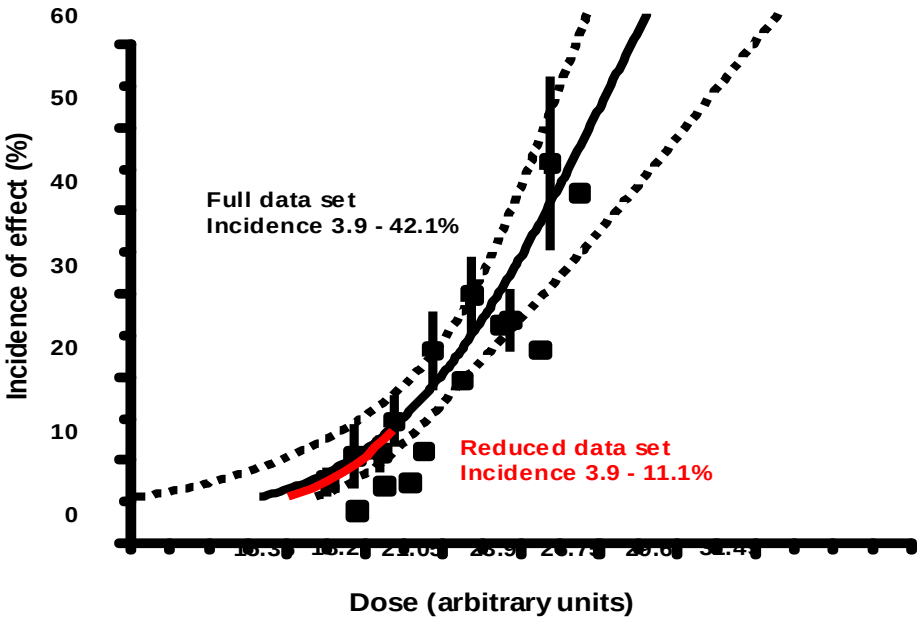
Table 8.1— Details of doses (arbitrary units), numbers of responders relative to those at risk for use in probit analysis.

Full data input

Dose	Responders	No. at risk	Probability
17.9	2	51	0.039
18.9	2	29	0.069
19.9	5	69	0.072
20.3	7	63	0.11
21.8	10	51	0.20
23.2	17	65	0.26
24.6	19	82	0.23
26.0	8	19	0.42

Reduced data input

Dose	Responders	No. at risk	Probability
17.9	2	51	0.039
18.9	2	29	0.069
19.9	5	69	0.072
20.3	7	63	0.11



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4751 **Fig.8. 1**. Dose-effect relationships fit to the complete data set and subset displayed in
4752 Table 8.1 are shown. Each data point is given with its associated standard error.
4753

For the full data set a clearly defined dose-effect curve was obtained with an associated 95% confidence interval. Dose values for an incidence rate of the complication of 10% or less are presented in Table 8.1. For the reduced data set, viewed in isolation, the impression of a trend for an increase in the response in relation to increasing dose is evident, however, no confidence interval was obtained since the differences in response between the minimum and maximum dose levels used did not reach an acceptable level of significance ($p > 0.05$). Therefore, on statistical grounds, an evaluation of a dose effect relationship CANNOT be made on the basis of such limited data. The only claim to be made from this reduced data set is that the average incidence was 7.5 ± 1.8% for the limited low dose range, even though a total of 212 cases were used in the evaluation. Also there would be no indication as to the relative position of these cases relative to higher doses where the risk increases very rapidly with increase in dose. In order to achieve a useful confidence level with data for doses below 20 (arbitrary units) the use of the full data set is required with the use of higher doses. This example based on patient data is typical of the results that would be expected from a NHP study of CNS damage. Thus, while restricted low-dose studies may suggest low-risk or even no risk of incidence of adverse events, they may not, even with a reasonable large number of cases involved, allow uncertainly estimates to be placed on that low risk. Since the complex dose response relationships (see figure 5.5) observed in many rodent experiments strongly suggest changes in mechanisms with increasing dose, it is unlikely that use of high dose points can be justified. Consequently "unreasonably" large numbers of animals exposed at low doses would be needed. These numbers will likely strain available resources for conducting NHP studies.

Thus, estimates for the number of NHPs required to design a potentially successful study would require careful planning when the endpoint(s) of greatest concern to be evaluated has be

agreed. Precise data from a research program using small animal models are essential for the design of NHP experiments to minimize the number of individuals needed and maximize the reduction of uncertainty in estimates of risk to humans.

Based on the current body of knowledge and nonhuman primate research efforts, the following recommendations are made:

1. An NHP program to confirm the magnitude of CNS risk should be considered only after there are sufficient rodent-based data to design a definitive protocol allowing a limited and focused experimental design.
2. When conducted, an NHP study should include validation of selected mitigators of CNS injury that have proven effective in rodent studies.
3. For these NHP studies, consider the development of an international consortium of research sites with appropriate radiation sources, animal populations and research expertise to address the two recommendations above.

Table 8.2 Percentage of incidence of an effect versus dose in arbitrary units, based on the fit to the full data set.

Probability	Dose	95% Fiducial Limits	
0.01	13.8	8.2	16.3
0.02	15.4	10.9	17.5
0.03	16.5	12.6	18.3
0.04	17.3	13.9	18.9
0.05	17.9	15.0	19.4
0.06	18.5	15.8	19.8
0.07	10.0	16.6	20.2
0.08	19.4	17.3	20.5
0.09	19.8	17.9	20.9
0.10	20.2	18.4	21.2

8.4 Recommendations

Based on the current body of knowledge and nonhuman primate research efforts, the following recommendations are suggested:

1. A NHP study to confirm the magnitude of CNS risk should be considered only after there is sufficient rodent based data to design a definitive protocol allowing a limited and focused experimental design.
2. When conducted, a NHP study should include validation of selected mitigators of CNS injury that have proven effective in rodent studies.
3. For these NHP studies, consider the development of an international consortium of research sites with appropriate radiation sources, animal populations and research expertise to address the two recommendations above.

9. Potential Interventions and Countermeasures

9.1 Acute Effects

9.1.1 Shielding

The radiation in space interacts with structural materials and added shielding in ways that modify the particle spectrum and often reduce the absorbed dose. However, the interactions are more complex than the processes which lead to exponential attenuation of photon radiation found in industrial and medical applications. Much of the energy of a cosmic ray particle is transferred to the electrons of the materials traversed, resulting in the collision stopping power. However, particles can also interact with atomic nuclei of the shielding materials resulting in a wide range of secondary radiations. The products of these "fragmentation" processes include other charged particles, neutrons, photons, and mesons. These products have different stopping powers, ranges, and mean free paths than the primary particles. The resulting absorbed dose distributions and radiation quality thus depends on the size, as well as the atomic composition, of the shielding structure. Consequently, precise estimates of absorbed dose and radiation spectrum require detailed information about the structure of the spacecraft as well as the spectrum of the incident radiation. However, some general characteristics of the effects of shielding can be provided for solar event particles and for galactic cosmic rays.

9.1.1.1 SEP. The particles produced by solar events, both impulsive events and coronal mass ejections, are primarily protons with a small fraction of He ions, but generally negligible numbers of heavier ions. The spectra of these particles vary significantly from event to event,

but are generally dominated by particles with kinetic energies less than a few hundred MeV/n. These particles have relatively short ranges and low stopping powers and the absorbed dose is generally reduced to acceptable levels by the inherent shielding of the structural materials of the spacecraft. In the case of an exceptionally large solar particle event additional shielding may be required in order to reduce the absorbed dose rate to an acceptable level, but this can often be accomplished by utilizing food and water stores to provide a small shielded volume. Solar event particles do have significant cross sections for interactions with nuclei of shielding materials, which result in production of target fragments including additional protons, heavier ions, and neutrons. The charged fragments all have lower velocity and shorter range than the proton that produced them, resulting in a shift in the radiation spectrum and possibly a change in the biological effectiveness of the radiation. The neutrons produced, being uncharged, deposit energy by creating recoil charged particles, primarily protons with still lower average energy. The neutrons have long mean free paths, Figure 9.1, so for small objects, the neutrons that are produced generally exit the object and do not deposit their energy in it. However, in objects with dimensions larger than the mean free path of the neutron, neutrons which leak out of the central region of the object are replaced by neutrons produced elsewhere. This situation is referred to as secondary particle equilibrium, and the energy deposited by neutrons is equal to the energy transferred to neutrons. Secondary particle equilibrium prevails for a volume which is equal to (or more than) the mean free path of the neutrons from the boundary of the object. Outside the region of secondary particle equilibrium the dose due to neutrons gradually decreases with distance until it reaches approximately half of the equilibrium value, at the surface of a large object.

The probability of producing a neutron in a fragmentation reaction depends on the proton velocity and the atomic structure of the target nucleus. Since normal hydrogen has no neutrons it can be used to absorb the energy of high energy protons without producing neutrons or other particles which would be likely to increase the biological effectiveness of the radiation beyond that which occurs due to the increased stopping power of protons near the ends of their paths.

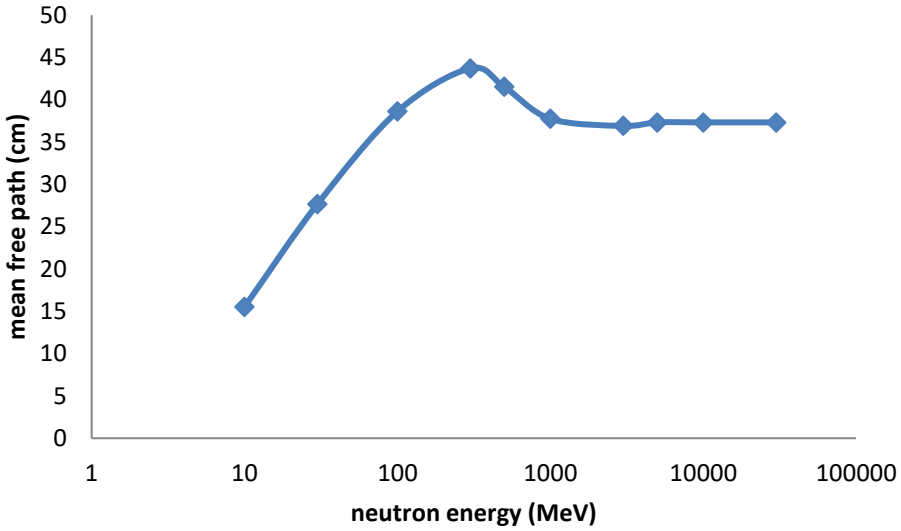

Fig. 9.1 The mean free path of neutrons in aluminum.

Consequently, shielding materials with a large hydrogen content, for example water or plastic, produce the least increase in radiation quality. As the Z of the target nucleus increases the average number of neutrons produced by fragmentation often increases, and various small nuclei may be produced. The ranges of the nuclei, often helium, are typically short and these particles are in secondary particle equilibrium, even in relatively small objects. Since their charge is often higher than that of the incident particle, and their velocity is lower, their stopping power is usually higher. For many biological endpoints this results in higher biological effectiveness, but this may not be true for all CNS endpoints.

9.1.1.2 GCR. The majority of the particles in the GCR spectrum are protons and He ions with energy distributions peaking around 1000 MeV/n. Heavier ions, with similar energy per nucleon, make up a significant fraction of the absorbed dose. These ions can fragment upon interaction with shielding nuclei, resulting in an increase in the number of particles. These fragment particles have lower Z and velocities up to nearly the primary particle velocity, so there is generally a decrease in the stopping power and therefore the absorbed dose. However, for aluminum (and higher Z) shielding the dose due to neutrons can become significant as the shielding thickness increases. Monte Carlo and analytical calculations have recently shown (Slaba et al 2017) that the dose equivalent produced by GCR incident on an aluminum shield decreases with shield thickness, up to about 20 g/cm^2, but increases at greater shield thickness, These results are depicted in Figure 9.2.

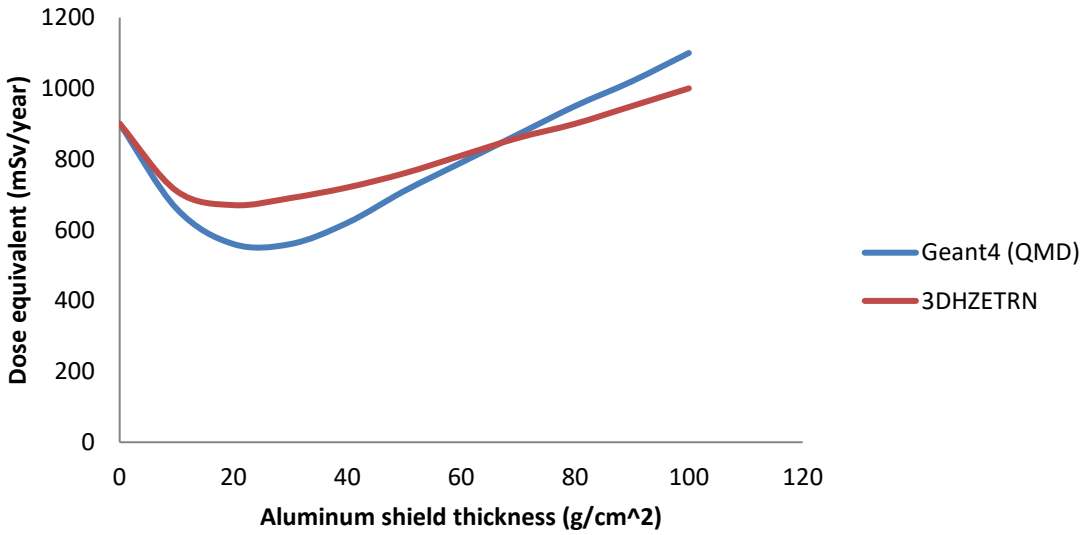

Fig. 9.2. Dose equivalent as a function of aluminum shield thickness as estimated by Monte Carlo (Geant 4, QMD) and analytical (3DHZETRN) calculations (Slaba et al 2017). The differences in the results are probably due to differences in the cross sections used as well as the difference in the computational methods.

The change in the distribution of particle types and particularly the buildup of relatively high LET recoil particles produced by neutrons, is likely to result in a change in the biological effectiveness with shielding thickness. The lack of data on CNS damage as a function of charged particle type and velocity makes it impossible to predict the effect of shielding on CNS risk.

9.1.2 Nutrition, Dietary Supplements, Exercise

9.1.2.1 Introduction. The use of countermeasures for mitigating potential CNS impairments from radiation exposure has been quite limited and not explored as fully as those for cancer. Many radioprotection strategies have been employed in clinical radiotherapy (reviewed in Kim *et al.* 2014) for a variety of tissue sites including the brain and serve as a guide for studies with charged particles. NASA funded studies involving countermeasures for CNS effects to date have largely focused on dietary supplementation (Rabin 2005, 2009) as well as the use of the antioxidant α-lipoic acid (Limoli 2007, Villasana et al 2013b). An important issue influencing CNS countermeasure identification is the realization that the effective target for radiation may be the elaborate cell processes of nerves and their dominant membrane component rather than or in addition to the DNA/chromatin target. This is similar to the situation with cancer where the importance of promotional effects of radiation exposure and influence of microenvironmental changes can drive the carcinogenic process and may be important targets for medical countermeasure evaluation in addition to countermeasures targeting DNA damage (Nelson et al 2016; Huff et al 2016). Also, the behavior/activity-driven responses of the CNS drive the tissue's functional status such that training, exercise, etc. represent unique modalities for

mitigating injury and environmental insults. However, in both situations the tissue-level environment, including inflammatory responses, extracellular matrix and networks of signaling molecules are recognized as important. Finally, the majority of NASA-funded work to date has focused on identifying the key biological and biochemical pathways regulating the CNS response to radiation required to lay the foundation for future studies that will have the primary goal of identification and/or development of mitigators and countermeasures (Nelson et al 2016). An example is the current work targeting neuroinflammation described in section 9.3 where a strong evidence base developed through previous research efforts and multiple investigators provided the groundwork allowing the performance of hypothesis driven medical countermeasure evaluation (Parihar et al. 2016, Krukowski et al 2018). The following illustrates some of the strategies employed for CNS countermeasures focused on dietary supplements and antioxidants, and captures many NASA funded or co-funded studies, but is not exhaustive for radiotherapy-related or other investigations using these categories of countermeasure agents.

9.1.2.2 Nutrition. Maintaining proper nutrition and an adequate food system are major concerns for exploration class missions where resupply will not be possible. Sustaining acceptable nutrient intake is essential for the maintenance of adequate physiological function and may be useful in protecting against maladaptive processes associated with the many stressors of spaceflight including risks associated with space radiation exposure. HRP supports research focused in these areas under the Risk of Performance Decrement and Crew Illness Due to an Inadequate Food System and Risk of Inadequate Nutrition (Douglas 2016, Smith and Zwart 2015). Nutrition research addresses issues related to determining the nutritional requirements for spaceflight, how nutritional status changes during spaceflight, and what the most important nutritional requirements are to address many of the health impacts associated with spaceflight (Smith and

Zwart 2008, Lane et al. 2013), while food research addresses issues related to food safety, processing, storage, nutrient content and acceptability (Perchonok et al. 2012). Research to date addressing the use of nutritional components and supplements to protect against adverse outcomes associated with space radiation exposure falls into the categories of dietary supplementation and antioxidant agents.

9.1.2.3 Dietary Supplementation. Work by Bernard Rabin, Barbara Shukitt-Hale, and the late James Joseph explored dietary supplementation using a variety of berries to provide anti-oxidant/anti-aging capacity (Rabin *et al*., 2005, 2009; Shukitt-Hale *et al*. 2007). Beginning in 2005 rats were placed on diets containing 2% blueberry or strawberry extract for 2 months prior to exposure to 1 - 2 Gy ^{56}Fe particles and strawberry diet was found to improve performance on an operant conditioning task after 6 months in irradiated animals. The supplementation was found to be ineffective when behavioral testing occured 13 – 18 months post irradiation. In 2007 the group extended observations to spatial learning and memory tasks using the Morris water maze system and measures of neurotransmitter secretion. They found that both strawberry and blueberry extracts were protective 8 weeks after 1.5 Gy of ^{56}Fe irradiation for behavior and protective for radiation-impaired potassium evoked dopamine release in brain slices at 1 month post irradiation. In 2009 the work was extended to determine if shorter dietary treatment was effective and generalizable to other behavioral measures. 2% and 4% berry extracts provided for 2 weeks prior to irradiation were found to improve radiation-induced impairments in novel object recognition memory in animals irradiated with 80 – 150 cGy iron particles (but not with 50 or 200 cGy, an example of an U-shaped dose response). In 2014 (Poulose et al. 2014) the group examined neurochemical changes at 36 hrs and 30 days after 1.5 Gy head-only iron particle exposure in animals fed berry diet for 8 weeks pre-irradiation. The irradiation produced

increases in numerous inflammatory and oxidative stress markers and anti-oxidation enzyme levels. The dietary countermeasure was found to reduce radiation effects on p62 and Beclin1 levels that mediate autophagy (to eliminate damaged proteins) and reduced radiation-induced accumulation of Tau proteins (related to Alzheimer's disease) in hippocampus. The diet also resulted in improved antioxidant enzyme levels at 30 days in irradiated animals. In 2017 the group looked at rats at early time points and showed that blueberry supplementation significantly reduced radiation-induced elevations in several measures of oxidative stress and inflammation as well as improving radiation-impaired novel object recognition behavior 24 – 48 hours after irradiation with 25 cGy of iron particles (Poulose et al. 2017).

9.1.2.4 Antioxidant Agents. Experiments with knockout and transgenic animals have implicated oxidative stress and free radicals in causing damage to biological systems and molecules. Accordingly, replacement or augmentation of enzyme activities that dampen oxidative stress and free radical formation have been tested. 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. 2011b, Rola et al. 2007) even though baseline neurogenesis was impaired. Enhancing H_2O_2 detoxification capacity using a catalase-overexpressing transgenic mouse (MCATtg) suppressed proton-induced impairment of neurogenesis (Liao et al. 2013) and cognition (Olsen et al. 2013, Parihar et al. 2015a). In related studies funded by NIAID/NIH the use of a metalloporphyrin compound EUK207, a catalytic free radical scavenger that acts as a mimetic to the enzyme superoxide dismutase, provided protection against radiation induced cognitive impairments in irradiated mice (Raberet al. 2017a).

In NASA and Alzheimer Foundation-funded projects the Raber group also tested the antioxidant α-lipoic acid (ALA) as a dietary supplement given beginning two weeks prior to (and continuing through behavioral testing) brain only irradiation of young adult male C57Bl/6 mice with 3 Gy of accelerated iron ions (Villasana et al. 2013b). The ALA prevented radiation-induced impairments in spatial memory retention measured by hippocampus and cortex-dependent water maze tests administered 3 months post-irradiation. Measures of anxiety were unaffected by the ALA. Tseng et al. (2013) and Limoli et al. (2007) 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 also radioprotective (Manda et al. 2008).

In a study of rat spinal cord irradiation, Peker et al. (2004) administered magnesium sulfate and/or the antioxidant Vitamin E for 5 days prior to irradiation of a thoracic spinal column segment with 6 MV x-rays at a dose of 20 Gy. They found that lipid peroxidation products such as malondialdehyde were reduced in agreement with a number of prior studies cited.

Using immortalized mouse hippocampal neuronal cells and the Zebra fish, Liao et al. (2016) investigated the efficacy of valproic acid, as a radioprotectant, acting through the antioxidant pathway controlled by the Nrf-2 transcription factor. Valproic acid has been previously shown to be neuroprotectant in several disease models. HT22 cells were irradiated with 6 Gy of 6 MV X-rays and wild type fish were head-only irradiated with 60 Gy in three consecutive daily fractions of 6 MV X-rays. Valproic acid was injected 3 hrs pre-irradiation in the fish and added to medium of cells. In both cells and hippocampus samples from fish the levels of reactive oxygen species, malondialdehyde were decreased and reduced glutathione and superoxide dismutase were increased in valproate treated samples relative to radiation only

samples. In valproate treated cells, Nrf-2 nuclear translocation and heme oxygenase-1 (principal target of Nrf-2) protein levels were elevated at 24 hrs post irradiation relative to untreated samples. Behavioral performance of animals in an exploration task 30 days post irradiation also exhibited radioprotection with valproate.

9.1.2.5 Exercise. Exercise has been shown to be an effective countermeasure to the rapid degeneration of musculoskeletal system that occurs following exposure to microgravity for even relatively short periods of time. The daily routine for astronauts aboard the international space station includes time designated for exercise using a suite of exercise equipment including a specially designed treadmill with a harness to hold the astronaut in place, an exercise bicycle and a resistive exercise device. For long duration space mission exercise is expected to be an effective countermeasure for microgravity associated physiological deconditioning for a number of organ systems including bone, muscle, cardiovascular, and sensorimotor systems (Ploutz-Snyder et al. 2015). Earth based research also clearly demonstrates the positive impacts of exercise on neurocognitive performance and mood, as well as its protective effects against processes associated with aging (Ratey and Loehr 2011, Hötting and Roder 2013, Garatachea et al. 2015). For radioprotection and mitigation, exercise is recognized as a potential strategy to counteract tissue damage associated with high dose radiotherapy (Berkman and Lakoski 2015). It is suggested as a potential candidate to ameliorate deleterious effects of space radiation exposure on both the CNS and the cardiovascular systems. However, supporting evidence, as summarized below, is limited at this time.

Exercise is suggested as a potential method to prevent cognitive impairments associated with childhood radiotherapy based on studies performed with juvenile rodents (Sahnoune et al. 2018, Naylor et al. 2008). Exercise has also been shown to offer protective effects against

radiation exposure when administered in older adult animals. In a study by Ji et al, (2014), rats received whole brain irradiation with 20 Gy and subjected to two daily sessions of running wheel exercise for three weeks at high, low or medium levels of exercise (wheel rotation rate). Behavior was assessed with open field and water maze tests, neurogenesis via anti-BrdU staining and immunofluorescence/Western blotting of BDNF neurotrophin pathway components. Forced running exercise significantly prevented radiation-induced cognitive deficits, ameliorated the impairment of hippocampal neurogenesis, and moderated the down-regulation of BDNF pathway components. Further investigation using radiation doses and qualities appropriate to the space radiation environment are necessary to determine the potential benefits of exercise for preventing behavioral and cognitive decrements associated with spaceflight.

9.1.3 Pharmacotherapeutics

9.1.3.1 Pharmacological Countermeasures for Space Missions. A broad range of drugs are supplied to the astronauts for medical purposes from headaches and nasal congestion to anesthetics for minor surgeries (Santy and Bungo 1991; Wotring 2015). Some of the drugs can be considered specific countermeasures for physical or physiological conditions such as space adaptation syndrome (SAS), sleep-wake cycle disturbances, pain, minor infections, skin conditions such as rash, allergy and asthma, gastrointestinal distress and heartburn, seizures, and eye conditions. Others drugs provided in the nine color-coded medical kits (NASA 2007) can be considered countermeasures for combating behavioral health issues such as concerns over alertness, depression, psychoses, and anxiety. None of the drugs currently supplied, however, are provided as a countermeasure for radiation exposure, even though some of the drugs available could be used to deal with specific components of an acute radiation syndrome (ARS)

such as nausea and vomiting. The reasons for the lack of countermeasures for space radiation are numerous. First and foremost, there are no FDA-approved drugs specifically for this purpose (Singh et al. 2014). Amifostine (WR2721, 2-(3-aminopropyl) aminoethylphosphorothioate), probably the most well-known and studied radioprotectant, is the only systemically-effective drug that has FDA approval for use in humans, but its use is limited to radiotherapy for head and neck cancers and it can produce debilitating adverse effects such as hypotension, nausea, and vomiting. Other reasons for the lack of radiation countermeasures include the paucity of data and knowledge surrounding, 1) potential short-term and long-term effects of mixed field, high LET exposures, 2) contribution of localized cellular and neuronal damage to functional deficits, 3) need for prophylactic versus mitigating (post-radiation) countermeasures, 4) mechanisms by which short-term exposures ultimately lead to late or delayed effects, 5) extent to which radiation exposures can synergize with factors associated with space travel or with behavioral health factors such as stress, and 6) extent to which some countermeasures might contribute to delayed effects.

9.1.3.2 Pharmacokinetics and Pharmacodynamics of Drugs. Regardless of the pharmacotherapeutic countermeasures that are eventually developed, the effectiveness of these countermeasures will be determined by both their pharmacokinetics and pharmacodynamics – just as these principles of drug action determine the effectiveness of the drugs that are currently used during space travel (Derendorf 1994; Gandia et al. 2004; Graebe et al. 2004; Kast et al. 2017; Lathers et al. 1989a). In short, the pharmacologic response of any drug depends on its concentration at the site of action; however, the capacity of a drug to get to its site of action depends on a number of pharmacokinetic factors such as its absorption and distribution (for a review of basic principles, see Backes and Moerschbaecher (2008)). These factors, in turn, can

be affected by a variety of variables. Absorption, for example, can be governed by the pH of the gastrointestinal tract, route of administration, first-pass metabolism, and gastrointestinal motility and integrity. Distribution, or the extent to which a drug can move from its site of administration to its site of action, can be governed by plasma protein binding, blood flow, specialized cell membranes or barriers, and the pH of various body compartments (plasma, tissue, and the intracellular space). Another pharmacokinetic factor that contributes to drug action, but after a drug has reached its site of action, is drug elimination. Drugs can be eliminated unchanged or can be converted by metabolic reactions (biotransformations) to metabolites, and the rate at which a drug is eliminated determines its duration of action. Elimination is generally controlled by the liver and kidney, though some drugs can be excreted in small amounts by other means (e.g., sweat glands).

In addition to all of the factors that can influence a drug's absorption, distribution, and elimination, there are also factors that can influence its capacity to produce a response at the site of action or its pharmacodynamics (Kenakin, 2014). Drugs produce their effects through an interplay with existing biochemical and physiologic systems within the body, and therefore, do not have inherent effects (i.e., they only modulate ongoing physiologic processes). For example, drugs that influence heart rate or blood pressure act on the part of the autonomic nervous system that controls those processes. The means by which drugs typically affect those processes is by binding to specific macromolecules or drug targets. These targets could be G-protein coupled receptors, ion channels, nuclear receptors, transporters, enzymes, or DNA. Moreover, drugs typically bind to the same binding sites that are used by endogenous neurotransmitters, and that is the means by which they alter ongoing physiological processes. If a drug is able to bind to a site and produce a response, it is considered to be an agonist because it has both affinity for the

site and efficacy. If a drug is only able to bind to the site, it is considered an antagonist because it only has affinity. Nonetheless, antagonists are able to modulate physiological processes by preventing other substances, including neurotransmitters, from activating a particular site. Agonists and antagonists along with some allosteric modulators, enzyme modulators, and transporter inhibitors comprise the bulk of the drugs supplied to the astronauts as countermeasures for both physiological and behavioral conditions that arise from space travel. Include the antibiotics and antivirals on this list and you have almost all of the drugs sent in the white, red, purple, and brown medical packs (NASA 2007).

9.1.3.3 Effects of Space Travel on Drug Disposition. Chief among the challenges to developing a pharmacotherapeutic countermeasure for radiation exposure during space travel is the fact that space travel alone markedly alters many of the physiological processes of the astronauts (Derendorf 1994; Graebe et al. 2004; Kast et al. 2017). Further, if drugs can only modulate ongoing physiological processes, the effects of space travel on those processes must first be clearly established before the effectiveness of a radioprotectant or mitigator can be determined (Santy and Bungo 1991; Williams et al. 2009). Take, for example, the manner in which microgravity can affect the pharmacokinetics of many drugs regardless of the route of administration (Brunner et al. 2000; Gandia et al. 2005). As shown by the classic NASA diagram, Figure 9.3, in Lathers et al. (Lathers et al. 1989a), blood flow and body fluids go through three distinct states during and after space flight compared with the distribution that occurs while under Earth's gravity. Immediately after entry into space, fluids shift toward the head and chest, whereas during adaptation to near weightlessness the distribution of fluids toward the head is maintained, but there is a notable reduction in blood volume and fluids. Both of these states also differ from the fluid distribution that occurs upon return, where fluids again

shift toward the feet and there can be a reduced flow to the head and upper body. Not surprisingly, each of these fluid phases could alter drug action by altering its distribution, which depends on blood flow, tissue perfusion, and the concentration gradient (Brunner et al. 1995; Gandia et al. 2004; Grindeland et al. 1990). This is not to say that these changes are insurmountable, or that simple changes in dose would not compensate for these shifts in fluid distribution, but to point out that this is just one variable produced by space flight alone that can alter drug action and the extent to which a drug is effective in treating a particular condition (e.g., pain in the lower extremities or inflammation in the brain).

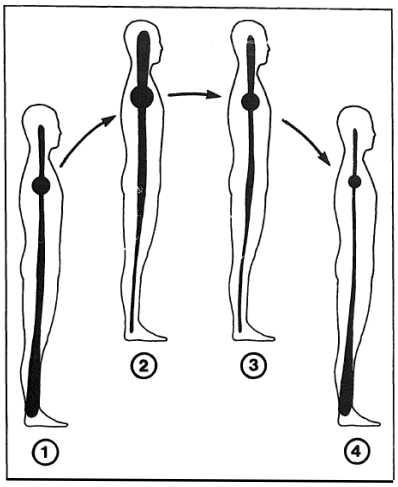

Fig. 9.3 Distribution of body fluids on Earth (1), shortly after launch (2), after adaptation to near weightlessness (3), and immediately after return (4).

Microgravity can also affect drug absorption by the oral route of administration, which is the route favored for most medications due to its safety and convenience. More specifically, as GI transit changes, so does the absorption of drugs from the stomach and small intestine (Amidon et al. 1991; Derendorf 1994; Tietze and Putcha 1994). Oral administration of drugs would also be complicated by space-induced motion sickness, which can persist in some astronauts beyond the initial space adaptation period (Santy and Bungo 1991; Thornton et al. 1987). Needless to say, frequent or even unexpected vomiting would alter the drug levels of any drug administered orally and potentially lower or raise the levels of any drug being taken chronically for a particular condition. Though some of these difficulties can be overcome by changing the route of administration, the particular drug will have to be supplied in a formulation or delivery system that allows for administration by alternate routes (e.g., i.v., i.m., or subcutaneous). Again, microgravity is just one factor of space flight that can affect the pharmacokinetics of a drug and affect it in multiple ways. Other factors associated with space flight that could clearly influence pharmacokinetics are: the amount of carbon dioxide (CO_2) in the environment, which could change pH in various body compartments and prevent drug movement due to ionization (Kellum 2000; Law et al. 2014); reductions in specific hepatic enzymes that are involved in hepatic metabolism (Merrill, Jr. et al. 1990), which could alter drug elimination; and reductions in immune function due to loss of hemopoetic cells (Crucian et al. 2008; Kaur et al. 2005; Pierson et al. 2005), which could increase susceptibility to asthma, allergy, or infection. Finally, there is also evidence that microgravity during space flight can affect pharmacodynamic mechanisms as well (Goldermann and Hanke 2001; Joseph et al. 1992). For example, Goldermann and Hanke (2001) using porin channels incorporated into crude membrane fractions of E. coli have shown that microgravity can influence the open-state probability of native ion channels, which could

potentially change the effectiveness of any drugs that were activated or inhibited by those channels. Among the drugs working at ion channels are some of the drugs provided for sleep (e.g., zolpidem) and anxiety (e.g., diazepam).

9.1.3.4 Determining the Effects of Radiation Exposure and Space Travel on Drug Disposition. As mentioned above, determining all of the potential pharmacokinetic and pharmacodynamic effects of space radiation in combination with space travel is an unmet challenge, and there are even less data on the combined effects than on space flight alone (Esposito et al. 2001a). Moreover, most of the laboratory models that were developed for use in animals and humans were developed to study the effects of microgravity, such as tail suspension in rodents (Brunner et al. 1995; Gandia et al. 2004) and head-down bedrest in humans (Kates et al. 1980). The use of animal models, in particular, needs to be expanded to include irradiations with the types of exposures that will likely occur in space. Although this may not be possible with the human models, the Animal Efficacy Rule (Crawford 2002) could provide a rationale for testing in some higher-order species, such as nonhuman primates if feasible (see section above). Just as pharmacologic countermeasures for most conditions in space must take into consideration the time after launch, so will pharmacologic countermeasures for space radiation (Gandia et al. 2005; Lathers et al. 1989a). For example, studies will need to investigate whether radiation exposures immediately after launch produce more damage due to the disruptions produced by space motion sickness [SMS] (Lathers et al. 1989b; Thornton et al. 1987), and whether pharmacologic countermeasures for radiation are as effective after administration of a drug or drugs used to treat SMS. Additional studies will also need to determine how the redistribution of fluids toward the head after launch, and compensatory loss of fluid shortly thereafter, affects the absorption and distribution of pharmacologic countermeasures for radiation (Klockowski and

Levy 1988; Moore and Thornton 1987). To date, the answers to these empirical questions are predominantly extrapolations from the fairly limited research that has been conducted.

Determining the types of pharmacologic countermeasures that will be required is particularly difficult to establish in the absence of data on the long-term effects of space radiation. Being able to prioritize the effects of GCR would, at least, help determine a therapeutic approach (e.g., prophylactically protect or mitigate after assessing exposure). Such a prioritization is no small matter, however, as research has already established that the radiation exposures expected in space could be potentiated by space travel. Thus, radiation could potentiate space-induced disruptions or damage to many systems and physiological processes: 1) the microvasculature, 2) ongoing peripheral or central inflammation, 3) protective barriers of the GI tract and brain, 4) demineralized bone, 5) plasma proteins or other proteins, 6) bone marrow and immune cells, and 7) oxidative stress. Each of these factors could then, in turn, modify the effectiveness of pharmacologic countermeasures by altering their pharmacokinetics or pharmacodynamics. Briefly, changes to the microvasculature or plasma proteins could reduce drug distribution and the effectiveness of heavily plasma protein bound drugs such as the antidepressants (Frazer 2001). Inflammation, for example, can change the pH of tissue and thereby the effectiveness of local anesthetics for dental or other minor surgical procedures, whereas loss of bone marrow and immune cells could reduce the effectiveness of anti-infectives for treating asthma, allergies or infections. If any of these particular radiation-induced effects persist after the astronauts return (Kaur et al. 2005), then some of these alterations in the pharmacokinetic and pharmacodynamic properties of certain drugs might persist as well. Another possibility is that the pharmacokinetics and pharmacodynamics are again modulated in response to the physiological changes that take place upon return (e.g., Fig 9.1). Investigators studying these changes will also have to

determine whether certain physiological systems actually return to the pre-flight levels (Bandstra et al. 2009; Miousse et al. 2014), and thus, a drug's preflight effectiveness. If the preflight baselines of some physiological systems are not re-established, as suggested by some papers and the "age-radiation parallel" hypothesis (Joseph et al. 1992), then this would permanently change the actions of the drugs that work through those systems.

9.1.3.5 Development of Pharmacologic Countermeasures for Exposure to Space Radiation. There are a number of excellent reviews and books on the progress that has been made in developing pharmacologic countermeasures for radiation (e.g., Koukourakis 2012; MacVittie et al. 1996; Seed 2005; Weiss and Landauer 2009), including the recent review by Singh (2014). There has also been a resurgence of research and interest in the nutritional, nutraceutical, and pharmacological means for countering the effects of radiation of all types. Nevertheless, a major challenge for the development of a pharmacologic countermeasure for space radiation is determining whether the pharmacologic countermeasures developed to date will be appropriate for the types of radiation encountered in space and can serve as a springboard. Researchers have tended to follow two distinct lines of reasoning. One line of reasoning posits that the pharmacologic countermeasures we have currently will not be of value as they were developed for high doses of low LET radiation and were not developed specifically for protection of the CNS. Thus, many have poor BBB penetration. The second line of reasoning posits that radiation damage is radiation damage and that its effects are dependent on the dosage, exposure rate, and quality of the ionizing radiation. Moreover, if radiation produces free radicals for example, a free radical scavenger could serve as a countermeasure in both the periphery and the brain.

The first line of reasoning is supported by the fact that the pharmacologic countermeasures that seem to have advanced the furthest toward FDA approval are those that have been

5251 developed to mitigate an ARS resulting from a nuclear accident, warfare, or terrorist bomb, and
5252 not the exposures likely to occur on a space mission (Allen et al. 2014; Hankey et al. 2015;
5253 Rabin et al. 1993). The major clinical components of ARS characterized by Singh (2014)
5254 include the hematopoietic (2-6 Gy), GI (6-8 Gy), and cerebrovascular (>8 Gy) sub-syndromes
5255 because these systems have cells that are highly susceptible to damage. Furthermore, as cancer
5256 patients typically receive fractionated high-dose exposures (Hopewell and van der Kogel 1999;
5257 Koukourakis 2012) some of these same countermeasures are now being approved or proposed as
5258 potential mitigators for the adverse effects of radiotherapy. The problem for researchers then is
5259 that barring a large SPE, they know that the exposures experienced by astronauts in space will be
5260 markedly lower than those experienced in these other situations. This knowledge, in turn, raises
5261 questions as to whether the same countermeasures would be appropriate for space radiation.
5262 Support for the second line of reasoning comes from the fact that even low-dose high LET
5263 radiation will likely affect susceptible cells; therefore, there will likely be some hematopoietic,
5264 GI, cardiovascular, and cerebrovascular effects given the overall susceptibility of the cell-types
5265 in these areas of the body (Boerma et al. 2015; Esposito et al. 2001b; Koukourakis 2012;
5266 Miousse et al. 2014), and brain in particular (Parihar et al. 2016; Tofilon and Fike 2000;
5267 Zlokovic 2011). If this is the case, the good news is that many of the radiation countermeasures
5268 currently under development may also have potential as countermeasures for space radiation, but
5269 they will have to be examined under the conditions likely to occur during space travel.
5270 Additional positives are that countermeasures for radiation exposure in space could simply
5271 require smaller doses of the various countermeasures, which could lessen any toxicities or
5272 neurobehavioral deficits associated with those countermeasures (Landauer et al. 1992; Maisin
5273 1988). One of the overarching concerns with some of the countermeasures under development

(i.e., for exposures that produce an ARS) is that they could be 'saving' cells that have radiation-induced DNA damage, as DNA mutations or altered genomic methylation patterns resulting in genomic instability could lead to delayed or late effects (Koukourakis 2012; Nelson et al., 2016). Another concern is that some countermeasures only ameliorate a subset of manifestations of radiation injury; thus, combinations of drugs may be needed as suggested many years ago (e.g., Maisin 1988) and recently reiterated (e.g., Singh et al. 2014). Based on the work by the radiation countermeasures program at AFRRI, the effectiveness of some pharmacologic countermeasures for radiation can also depend on the radiation quality (Cary et al. 2012). Therefore, the countermeasure would ideally need to be tested against radiation qualities appropriate for specific exposure scenarios before they become part of a response plan. Pharmacological studies of the effectiveness of potential countermeasures must also evaluate more than a single dose unless previous studies have already determined an effective, non-toxic, range of doses. Unfortunately, the literature is filled with single-dose studies, and in many instances this is all the information we have on some countermeasures. The results from single-dose drug studies are also particularly difficult to interpret when they involve an inverted u-shaped curve as has been proposed for some of the effects of space radiation. More specifically, when the curve is an inverted u-shape, there is little way of knowing whether or not the change produced by the interaction was a leftward or rightward shift in the radiation curve.

Currently, both drugs and biologics have been proposed as pharmacologic countermeasures for radiation exposures capable of producing an ARS. In general, "biologics" refer to genetically-engineered proteins derived from human genes, and they produce their effects by modifying specific components of the immune system. Given that many biologics are antibody-based, they follow the principles of immunology rather than the pharmacologic principles that

guide drug action. Nevertheless, whether drug or biologic these countermeasures fall into two broad categories, either prophylactic or palliative, with the realization that some of countermeasures would be appropriate for both categories. Simply put, this is because an individual will need to be protected from the same deleterious effects that will require mitigation if there is an exposure (e.g., inflammation, oxidative stress, microvascular or cerebrovascular damage, myelosuppression, and any behavioral or cognitive disruptions). What could differ, however, is the dose, frequency, and duration of treatment or countermeasure. Take anti-inflammatories, for example, the dose of non-steroidal or steroidal anti-inflammatory necessary for prophylaxis could be quite different from the dose necessary to alleviate inflammation that is already present or injury induced. Likewise, the prophylactic dose of one of the three types of free-radical scavengers (i.e., polyamines, superoxide dismutase promotors or organosulfur compounds) would probably be much lower than the dose needed to treat the free radicals generated from a large exposure. In the ideal situation, there would be enough prophylactic anti-oxidant activity from dietary supplements (e.g., strawberries and blueberries) or vitamins (A, C, and E) that other drugs would not be required. Some of these substances, or analogs of these substances, are also noteworthy because they could serve as palliative countermeasures if necessary (Krager et al. 2015). For example, the vitamin E analogs delta-tocotrienol (DT3) and gamma-tocotrienol (GT3) have been shown to have significant protective and mitigative capacity against the detrimental effects of doses of ionizing radiation above 10 Gy (for a review cf. Singh et al. 2013).

Underlying many of the most promising pharmacologic countermeasures is the capacity to serve as leukocyte growth factors by stimulating granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF). Leukocyte growth factors,

such as filgrastim, sargramostim, pegfilgrastim, and the new biosimilar pegfilgrastim-jmdb, have FDA approval for the treatment of chemotherapy-induced myelosuppression; however, they have not yet received approval for myelosuppression resulting from acute radiation exposure even though they have been used to treat the hematologic symptoms of ARS after radiation accidents (Hankey et al. 2015). Approval for treating the effects of radiation exposure had been delayed mostly due to the fact that the development and testing must be in accordance with the Animal Efficacy Rule. Nevertheless, filgrastim (Neupogen) and sargramostin (Leukine) are expected to obtain FDA Emergency Use Authorization and both are available in the Strategic National Stockpile (SNS, Singh et al. 2014). With their potential approval by the FDA, these drugs clearly mark an advance in our options for treating the hematologic effects of radiation exposures, but their applicability and value for exposures to space radiation remain largely unknown. The same is true for the seven radiation countermeasures with IND status (i.e., androstenediol [5-AED/Neumune], OrbeShield/BDP, BIO 300/genistein, CBLB502/entolimod, HemaMax/NMIL 12-1, and ONO1210/ExRAD), and the four substances in advanced stages of development (i.e., amifostine/ethylol, GT3, AEOL 10150, and myeloid progenitor cells/CLT-008). Information on each of these 11 potential countermeasures can be found in the most recent review by Singh et al. (2014), as well as what is currently known about 16 other potential countermeasures that have been proposed for development. Although much less is known about these 16 substances, there are clearly some underlying themes: growth factors to compensate for hematopoietic damage (fibroblast growth factor peptide, insulin-like growth factor, 17-DMAG, and the thrombopoietin mimetic ALXN4100TPO) as well as GI damage (palifermin, somatostatin, and R-spondin1), antioxidants for oxidative stress (superoxide dismutase and oltipraz), DNA stabilizers for preventing DNA instability (phenylbutrate), and anti-inflammatories for acute or chronic

inflammation (anti-ceramide antibody). Outside of these 16 potential countermeasures are also some drugs that could help preserve the cardiovascular and cerebrovascular systems. For instance, dexrazoxane (Zinecard) is an iron chelator that can serve as a cardioprotectant, difluoromethylornithine (DFMO) is an ornithine decarboxylase inhibitor that can reduce cerebral ischemia by reducing vascular permeability and vasogenic edema (Allen et al. 2014), and various thiol donors. A review of the literature will also show that countless drugs have been proposed and tested as countermeasures for radiation exposure, but the aforementioned drugs are some of the most promising and those that appear to affect many of the same biochemical pathways that are affected by radiation. Given the overall concern with protecting the CNS during the proposed mission to Mars increased attention will have to be devoted to countermeasures that cross the BBB and that protect not just neurons, but also their dendritic projections (Duman et al., 2018; Parihar et al., 2015; Wilke et al., 2017). The n-methyl-d-aspartate (NMDA) glutamate receptor antagonist memantine, for example, has been suggested as a countermeasure for the radiation-induced increase in glutamate observed after high-dose exposures in brain areas such as the hippocampus (Duman et al., 2018). Whether or not a drug such as memantine could be used as a protectant rather than mitigator remains to be seen. Not surprisingly, glutamate transmission is also critical for learning and memory processes in the CNS and blocking its receptors chronically for radioprotection could be problematic for these processes.

9.2 Countermeasures

9.2.1 Impact of Habitat and Vehicle Design on late risks to the CNS

During extended-duration exploration class space missions, astronauts will be exposed to long periods of isolation and confinement, with an overall reduction in sensory stimulation. Extended timeframes spent within this type of stressful environment, along with disruption of

sleep and circadian rhythms, may lead to potential adverse psychological and behavioral outcomes that have the potential to negatively impact inflight operations and jeopardize success of the mission (Pagel and Chouler, 2016). There is also concern that if these behavioral conditions remain undetected and unmitigated that they may lead to the development of mental disorders, which could influence long term health outcomes and quality of life for the astronauts.

Anecdotal evidence from spaceflight experience and research using ground based spaceflight analogs show that habitat modifications can have significant benefits on the behavioral risks posed by extended exposure to the harsh spaceflight environment (Stuster, 2010; Palinkas and Suedfeld, 2008). Research conducted by the NASA Human Factors and Behavioral Performance element aims to identify and validate the most effective methods for modifying the habitat/vehicle environment as mitigation strategies for these behavioral health risks. Current strategies under investigation include techniques using virtual reality, plant growth, methods for sensory augmentation and optimization of habitat/spacecraft lighting. A full description of the available evidence and research plans can be found in the NASA Human Research Roadmap under the Risk of Adverse Cognitive or Behavioral Conditions and Psychiatric Disorders (NASA, 2016; Human Research Program Integrated Research Plan).

9.2.2 Shielding

The effects of shielding on the radiation spectrum are described in section 9.1.1. Since the relative biological effectiveness of each component of the radiation spectrum may be different for prompt and delayed damage, the consequences of the change in the radiation spectrum resulting from shielding are likely to differ. Therefore, the characteristics of shielding required to minimize the risks from prompt and delayed health effects may differ, and it may not be possible to simultaneously minimize the risk of prompt and delayed health effects. Precise

information on the dependence of each biological effect on the charge and velocity of the radiation, as well as the absorbed dose and dose rate, will be necessary in order to properly evaluate the effects of shielding and establish criteria for shielding.

9.3.3 Pharmacotherapeutics

9.3.3.1 Challenges to developing pharmacologic countermeasures for the late effects of radiation exposure. Determining whether or not appropriate pharmacotherapeutics exist (or will exist) for the late effects of space radiation is even more difficult than determining what might be appropriate for the acute or short-term effects, because even less seems to be known about the ontogeny of the late effects. Moreover, knowledge concerning the appropriate pharmacotherapies will largely have to be derived from secondary sources, such as data from studies employing the Animal Efficacy Rule (e.g., Hankey et al. 2015) and from patients undergoing radiotherapy for various cancers (e.g., Hopewell and van der Kogel 1999; Sokol et al. 1978), which do not often approximate the exposures that are likely to occur during lengthy space missions (Cucinotta et al. 2008). This complication alone will inevitably slow the development and implementation of countermeasures that can protect or mitigate against the late effects. Another complication that thwarts many treatments for neuropathological disorders is knowing exactly when to treat the disorder if there is no overt neurobehavioral disruptions to indicate that damage has occurred. This is the same impediment that obscures our understanding of the origins of progressive neuropathological diseases such as Parkinson's (i.e., by the time symptoms appear there is pervasive underlying damage to the extrapyramidal system). Due to this impediment, treatment of Parkinson's is limited to palliative pharmacotherapies that fail to stop the progression of the disease, a less than ideal approach or situation. In radiation research, this impediment to treatment is exemplified by the Casarett model of late tissue effects, which

posits that significant effects will occur at lower doses, but with increased latency compared to higher doses (Cox et al. 1983). Take as examples, 1) the increased incidence of cataracts observed long after low doses of space radiation where only a small fraction of cells in the tissue were damaged (cf. Cucinotta et al. 2014), 2) the persistent GI disruptions that occur following abdominal radiation therapy (McDowell and Brown 1965; Sokol et al. 1978), and 3) the apparent radiation-induced senescence of fibroblast cultures from the skin biopsies of rabbits and monkeys (Bergtold et al. 1983). According to Cucinotta (2014), the data for cataracts alone would suggest that scientists consider new paradigms for the deterministic effects of space radiation. Until then, however, countermeasures for the late effects of space radiation could well be limited to mitigating pharmacotherapies, as it has for Parkinson's.

9.3.3.2 Pharmacologic countermeasures in the face of permanent radiation-induced effects. The insidious nature of the late effects could also limit the effectiveness of any palliative or mitigating pharmacotherapeutic countermeasures. For instance, persistent GI disruptions have been shown to adversely affect the absorption of at least two orally-administered drugs, digoxin and desmethyldiazepam (Sokol et al. 1978). Granted, these disruptions were produced by relatively high-dose exposures, and using other routes of administration could bypass this particular issue; however, these data indicate that additional research will be needed to determine the most effective doses and routes of administration for countermeasures of space radiation. As mentioned above, the effectiveness of most treatments for the late effects of space radiation will likely vary as a result of many variables (dose, dose rate, quality of radiation, and organ system affected), not the least of which is the time since the exposure. This is relatively clear from the data that is being generated for the pharmacologic countermeasures proposed for treating a radiation-induced ARS (cf. Singh et al. 2014). That is, some countermeasures are more effective

prior to irradiation than after radiation (e.g., 5-AED), and some are as effective after a single dose (GT3) than after multiple doses of another (G-CSF). Whether the same will be true for potential countermeasures for space radiation has yet to be determined. One severe limitation would be if space radiation, either alone or in combination with space travel, permanently reduced the resiliency and function of certain physiological systems, which could reduce the capacity of any countermeasure to treat pathologies of those systems. If this is the case, leucocyte growth factors may be less effective for stimulating hematopoiesis (Miousse et al. 2014), anti-infectives less effective for infections (Kaur et al. 2005), cholinesterase inhibitors for memory dysfunction (Joseph et al. 1992; Parihar et al. 2016), chemotherapeutics for cancer (Durante et al. 2001), bisphosphonates for weakened bone (Bandstra et al. 2009; Willey et al. 2010), anti-inflammatories for inflammation (Williams et al. 1999), and free-radical scavengers for free-radical production (Eisbruch 2011). Also, without additional data, there is currently no reason to believe that even more advanced countermeasures such as stem cell transplantation will be able to fully restore these systems to their pre-irradiation functionality (Acharya et al. 2011a; Acharya et al. 2011b; Baulch et al. 2016).

9.3.3.3 Future directions and approaches for the development of pharmacologic countermeasures. A review of the literature would seem to indicate that we are even further from having a pharmacologic countermeasure for the short-term and delayed CNS effects of space radiation than we are of having a FDA-approved pharmacologic countermeasure for the short-term and delayed effects of radiotherapy for cancer patients. This situation also exists despite the many new concepts regarding cytoprotection that have been derived from molecular biology and medicine, and the large array of substances that have been proposed to have cytoprotective effects (Koukourakis 2012; Singh et al. 2015). One area where there seems to have been some

progress is in protectants or mitigators for a total body irradiation resulting from an accidental exposure or nuclear weapon, because some of the most effective drugs have been stockpiled for those types of exposures (Singh et al. 2014). However, other than amifostine, none of these substances have FDA approval for use with radiotherapy or have been used in enough scientific studies to qualify them for use against the low-dose high-LET exposures expected to occur during lengthy space expeditions – not to mention as protectants or mitigators for the potential CNS effects produced by these exposures.

One general approach could be to use the same translational models for drug development that are being sought for understanding the CNS effects of space radiation (see sections 8 and 11). If some consensus can be reached regarding the most useful translational models for establishing significant short-term and delayed CNS effects, then those same models should be used to test potential pharmacologic countermeasures to save critical time and effort. From a pharmacological perspective, these models must provide information about both the effects of space travel *and* exposure to space radiation, because the effectiveness of all pharmacologic countermeasures for radiation will be contingent on specific physiological processes that may be altered as a result of space travel.

Moving forward, additional *in vitro* approaches will also be needed to more rapidly screen promising new drugs and biologics for preventing radiation-induced CNS effects. These approaches will not be able to replace whole-animal approaches at this time, but they could certainly reduce the time and resources spent testing compounds with only marginal or equivocal effectiveness. Savings in time and effort could also be achieved if drugs already approved for use were found to be effective countermeasures for space radiation (e.g., memantine), as the repurposing of drugs often requires fewer steps for approval. For example, there are many drugs

and drug classes that have received FDA approval for more than one indication (e.g., serotonin and norepinephrine reuptake inhibitors for major depressive disorder (MDD) and fibromyalgia, benzodiazepines for anxiety, insomnia and convulsions, selective serotonin reuptake inhibitors for MDD and premenstrual dysphoric disorder (PMDD), and dopamine receptor agonists for Parkinson's and restless leg syndrome). An even simpler approach than repurposing already-approved drugs may be to test lower doses and dosing regimens of existing radioprotectants. There is certainly the possibility that some of the drugs that are currently used for acute high-dose low-LET exposures may be appropriate for acute low-dose high-LET exposures or chronic exposures when administered in much lower doses and/or less frequently. A strategy of this sort has resurrected interest in a whole class of drugs previously abandoned due to their hallucinogenic effects; namely, the 5-HT2A agonists, which are very potent anti-inflammatories and not hallucinogenic when administered in microdoses (Szabo 2015; Yu et al. 2008).

Another, still unanswered question is whether nonhuman primate studies can be eliminated from the process of developing pharmacologic countermeasures. This report has identified many of the difficulties that would be encountered in trying to use data from nonhuman primate studies as the predominant indicator of GCR-induced CNS effects; however, these studies could be invaluable for confirming the results obtained from rodent studies or for determining the pharmacokinetic and pharmacodynamic properties of countermeasures for space radiation exposure. Ideally, if nonhuman primates were to be used in a space radiation study, they would employ a within-subject design to concurrently confirm both the CNS effects of these low-dose high-LET exposures, and the effectiveness of specific countermeasures for these exposures.

10. Managing Risk

The risk resulting from radiation exposure of the CNS can be managed in either of two ways. Since most CNS effects are tissue reactions, that is, the accumulation of stochastic damage to individual targets leads to increasing levels of impairment, it may be possible to determine a minimum level of CNS damage, and therefore radiation dose, which can be operationally significant. At lower levels of CNS damage, and radiation dose, the reduced performance of the individual would remain within the range of normal variability for crew members or, though outside the normal range, sufficient to complete necessary functions. Furthermore, some damage may be latent, remaining undetected until revealed by the action of other stressors such as sleep deprivation, communication delays, elevated CO_2 etc. Unfortunately, there is no clear way to define when the level of impairment becomes operationally significant. Ideally a comprehensive risk management system would incorporate information on changes in CNS performance resulting from radiation exposure, and other forms of stress, with the characteristics of the spacecraft and the mission plan to minimize the risk of mission failure and long term crew health effects. Implementation of such a comprehensive risk management system requires extensive knowledge of the effects of radiation on the human CNS, which is not currently available. The best that can be done at this time is to consider other, more familiar, causes of impairment and evaluate the level at which they become operationally significant. Probably the most familiar cause of CNS impairment is ethanol.

10.1 How Should "Significant Impairment" Be Defined?

In December 2011, NASA issued NASA/SP-2011-3421, the NASA Risk Management Handbook (NASA, 2011). The handbook addresses NASA's Risk Management Process, including both the Risk-Informed Decision Making (RIDM) and the Continuous Risk

Management (CRM) processes. The RIDM process described in the handbook attempts to respond to some of the primary issues that have derailed programs in the past, namely:

1. The “mismatch” between stakeholder expectations and the “true” resources required to address the risks to achieve those expectations;

2. The miscomprehension of the risk that a decision maker is accepting when making commitments to stakeholders; and

3. The miscommunication in considering the respective risks associated with competing alternatives.

The new CRM process described in the handbook is an enhanced version of NASA’s traditional CRM paradigm. Though the new CRM process maintains the traditional core elements, it builds on the foundation of quantitative parameters and data provided by the RIDM front-end to the Risk Management Process. This approach fundamentally changes the focus from qualitative assessments to quantitative analyses. The approach moves from the management of individual risks to the management of aggregate risk. Rather than eliminating or reducing the impact of single unwanted events the CRM process becomes the management of risk drivers. This quantification is intended to allow managers to discover the drivers of the TOTAL risk and find the interactions/dependencies among their causes, mitigations, and impacts across all parts of the program.

Managing risk using the CRM process assures that risk management decisions are informed by their impact on specific objectives rather than in abstraction. Risk is, therefore, characterized as a set of triplets:

1. The scenario(s) leading to degraded behavioral and/or cognitive performance with respect to one or more performance measures (e.g., scenarios leading to injury, fatality, destruction of key assets, etc.).

2. The likelihood(s) (qualitative or quantitative) of those scenarios.

3. The consequence(s) (qualitative or quantitative severity of the degradation) that would result if those scenarios were to occur.

Given the complexities and uncertainty inherent to long-duration manned space flight, "significant impairment" should be defined in terms of the consequence of the behavioral and/or cognitive impairment on the mission and long-term health of the crew. In the presence of NASA's enlighten integrated approach to Risk Management, it seems inappropriate to a priori define "significant behavioral and/or cognitive impairment". Instead, it would be better to provide NASA risk managers with a unified database of existing empirical findings and a document clearly articulating the known direct and indirect effects of cosmic ionizing radiation on the Central Nervous System, as well as the areas of uncertainties and of the unknown.

10.2 How Might Existing Empirical Findings Be Best Utilized?

Long-duration manned space flight is a dynamic problem created by the tight interconnection between the humans onboard and the various subsystems of the spacecraft (e.g., navigation, propulsion, power systems, etc.). Numerous minor incidences can cascade into significant risks to the mission and risk the lives of the crew. The complex interconnections and dependencies between various factors may require a non-reductionist approach to understanding the risks involved. To that end, for the remainder of this section, we will discuss risk in the context of the Cynefin framework (Kurtz and Snowden, 2003).

10.2.1 Introduction to the Cynefin Framework

The Cynefin framework was first developed by Dave Snowden in the 1990's in the context of knowledge management and organizational strategy. The word "Cynefin" (pronounced ki-neh-vin) is a Welsh word commonly translated as 'habitat' or 'place' that profoundly influence who we are, but of which we are only partially aware. In the early 2000's, it was further developed with Cynthia Kurtz to include complex adaptive systems theory (Kurtz and Snowden, 2003). Depicted in Fig 10.1, the Cynefin framework is a characterization of the continuum of the possible environmental states that the crew could find themselves in (from simple to chaotic) and the approaches they should take.

The 3 basic types of systems involved in Cynefin framework are; ordered (right side of the diagram), complex and chaotic (left side of the diagram). The ordered systems are further sub-divided into 2 domains; simple and complicated. There are 5 regions in total: 1) Simple, 2) Complicated, 3) Complex, 4) Chaotic, and 5) Disorder. During the course of space flight, the environment of the crew and spacecraft may be characterized as a point continuously moving between the regions on the continuum.

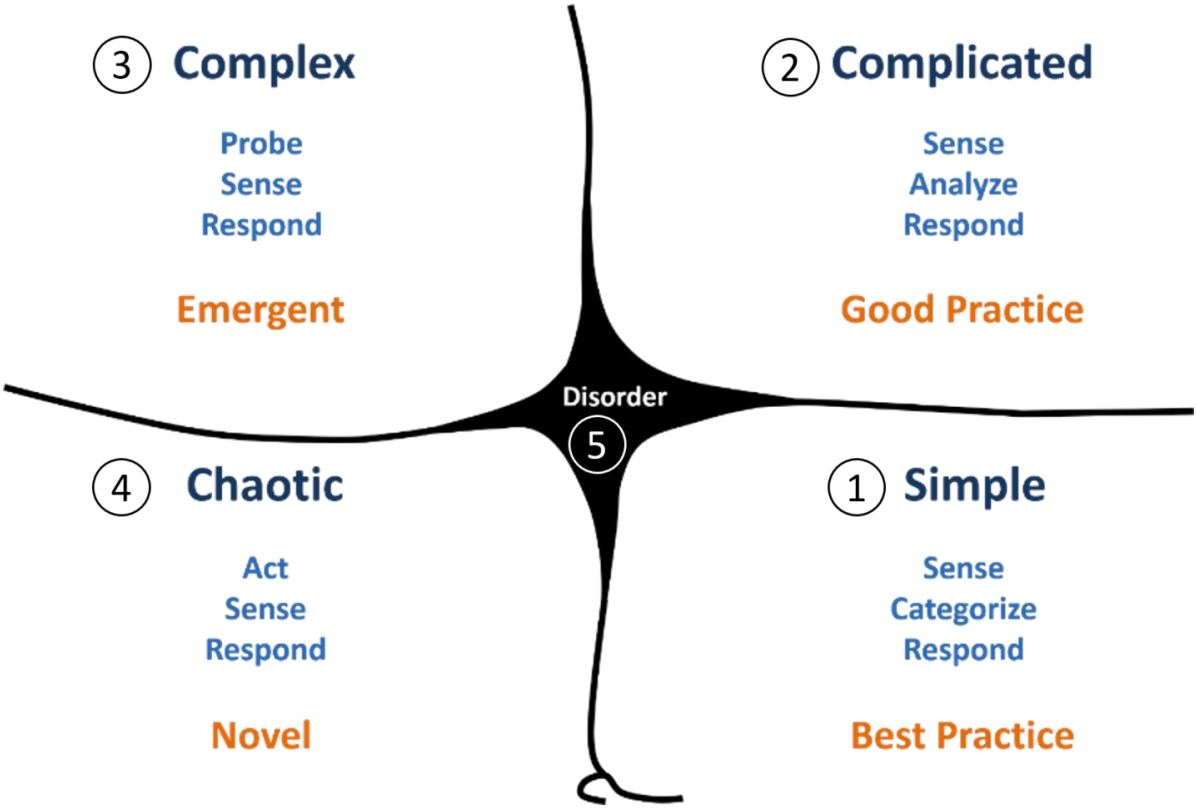

Fig. 10.1. Diagram of the Cynefin Framework.

Before we describe the various regions, it is important to note here that there is no one-size-fits-all approach and the actions one should take depends greatly on the environmental conditions one finds oneself in.

1) The Simple domain

In the simple domain, the crew is in an ordered system. The relationship between cause and effect is predictable in advance and self-evident to any reasonable person. We need to apply best practices and the approach is to:

a) Sense: See what is coming in.

b) Categorize: Make it fit predetermined categories.

c) Respond: Decide what to do.

Best practice is applied in terms of predefined formulae and given an input the results are always the same.

2) The Complicated Domain

In the complicated domain, the crew is in an ordered system where a relationship does exist between cause and effect, however, the answer is not self-evident and requires analysis and/or the application of expert knowledge. There can be several different ways of doing things in this domain, with the right expertise. We need to apply good practice and the approach is to:

a) Sense: See what is coming in.

b) Analyze: Investigate or analyze, using expert knowledge.

c) Respond: - Decide what to do.

While there is no best practice, there are several good practices available to those with the right expertise.

3) The Complex Domain

In the complex domain, the crew is in an unordered system. The relationship between cause and effect can only be perceived in retrospect and the results are unpredictable. Complex systems are therefore dispositional and not causal. Here, the crew and ground control need to create safe to fail experiments and not attempt to create fail safe design. Complex problems cannot be solved with best or good practices alone. While conducting safe to fail experiments, the crew and ground control must dampen the parts that fail and amplify the parts that succeed. In this domain we get emergent order and practice that is often unique. We need to apply emergent practice and the approach is to:

a) Probe: Experimental input

b) Sense: Failures or successes

c) Respond: Decide what to do to either amplify or dampen

In this domain, professional training or best practices are replaced with the probing of different approaches and ideas, sensing the results/feedback to see what works and what does not, and responding appropriately by amplifying and dampening the probes.

4) The Chaotic Domain

In the chaotic domain no cause and effect relationship can be determined. The goal is to stabilize the situation as quickly as possible! The approach is to:

a) Act: Attempt to stabilize

b) Sense: Failures or successes

c) Respond: Decide what to do next

In this domain, the adage of “crawl, walk, run” is replaced with “falling forward” and stabilize the situation.

5) The Domain of Disorder

This domain includes the state of not knowing what type of causality exists. In this domain, people tend to assess the situation that they are in based on their personal preferences for specific actions, reverting to their own comfort zones. Here, "people might not know what they like, but they sure like what they know."

The Cynefin framework presents general strategies for regulating the different systems, with the exception of disorder as that area describes an environment without any internal network behavior.

10.2.2 The Cynefin framework's Implication to Risk Management

The Cynefin framework can be a powerful tool for identifying the proper courses of action in a situation. The recommended actions are dependent on the environment or system, which will fall within one of the five areas described above. The framework not only characterizes, but tracks the complexity of a system as it changes. A system that starts simple can move to the other areas as more factors and variables are introduced, and vice versa. This approach to managing a system is dependent on the system's dynamics.

Within this section, cosmic ionizing radiations will be treated as a specific class of behavioral performance- and/or cognitive performance-modifying stimuli. The adverse behavioral and cognitive outcomes will be treated as a specific class of human error within the Cynefin framework. Human error taxonomies have been implemented in numerous safety critical industries. These taxonomies have provided invaluable insight into understanding the causes of human error. Examples include:

- Model of Internal Human Malfunction – Rasmussen (1982)
- Information Processing Model – Wickens and Flach (1988)
- Model of Unsafe Acts – Reason (1990)
- Situation Awareness (SA) – Endsley, et.al (1998)

However, these human error taxonomies are limited in their ability to capture the interconnectivities found in the Cynefin emergent orders (i.e. in the complex and chaotic regions). These taxonomies have difficulties accounting for outcomes such as temporary lapses in judgement that can lead to cascading effects with catastrophic results.

The causal relationships between space radiation exposure and behavioral and cognitive modifications are critical to the prediction of downstream consequences. Though controlled randomized experiments are the ideal tools for identifying the causal relationships, such experiments in many of our cases are cost prohibitive, unethical, and even technically impossible. Therefore, the causal relationships must be inferred from clinical data, accidental/unintended exposures, and/or animal studies. Unfortunately, when designing real-world systems, anyone who has ever attempted to obtain guidance from the literature is all too familiar with the challenges and frustrations that come with the mountains of empirical data and a jumble of micro-theories. Many of these micro-theories are inextricably linked to specific experiments, conducted under carefully controlled conditions. So controlled are these experiments that they become contrived and limited in their ecological relevance.

To make the mountains of psychological and physiological data usable to support practical engineering, NASA's System-Wide Accident Prevention Program has been developing new technologies, over the last several decades, to reduce accidents in aviation (Leiden et al., 2001). The Human Error Modeling element within this program has supported several efforts to

develop computer simulation and modeling tools to predict human error. The integration of Task Network Modeling with Cognitive Modeling is a very promising approach.

10.2.2.1 Task Network Model. Task Network Models use human/system task sequence as the primary organizing structure and are often considered top-down approaches (NAS/NRC, 1998). In a Task Network Model, human cognitive performance within a complex system is decomposed into smaller-and-smaller elements until the human-system interactions can be described as a closed-loop function. The individual elements of human cognitive performance are then connected to tasks from a task analysis, and organized according to task sequences. The level of system decomposition (i.e., how finely to decompose the tasks) and the amount of the system simulated (i.e., how much of the system) depends on the particular question.

10.2.2.2 Cognitive Model. Cognitive Models focus on the structural components/properties of the cognitive system and are often considered bottom-up approaches. In a cognitive model, human cognitive performance is simulated at the level of the underlying architecture and mechanisms. The predictions are often provided in the forms of response times and actions, as well as the ratio of correct-detection and false-alarm. In recent years, researchers have extended the predictive capabilities of cognitive models to functional magnetic resonance imaging (fMRI) data (Staal, 2004). The cognitive model components have been associated with specific fMRI activation patterns. The researchers suggest that fMRI data can now be used to inform and constrain the cognitive models, and the cognitive models can be used to interpret fMRI data in a theory-driven manner. Similarly, these models can be used to interpret pathway data or data showing neuronal activation as assessed by molecular or electrophysiological approaches.

10.2.3 Design of Mission Hardware and Procedures to Minimize Impact on Missions

By integrating Task Network Models with Cognitive Models, in principle, NASA managers can be provided with links between existing empirical findings and down-stream mission outcomes. However, the nature of exploration means that astronauts are likely to encounter conditions that were not and could not be foreseen (i.e., the Cynefin complex and chaotic regions). A priori defined safety thresholds based on similitude, margins-of-safety heuristics, and worst case scenarios will likely be insufficient.

Fortunately, the revolutionary approach known collectively as Digital-Twins is now underway. NASA, the military, and a number of key U.S. industries are in the forefront of its development. Digital Twin, originally conceived by the Defense Sciences Office at the Defense Advanced Research Projects Agency (DARPA), has historically been defined in the context of Product Lifecycle Management. In this context, a digital twin is a digital representation of a physical entity and the dynamic interaction(s) of how that entity acts on and is acted upon by its physical environment.

As far back as the Apollo era, NASA was already using the concept of twins. When disaster struck Apollo 13, it was the presence of a mirrored system on earth that allowed the ground-crew and astronauts to work together to determine the best course of action. The “2015 NASA Technology Roadmaps Technology Area 11” document unveils NASA’s audacious plan to not only create digital twins for the spacecraft, but also human digital twins for the crew (Gaessgen and Stargel, 2012; Shafto et al., 2012).

Human digital twins will have the greatest impact on vehicle and mission design, if they can be programmatically integrated into the mission modeling effort. During the mission, if the digital twins can properly reflect the astronauts’ performance and responses to off-nominal situations, such as exposures to ionizing radiation, then sensors onboard a spacecraft in concert

with the digital twin can properly diagnose the adverse impact of the exposure. Due to the complex interactions of the instruments onboard the spacecraft, perhaps hundreds of simulations with the digital twins will be necessary to provide the astronauts with the optimum remediation course of action. Like the predictive complex weather models of today, the Digital Twins can simulate the interactions between the human crew and their dynamic environment; predicting the complex risk factors to mission outcomes by linking the cognitive-behavioral performances of the crew with physical states of the space craft. Furthermore, the crew's human digital twins may provide the onboard artificial-agents with the "empathy" necessary for them to behave as partners for the human crew.

10.2.4 Development

Development of human digital twins is an iterative process that must have a foundation of physiological and psychological data collected from human and animal experiments. Current cognitive models are based on a myriad of psychological research that has informed the architectures from which they are created. As more information is presented these models are augmented both by psychological and physiological findings (e.g., integration with fMRI, pathways, and neuronal activation as assessed by molecular or electrophysiological data).

Human digital twins constructed to inform vehicle and mission design should be based on available data and to inform the initial development. From there additional experiments and empirical studies can augment and refine digital twins to better capture performance and behavioral changes. In addition, to foster creation of a model dealing with hazards such as ionizing radiation; it would be valuable to develop animal digital twins, for the purposes of refining human digital twins, through available data and additional experiments for which it

would not be possible to include humans. It is important to note here that while the digital twins concept shows great promise, it is still an area of active developmental research.

10.3 Recommendations

NASA has been making wise S&T investments and engaging in fruitful partnerships. Integrating the committee's findings into NASA Risk-Informed Decision Making (RIDM) and the Continuous Risk Management (CRM) process would be the most logical course of action. The following three recommendations are proposed:

1. Utilize the Committee's findings to inform the initial development of rodent digital twins, with follow-on rodent experiments to evaluate and improve the models. A rodent digital twin is an in-silico quantitative mechanistic model of the animal. Following Karl Popper's logic of falsification, this should allow NASA to reject false claims and avoid non-scientific claims.
2. Focus future NASA's Human Research Program investments toward applied research; insisting on quantitative claims and demanding the investigators make explicit the warrants that link the experimental data to the claims. More importantly, the claims should be directly relevant to identified mission variables/parameters (e.g., those from Dr. Jack Stuster's "Task analysis for an Expedition to Mars"). These psychological and physiological findings will support the construction of human digital twins.
3. Re-enforcing NASA's investments toward creating in-silico quantitative mechanistic models of the vehicles, to act as the simulated space flight environment. Finally, integrate the in-silico vehicle models with the human digital twins to create non-linear dynamic models to inform vehicle and mission design.

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5777 **10.4 Ethanol as a Model for Radiation Induced CNS Impairment**

5778 While at the superficial level, there seem to be many similarities between human
5779 responses to consumption of ethanol and responses in animals exposed to low doses of HZE
5780 radiation. Those similarities include slowed reflexes, inhibited memory, degraded executive
5781 functions as well as increased risk of late effects such as dementia. However, the differences in
5782 the spatial and temporal distribution of ethanol and the energy deposited by HZE irradiation are
5783 so dramatic that similarities in the mechanisms leading to those effects seem to be unlikely. To
5784 the extent that impairment due to ethanol consumption and due to radiation exposure are
5785 effectively similar (i.e. with face validity), the effects of ethanol may be useful for evaluating
5786 operationally significant impairment. The effects of ethanol on human cognitive and behavioral
5787 functions are well understood, and the impacts of small doses of ethanol on functions which may
5788 be critical to mission performance are predictable. If rodents show similar response to ethanol,
5789 the performance degradation produced by a radiation exposure may be characterized by the
5790 ethanol level needed to produce the same decrement. This may make it possible to predict the
5791 impact of a radiation exposure on human performance. The critical step in making this
5792 association would be the quantitative relationship between radiation exposure and ethanol
5793 concentration to produce the same cognitive and behavioral decrement, and establishing that
5794 relationship for humans as well as for rodents. This approach, and its assumptions, has been
5795 applied to impairments produced by organic solvents (such as toluene) and ethanol (Benignus, *et*
5796 *al*, 2005, 2007; Bushnell *et al*., 2007).

5797 Furthermore, the acute effects of ethanol are the result of its binding to a limited number
5798 of proteins involved in neurotransmitter activity (Tabakoff and Hoffman, 2013, Rusyn and

Battaller, 2013), whereas radiation induced damage generally impacts a much wider range of target molecules. However, it is possible that a detailed analysis of the adverse outcome pathway for some CNS effects may reveal key events and key event relationships which are the same for metabolic products of ethanol and radiation chemistry initiated by HZE events. If such common key events exist, they may be instrumental in leading to a full understanding of the relationship between physical patterns of energy deposition and biological consequences. Unfortunately, unless the key event occurs at an early stage of the adverse outcome pathway there may be insufficient information to relate it to track structure or other physical characteristics of the irradiation that produce acute effects. Key events occurring late in the AOP may contribute to effects of both ethanol consumption and radiation exposure but would be unlikely to relate to radiation quality.

11. Conclusions and Recommendations

11.1 Conclusions

The report's conclusions are formulated in terms of answers to the 8 questions posed in NCRP's proposal to NASA (see Section 3.3.2).

1. How should a "significant impairment" in performance be defined? What are the performance domains that could be significantly affected? What constitutes a "significant impairment" in the context of actual performance and operation?

A significant impairment can be defined as an impairment in performance of an individual astronaut, or impairment in performance of the astronaut team, that negatively affects the goal and success of the mission. Performance domains that could be significantly affected include attention, acquisition of new tasks, memory formation, memory retrieval, memory extinction, spatial navigation, ability to detect changes (novelty) in the environment, and sensorimotor function. Additional behavioral and cognitive alterations that can affect performance include depressive behaviors, anxiety, alertness, processing speed and executive function, and the ability to work well with others as part of the team, including interactions with ground control.

For any specific performance domain, or grouping of domains, several approaches could be taken to specify objective measures, such as (i) if available, a clinically defined adverse effect (e.g. 10% increase in nerve conduction velocity relative to a comparable population), (ii) some specified percentage change from the astronaut population norm in performance (for matched sex and age) or (iii) some percentage change from the individual's own baseline. These approaches would require defining the behaviors and cognitive performances of interest and accurately quantifying them.

The level of impairment that is significant dependent on the specific requirements of the mission and hardware being used. The ideal approach would be to determine the functional relationship between radiation exposure and risk to the mission and perform risk management procedures to minimize the total risk. This will result in different limits for radiation-induced impairment depending on the magnitudes of other risks. Determination of the required functional relationship will require development and testing of biological response models for animals and humans, and will likely require many years. An interem approach to determining the boundary of "significant impairment" would be to determine the acceptable level of impairment due to a relatively well known chemical agent such as alcohol, and adopt that as the maximum acceptable level of radiation-induced impairment for the specific mission.

2. Do risk assessments for chemical toxicity, including neurotoxicity, provide guidance? Are the IARC evaluation schemes for chemical neurotoxicity of value in assessing health risks?

Risk assessments for chemical toxicity can provide useful guidance in terms of the adverse outcomes pathway (AOP) approach discussed in question 3 below.However, the evaluation scheme of the International Agency for Research on Cancer (IARC) (http://monographs.iarc.fr/ENG/Classification/) is not of direct value for assessment of risks of behavioral and cognitive effects from radiation exposure of the CNS. The IARC evaluation scheme is designed specifically for classification of chemical and other agents according to the strength of evidence that they are capable of causing cancer and irrespective of their potency. Thus, it identifies agents as cancer hazards, but is not concerned with magnitude of risk. Ionizing radiations such as X- and gamma-radiation, neutrons and a wide variety of internally-

deposited radionuclides have been classified as carcinogenic to humans (Group 1) (IARC 2000, 2001, 2009). Although others have suggested applying a similar evaluation scheme for classification of chemicals according to the degree of evidence that they are neurotoxic (hazard identification), with an added 3-level concentration-based potency classification (Simonsen *et* al. 1994), this approach has not been widely applied. Ionizing radiations such as X- and gamma-rays are, in any case, well recognized as neurotoxic hazards.

3. Is the adverse outcome pathway approach used by EPA useful in the context of space radiation and CNS effects? What are the key events related to adverse outcome in behavior/performance impairment that might affect mission and, conceivably, be related to late effects such as dementia?

The adverse outcome pathway approach (AOP) is a useful framework for considering space radiation-induced CNS effects. Although developed for chemical-induced biological changes, the AOP framework could just as well be used to describe radiation-induced biological changes, as has been demonstrated for radiation cancer risk (Brooks *et al*. 2016). Furthermore, the AOP approach provides a direct method for consideration of the interactions between a wide variety of environmental stressors. In the context of space radiation, the molecular initiating events (MIE) result from interaction of radiation with biological matter. As discussed in Section 7.1, the AOP framework offers a means to organize knowledge about relationships between the initial biological perturbation and an apical outcome. This framework could facilitate detailed characterization of the complex processes that lead from energy deposition by radiation to specific CNS impairments. There seem to be some events in common in the AOPs of some

neurotoxins and radiation, but currently there is insufficient information to identify initiating events for radiation-induced CNS effects.

Relevant features of the AOP approach include description of a biological pathway that is agnostic to which physical/chemical stressors produce the initial biological alteration and description of the weight of evidence for the different portions of the biological pathway, thereby facilitating identification of knowledge gaps and indication of priority areas for future research. A well-defined, quantitative, AOP may assist with the reduction of default uncertainty factors. In addition, a well-defined AOP may allow the use of assays for Key Events, instead of apical Adverse Outcomes, in risk assessment. However, development of quantitative AOPs is challenging. Defining critical Key Events will require determining which Adverse Outcome is of interest (since Key Events can vary between pathways). It will be extremely difficult to define the critical Key Event for CNS effects, as pathways for complex functional outcomes (reaction time, memory) have not been defined by the neurobiological community. Some potential key events are indicated in Section 6, but it should be noted that the majority of performance studies to date have been limited to an identification of single pathways. Unbiased studies involving large data might reveal other pathway changes following space irradiation that are pertinent to behavioral and cognitive performance during the mission, as well as risk to developing neurodegenerative conditions, including dementia, following the mission. It should be noted that adverse does not mean that the pathway has to have immediate detrimental effects. Compensatory changes that have long term negative consequences for the astronaut are also considered adverse outcomes

4. Are non-human primate (NHP) experiments necessary, and if so, how should they be considered?

The closest animal model to humans is a NHP. Nonhuman primates would be valuable not only to confirm the findings from rodent studies on the risk of CNS effects, but ultimately also to confirm the effectiveness of potential pharmacological countermeasures for those effects. Well-designed NHP studies with large enough animal populations for specific targeted research questions would reduce the uncertainty, but it would probably require more than 5 years to produce significant results (taking into account the time to establish: a) a sufficient population of mature animals, b) development of the model with validated endpoints, c) the optimum radiation dose, dose rate, quality and geometry, e) the clinical time course for development of delayed effects following exposure and f) the research site(s) and qualified personnel to conduct the studies). In considering a focused study with NHP, it will be critical to limit the exposure paradigm and other experimental conditions for reasons of time and budget. The focus on low-dose HZE effects will also affect the time frame as low-dose studies are more difficult to design and carry out in short time-frames.

Great progress has been made in comparing tests used in the clinic in humans with tests used in animal models. Examples include eye blink conditioning, analysis of the visual system, analysis of the motor system, analysis of spatial learning and memory, emotional learning and memory (for example as assessed in fear conditioning), conditioned place preference, psychomotor sensitization, and the representation of uncertainty in the human and animal brain. All these examples are included in a Neuromethods book published as part of Springer Protocols. However, current methods have not enabled these observations in irradiated rodents to be closely

related to relevant behavioural or cognitive parameters in humans, particularly in regard to "significant effects" for mission operations or for quantitative risk relationships with dose or radiation quality.

5. Are the CNS effects, including the behavioral impairment domains, deterministic or stochastic? That is, assuming that there is sufficient evidence for concern, are there threshold levels below which the concern is minimal?

Most CNS effects are considered to be tissue reactions, with unknown thresholds. The threshold may be altered by additional stressors (such as sleep deprivation). The CNS is considered to have some level of redundancy and adaptive ability, leading to thresholds for the apical behavioral and cognitive outcomes. For deterministic effects it is expected that as detection techniques become more sensitive, effects will be detectable at lower radiation exposures. However, statistically significant effect does not necessarily imply operational significance. An individual may suffer impairment and still be within the normal range of individuals performing a specific task. Overall, the dose response curves reported to date for CNS effects of space irradiation in rodents have often been complex (e.g., Figs. 5.4 and 5.5). Generally different animal models and exposure conditions have been used in each study, so there has been little opportunity to assess reproducibility within or across laboratories and studies. For example, while in some instances only the higher doses used showed significant effects, suggesting a safe threshold, in many other cases the opposite pattern was revealed, with the lower doses causing pronounced effects that were not seen at higher doses. Under such circumstances, it would be premature to suggest safe threshold doses without additional studies confirming that doses below the threshold indeed do not cause any detrimental effects. As

discussed in more detail later, the CNS effects might also be modulated by age at exposure and assessment, and by sex of the exposed individual. Obviously, it would be important to weight the severity of the effects and the probability of them occurring during or following space missions in these considerations. In consideration of the "rigor and reproducibility guidelines" NIH has initiated, it will be important for NASA to support reproducibility assessments for all studies, including those involving omics approaches, utilizing identical samples in different institutions.

6. How might space radiation "interact" with other aspects of a mission that would impair performance, both for the individual and the team, such as sleep deprivation, medications, zero gravity, constant close quarters, reduced communications, absence of windows to the world, and other?

Many features of the work environment in space produce behavioral and cognitive effects similar (or identical) to those produced by radiation. The effects could be independent, additive, or synergistic, depending on the features of the biological pathway for each cause; all may be expected to be involved in the variety of pathways for individual and team performance. Currently there is insufficient evidence to determine the nature of interactions. Sleep deprivation is an especially profound environmental challenge that may be expected to interact with measures of behavioral and cognitive performance. Increased CO_2 levels might also affect behavioral and cognitive performance.

7. What is the relative balance between the likelihood of neurobehavioral effects that would impair operational performance and adversely affect the mission, and the likelihood that

serious neurodegenerative disease develop such as Alzheimer, Parkinson, Huntington, amyotrophic lateral sclerosis (ALS), and dementia?

As yet there are insufficient data to estimate the relative balance between in-mission and long-term health effects. It is likely that the biological pathways for the variety of effects, in-mission and long-term, are different, potentially even in those instances for which the initial event in the pathways is the same. Such differences will generally result in different relative balances for the variety of acute and long-term effects. In-mission effects could be estimated (for example, slowed reaction time, lapses of attention) based on what is considered a risk to the mission and these changes may be mitigated by engineering modifications. The rodent experimental data suggest that rather than the biological processes resulting in distinct early and late effects, there may be more of a continuum of effects. Even if neurobehavioral and/or neurocognitive effects during a mission are not detected, there may still be risks of developing late effects pertinent to neurodegenerative conditions. Similarly, there might be neurobehavioral and/or neurocognitive effects during a mission without resulting in long-term effects pertinent to neurodegenerative conditions following missions. It should be kept in mind that detrimental CNS effects are probably not limited to high LET exposure

8. True to all human tissues, the brain is designed to respond rapidly to changes and then compensates for survival. Do compensation mechanisms exist that would influence the likelihood of getting to a level of significant impairment that would adversely affect performance and the mission?

Based on the currently available data, there are insufficient grounds to assume that there would be compensatory mechanisms robust enough to prevent significant effects of space

irradiation on neurobehavioral and/or neurocognitive performance.Such compensation mechanisms likely do exist. This would be related to the threshold for adverse outcome, although compensation for "survival" does not necessarily imply good performance. An additional complicating problem is potential interaction with other non-radiation stressors that could lower thresholds. Well-performed dose-response studies in animals, coupled with transfer functions to humans may be able to provide some estimates of the thresholds. It would be necessary to include some safety factors, and be sure analogous functions are evaluated in animals and humans.

If the CNS effects of interest are transient, fading on time scales of weeks, as sometimes seen in rodent studies, this might suggest that at low dose rates the level of impairment during space missions may be reduced. Medical experience suggests that many delayed effects become progressive above a dose level; at low doses those effects generally disappear with time. On the other hand, while compensatory mechanisms might help to offset the earliest changes and related significant impairments, with continued challenges these compensatory mechanisms might not be able to prevent the occurrence of significant impairments during the mission. Sleep deprivation and alcohol exposure are good examples showing that there can be clear limits to what compensatory mechanisms can protect against.

11.2 Recommendations

Individual Sections of this report include recommendations related to their particular areas of study. In addition, the committee wishes to highlight three long term (more than 5 year) recommendations, and short term (potentially accomplished in less than 5 years) recommendations. A summary of the additional recommendations follows.

Priority long term recommendations

1. The committee recommends establishing ongoing collaborations with the charged particle radiotherapy cancer centers globally to harness the world-wide data base of patients to enable follow-up of endpoints related to short- and long-term behavioral and cognitive effects potentially associated with charged-particle exposures to the CNS in this patient population.

2. **The committee reiterates the recommendations of the Phase I Report, that life-long medical follow-up of crew members to evaluate potential CNS changes is essential.**

- Comparison of the assessment results for exposed crew members with those for other non-exposed groups is essential (providing controls matched as closely as possible to exposed crew-members for follow-up studies).

- There is a critical need for long-term, regular follow-up of individuals who have flown in space, including appropriate assessments of potential detrimental CNS effects. While understanding that there are privacy and ethics concerns, a search should be made for a means to make relevant health information available to appropriate researchers**.**

3. Priority should be given to development of methods for translation of results from studies in rodents, nonhuman primates, and other biological models, to humans. This should include identification of key molecular and tissue changes and pathways linking the early radiation damage to relevant short-term behavioral or cognitive changes and/or to late effects on behavioral or cognitive performance or altered risk of developing neurological conditions. This should also include elimination of dose rate as a potential confounder of the

findings based on ground-based studies. The Adverse Outcome Pathway (AOP) approach may provide a helpful formalism to guide research, with emphasis being put on conservation between rodents and humans and on quantitative as well as qualitative relationships. For those pathways where there are major limitations comparing pathways in rodents and human, comparing the AOPs in nonhuman primates and humans will be critical.

Short term recommendations

4. Support research on the combined effects of space irradiation and a limited number of other environmental challenges pertinent to astronauts during space missions (CO_2, crowding, etc.).

5. Support more studies comparing performance of females and males in space radiation studies.

Additional recommendations

6. It is also recommended that NASA monitor developments in the area of airline crewmember health. Crewmembers are exposed to various forms of stress, including variable air quality, disrupted sleep and elevated radiation levels, primarily neutrons. Recent data demonstrates airline crews have higher prevalence of cancer, and may be at increased risk for cognitive impairment and brain white matter abnormalities. Although this literature is too premature to make solid conclusions, enhanced research efforts could provide useful data that generalizes to astronaut experiences.

7. Initiate clinical studies of workers exposed to polonium, radium, plutonium, uranium and americium. This population is limited but may be useful for evaluating tests for CNS function as well as mortality from dementia or other motor neuron diseases that might be associated with

high-LET alpha particle dose to brain tissue following years of exposure.8. Promote analyses of results from head and neck cancer patients as they can be of interest to NASA. While therapeutic target doses to the cancer tissues are much higher than the estimated doses in space, lower scattered doses to the neighboring normal tissue may be in the dose ranges that are relevant to NASA. Due to the favorable depth-dose characteristics of particle beams and its utility in radiotherapy, the patient populations that were either treated or are currently undergoing therapy are increasing both in the U.S and internationally.

9. Use chronic exposures to complement the acute exposures and fractionated doses used so far (section 5& 6).

10. Increase use of mixed beam exposures as the interaction of effects of different particles is largely unknown (section 5 & 6).

11. Support research in the effects of space irradiation on emotional behaviors, including depressive-like and social behaviors (section 5).

12. Support assessment of effects of pharmacologic and nonpharmacologic mitigating strategies to treat or even prevent detrimental effects of space irradiation on the brain.

13. Support assessment of effects of multiple exposures over time to mimic multiple space missions to which astronauts are exposed.

14. Encourage studies combining behavioral and cognitive assessments with unbiased “omic” strategies to determine pathways altered following space irradiation and gain insight into mechanisms underlying space radiation effects and to guide the selection of mitigators for space radiation-induced CNS injury.

15. Support studies of CNS effects in the context of space radiation interactions with physiological systems, such as the cardiovascular and immune systems, which are intimately linked to proper brain function.

16. Encourage application of new tools in neuroscience such as high density in vivo recordings, optical imaging, optogenetics and pathway tracing to probe space radiation effects beyond local circuits.

17. Conduct a systematic review of existing literature and data, organized in an AOP framework, to identify the strength of converging evidence for radiation-induced changes in various biological pathways. Although limited to what is published in the literature, this process may lead to identification of knowledge gaps for research prioritization and targets for mitigation of detrimental radiation-induced effects on the brain.

18. Clearly define the adverse outcome(s) of concern. This will assist in determining what a biologically-significant change is (such as 10% increase in reaction time). This process will focus work on the biological pathways (AOPs) that may be the most important.

19. Fund research into the similarity of biological pathways between multiple species for radiation-induced changes in neural function. This will assist in constructing AOPs to organize data which may be collected in other species (mice, NHPs), and help determine the relevance of the data to describe biological changes which produce the adverse outcome(s) of concern.

20. Develop methods for translation to humans of results from studies in rodents and other biological models, including by identification of key molecular and tissue changes and pathways linking the early radiation damage to relevant short-term behavioural or cognitive changes or to late effects. The Adverse Outcome Pathway approach (see Section 7.1) may provide a helpful

formalism to guide research, with emphasis being put on conservation between rodents and humans and on quantitative as well as qualitative relationships.

21. Concentrate experimental research efforts on obtaining reproducible dose response relationships for protons and two specified heavy ions (possibly Si and Fe) at 3 specified velocities, as well as corresponding results for gamma- or X-ray photons which will provide a potential linkage to human data. This will facilitate the development of quantitative risk projection models of the type discussed in Section 7.2,1 and will be necessary in order to estimate the dependence of relevant behavioural, cognitive and long-term health changes on radiation parameters (for specification of radiation exposure in terms of quantity and quality).

22. Conduct tissue equivalent proportional counter (microdosimetry) measurements of proton beams used for CNS effects research. Some experiments have shown proton beam exposure to be significantly more effective than photon exposure. Since TEPC measurements will detect any high lineal energy component in the proton beam they will be able determine if beam contamination or a unique property of proton tracks is responsible for the unexpected effectiveness.

23. Use low dose rate neutron exposures, comparing the results to high dose rate exposures with nearly identical neutron spectrum, to estimate the effects of dose rate on CNS effects. Although the secondary radiation produced by neutron exposure cannot produce the long tracks of cells affected by a single GCR particle, they are a reasonable simulation in other respects.

24. An NHP study to confirm the magnitude of CNS risk should be considered only after there is sufficient rodent based data to design a definitive protocol allowing a limited and focused experimental design.

25. When conducted, an NHP study should include validation of selected mitigators of CNS injury that have proven effective in rodent studies.

26. For these NHP studies, consider the development of an international consortium of research sites with appropriate radiation sources, animal populations and research expertise to address the two recommendations in 24 and 25 above.

27. Utilize the available research findings to inform the initial development of rodent digital twins, with follow-on rodent experiments to evaluate and improve the models.

28. Focus NASA's future Human Research Program investments toward applied research; insisting on quantitative claims and demanding the investigators make explicit the warrants that link the experimental data to the claims

29. Re-enforce NASA's investments toward creating in-silico quantitative mechanistic models of the vehicles, to act as the simulated space flight environment.

30. Integrate task network models with human digital twins to create non-linear dynamic models to make predictions and inform vehicle and missions design.

Glossary

absorbed dose (*D*): The mean energy imparted by ionizing radiation to an irradiated medium per unit mass defined as the quotient of dE by d*m*, where dE is the mean energy imparted to matter of mass d*m* (*i.e.*, *D* = dE /d*m*). The unit for *D* is joule per kilogram (J kg–1) with the special name gray (Gy).

acute (effect or injury): Effects or injuries expressed in days to weeks after irradiation.

acute (exposure): Refers to an irradiation that occurs over a short term (*e.g.*, hours or less).

Alzheimer's disease: A progressive disease that destroys memory and other important mental functions. These changes are severe enough to interfere with day-to-day life. In Alzheimer's disease, the brain cells themselves degenerate and die, causing a steady decline in memory and mental function.

axon: The long threadlike part of a nerve cell along which impulses are conducted from the cell body to other cells.

cancer: A general term for >100 diseases characterized by abnormal and uncontrolled growth of cells.

carcinogenesis: Induction of cancer by radiation or any other agent (a somatic effect).

charged particle: An atomic or subatomic quantity of matter (*e.g.*, electron, proton, alpha particle, ionized atom) having a net positive or negative electrical charge of one or more elementary units of charge.

chronic (effect or injury): Refers to effects or injuries of long duration.

chronic (exposure): Refers to an irradiation that occurs over an extended time (*e.g.*, days to years).

cognitive impairment: When a person has trouble remembering, learning new things, concentrating, or making decisions. Cognitive impairment ranges from mild to severe.

confidence level: In this Commentary, refers to the 95 % confidence level on the NASA standard of 3 % risk of exposure-induced death for cancer, as implemented operationally by NASA.

crew member: Any individual that is a flight crew member in a NASA human space flight mission.

delayed (early) (effect or injury): Effects or injuries that occur one to six months after irradiation.

delayed (late or long term) (effect or injury): Effects or injuries not observed until more than six months after irradiation.

dementia: A group of symptoms affecting memory, thinking, and social abilities severely enough to interfere with daily functioning.

dendrite: Receives synaptic input from axons (and occasionally other dendrites) of other neurons.

deoxyribonucleic acid (DNA**):** Genetic material of cells; a complex molecule of high molecular weight consisting of deoxyribose, phosphoric acid, and four bases which are arranged as two long chains that twist around each other to form a double helix joined by hydrogen bonds between the complementary components.

dose: A general term used when the context is not specific to a particular dose quantity. When the context is specific, the name for the quantity is used (*e.g.*, absorbed dose).

dose rate: Dose delivered per unit time. Can refer to any dose quantity (*e.g.*, absorbed dose).

effective dose: The sum over specified tissues of the products of the equivalent dose in a tissue or organ and the tissue weighting factor (that accounts for the radiosensitivity of that tissue or organ). Equivalent dose is the product of the radiation weighting factor (that accounts for the relative biological effectiveness of the radiation type) and the mean absorbed dose in a tissue or organ (organ dose).The International System (SI) unit for both effective dose and equivalent dose is joule per kilogram (J kg^{-1}), with the special name sievert (Sv) (see *organ dose*).

electrons: Small negatively charged subatomic particles that can be accelerated to high energy and velocity close to the speed of light.

executive function: The cognitive or mental abilities that people need to actively pursue goals.

exposure: In this Commentary, exposure is used in a general sense meaning to be irradiated.

fluence (Φ): A stream of particles crossing a unit area defined as the quotient of dN by da, where dN is the number of particles incident on a sphere of cross-sectional area da (*i.e.*, $\Phi = dN/da$). The unit for fluence is m^{-2}, usually given in cm^{-2}.

fluence rate: The quotient of dΦ by dt, where dΦ is the increment of the fluence in the time interval dt. The unit for fluence rate is $m^{-2}\ s^{-1}$.

galactic cosmic radiation (GCR**):** The charged-particle radiation outside the magnetosphere comprised of 2 % electrons and positrons, and 98 % nuclei, the latter component consisting (by fluence) of 87 % protons, 12 % helium ions, and 1 % high atomic number, high-energy (HZE) particles.

gamma rays: Short-wavelength electromagnetic radiation of nuclear origin (approximate range of energy: 10 keV to 9 MeV).

gray (Gy**):** The SI unit of absorbed dose of radiation, 1 Gy = 1 J kg^{-1}.

gray equivalent (Gy-Eq): A dose weighted for relative biological effectiveness (RBE). Dose limits for tissue reactions (deterministic effects) are expressed as the organ dose in gray multiplied by the relevant RBE for the specific organ and radiation.

heavy ions: Nuclei of elements heavier than helium such as nitrogen, carbon, boron, neon, argon or iron which are positively charged due to some or all of the planetary electrons having been stripped from them.

high atomic number, high-energy (HZE**) particles:** Heavy ions with an atomic number greater than that of helium (*e.g.*, boron, carbon, nitrogen, neon, argon or iron ions) that are positively charged and have high kinetic energy.

incidence: The rate of occurrence of a disease, usually expressed in number of cases per million.

ionization: The process by which a neutral atom or molecule acquires a positive or negative charge through the loss or gain of an orbital electron.

ionizing radiation: Any electromagnetic or particulate radiation capable of producing ions, directly or indirectly, in its passage through matter.

linear energy transfer (LET**):** Average amount of energy lost per unit of particle track length and usually expressed in keV μm^{-1}.

low-LET: Radiation having a low linear energy transfer (<10 keV μm^{-1}) (*e.g.*, electrons, x rays, and gamma rays).

high-LET: Radiation having a high linear energy transfer (>10 to 100 keV μm^{-1}) (*e.g.*, low-energy protons, alpha particles, heavy ions, and interaction products of fast neutrons).

myelin: A sheath which helps to increase the conduction velocity of the axon.

neuron: The fundamental nerve cell of the brain.

neutrons: Particles with a mass similar to that of a proton, but with no electrical charge. Because

they are electrically neutral, they cannot be accelerated in an electrical field.

noncancer: Health effects other than cancer (*e.g.*, cataracts, cardiovascular disease, dementia).

permissible exposure limits: Current NASA standards for ionizing radiation exposure that have to be met to maintain the health of crew members in-flight. Permissible exposure limits have the primary functions of preventing in-flight risks that jeopardize mission success and of limiting chronic risks to acceptable levels based on legal, ethical or moral, and financial considerations.

protons: The nucleus of the hydrogen atom. Protons are positively charged.

quality factor (*Q*): The factor by which absorbed dose (*D*) at a point is modified to obtain the dose equivalent (*H*) at the point (*i.e.*, $H = Q\,D$), in order to express the effectiveness of an absorbed dose (in inducing stochastic effects) on a common scale for all types of ionizing radiation. Stochastic effects are those for which the probability of occurrence, rather than their severity, is a function of radiation dose without threshold (*e.g.*, cancer).

radiation: (1) The energy propagated as waves; radiation or radiant energy, when unqualified, usually refers to electromagnetic radiation; commonly classified by frequency (*e.g.*, infrared, visible, ultraviolet, x rays, and gamma rays), and (2) corpuscular emission, such as alpha and beta particles, or galactic cosmic radiation (see *galactic cosmic radiation*).

radiation quality: A general term referring to the spatial distribution of absorbed dose. For example, an exposure to neutron radiation may be quantitatively the same as an exposure to gamma rays, in the sense that, for large volumes of tissue on the order of 1 cm^3, the absorbed energy is the same, yet at resolutions of a few micrometers the ionizing events will be more uniformly dispersed for the gamma-ray radiation than for the neutron radiation, producing quantitatively different biological effects (see *relative biological effectiveness*).

relative biological effectiveness (RBE**):** A factor used to compare the biological effectiveness of absorbed doses from different types of ionizing radiation, determined experimentally. RBE is the ratio of the absorbed dose of a reference radiation to the absorbed dose of the radiation in question required to produce an identical biological effect in a particular experimental organism or tissue.

risk: The probability of a specified effect or response occurring.

solar maximum: The period of the 11 y solar cycle during which the solar wind is at its most intense resulting in lower levels of galactic cosmic radiation about Earth.

solar minimum: The portion of the 11 y solar cycle during which the solar wind is at its least intense resulting in higher levels of galactic cosmic radiation about Earth.

solar particle event (SPE**):** An eruption at the sun that releases a large number of particles (primarily protons) over the course of hours or days.

soma: The nucleus and its surrounding cytoplasm a neuron, which contains a substantial portion of the biosynthetic machinery of the neuron. Most protein and other macromolecular synthesis occurs in the soma.

synapse: (noun) The junction between the terminal of a neuron and either another neuron or a muscle or gland cell, over which nerve impulses pass. (verb) To form a synapse.

Abbreviations, Acronyms and Symbols

3-NT	3-nitrotyrosine
5mc	5-metylcystosine
5hmc	5-hydroxymetylcystosine
53BP-1	p53-binding protein 1
ADC	apparent diffusion coefficient
ALL	Acute Lymphoblastic Leukemia
APPswe/PS1dE9	Amyloid precursor protein, Swedish mutation/Presenilin-1 deleted in exon9
AO	adverse outcome
AOP	adverse outcome pathway
AVM	arteriovenous malformation
BBB	blood brain barrier
BDNF	brain derived neurotrophic factor
BrdU	Bromodeoxy-uridine
CA1	Cornu ammonis subfield 1
CA3	Cornu ammonis subfield 3
CB_1BC	Cannabinoid receptor type 2 –expressing basket cells
CCL11	C-C motif chemokine 11
CCL22	C-C motif chemokine 22
CCR2	C-C chemokine receptor type 2
CD11c	Cluster of Differentiation 11c
CD68	Cluster of Differentiation 68
CFPnuc	Cyan fluorescent protein with nuclear localization signal

6299	CNS	central nervous system
6300	DCX	doublecortin
6301	DG	dentate gyrus
6302	DHE	dihydroethidium
6303	DHR	differentially hydroxylmethylated regions
6304	DNA	deoxyribonucleic acid
6305	DNMT	DNA methyltransferase
6306	DWI	diffusion weighted imaging
6307	E	energy
6308	EM	exploration mission
6309	FDG	fludeoxyglucose
6310	FDR	false discovery rate
6311	GABA	gamma-Aminobutyric acid
6312	GCR	galactic cosmic radiation
6313	GFAP	glial fibrillary acidic protein
6314	HERA	
6315	HexCer	glycosylated ceramides
6316	HO-1	heme oxygenase-1
6317	HRP	human research program
6318	HZE	high atomic number, high energy
6319	Iba-1	ionizing calcium-binding adaptor molecule 1
6320	ICAM-1	intercellular adhesion molecule 1
6321	IFNγ	interferon γ

6322	IHC	immunohistochemistry
6323	IL-1β	interleukin-1β
6324	IL-4	interleukin-4
6325	IL-6	interleukin-6
6326	IL-12p70	interleukin-12 heterodimer
6327	IQ	intelligence quotient
6328	ISS	International Space Station
6329	KEGG	Kyoto encyclopedia of genes and genomes
6330	KE(R)	key event (relationship)
6331	Ki-67	marker of proliferation Ki-67
6332	LEO	low-Earth orbit
6333	LET	linear energy transfer
6334	LPS	lipopolysaccharide
6335	LTP	long term potentiation
6336	MCAT	human cutulase in mitochondria
6337	MCM	medical countermeasures
6338	MIE	molecular initiating event
6339	MHC-II	major histocompatibility complex II
6340	MRI	magnetic resonance imaging
6341	NeuN	neuronal nuclear antigen
6342	NG2	cells expressing the NG2 chondroitin sulfate proteoglycan
6343	NHP	non-human primate
6344	NPC	neural precursor cell

6345	NSRL	NASA Space Radiation Laboratory
6346	OPC	oligodendrocyte precursor cell
6347	PET	positron emission tomography
6348	PlsEtn	phosphatidyethanolamine plasmalogens
6349	PMA	phorbol 12-myristate 13-acetate
6350	PSD-95	postsynaptic density protein 95
6351	PVIN	parvalbumin inhibitory interneurons
6352	QF	quality factor
6353	QTL	quantitative trait loci
6354	RBE	relative biological effectiveness
6355	REID	risk of exposure induced death
6356	RNS	reactive nitrogen species
6357	SIV	
6358	ROS	reactive oxygen species
6359	SPE	solar particle event
6360	SPEL	Space Permissible Exposure Limit
6361	ST	sulfatides
6362	STLV	
6363	SGZ	subgranular zone
6364	TCA	tricarboxylic acid
6365	TET	ten-eleven translocation enzymes
6366	Thy-1	thymocyte differentiation antigen 1
6367	TNFα	tumor necrosis factor α

6368	UPLC-HDMSE	ultra-performance liquid chromatography-high definition mass
6369		spectrometry
6370	WinSCAT	Space Flight Cognitive Assessment Tool for Windows
6371	Z	atomic number

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