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Impact of Spaceflight Stressors on Behavior and Cognition: a molecular, neurochemical, and neurobiological perspective

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Abstract

The response of the human body to multiple spaceflight stressors is complex, but mounting evidence implicate risks to CNS functionality as significant, able to threaten metrics of mission success and longer-term behavioral and neurocognitive health. Prolonged exposure to microgravity, sleep disruption, social isolation, fluid shifts, and ionizing radiation have been shown to disrupt mechanisms of homeostasis and neurobiological well-being. The overarching goal of this review was to document the existing evidence of how the major spaceflight stressors, including radiation, microgravity, isolation/confinement, and sleep deprivation, alone or in combination alter molecular, neurochemical, neurobiological, and plasma metabolite/lipid signatures that may be linked to operationally-relevant behavioral and cognitive performance. While certain brain region specific and/or systemic alterations titrated in part with neurobiological outcome, variations across model systems, study design, and the conspicuous absence of targeted studies implementing combinations of spaceflight stressors, confounded the identification of specific signatures having direct relevance to human activities in space. Summaries are provided for formulating new research directives and more predictive readouts of portending change in neurobiological function.

1. Introduction

Astronauts must maintain a stable and high level of performance efficiency over the course of their stay in space. During deep space missions, astronauts are exposed to a hazardous environment that can induce detrimental effects on the central nervous system (CNS) to impact operationally-relevant neurocognitive performance. Behavioral and neurocognitive problems occurring in space are predominantly related to four different sources: (1) physical factors, including acceleration, microgravity, radiation and light/dark cycles; (2) habitability factors, including vibration, noise, temperature, light and air quality; (3) psychological factors, including isolation, danger, monotony and workload; and (4) social or interpersonal factors, including gender issues, cultural effects, crew size, leadership and social dominance issues, and personality conflicts (De la Torre, 2014). Travel to Mars will involve continuous exposure to all of these challenges for up to 3 years, including time spent on the planet. Notably, the duration and distance of this mission will far exceed any prior deep space mission, subjecting these astronauts to unprecedented levels of exposure to these spaceflight hazards. Consequently, it is vital that we understand how these spaceflight stressors alone and in combination not only impact overall CNS-related behavioral and cognitive function in-flight, but also how they impact risk of manifesting neurodegenerative conditions when astronauts return to earth. Unfortunately, little information is currently available on how the combined exposure to these spaceflight stressors alter molecular, neurochemical, and neurobiological signatures in the brain to impact behavior and cognition. This information gap has significantly hindered our ability to realistically estimate spaceflight stressor risk to the CNS associated with human deep space exploration and,

consequently, impeded the development of future human deep space missions. Given this backdrop, our overarching objective in this review is to provide a better understanding of the impact of spaceflight stressors alone or in combination on brain molecular, neurochemical, and neurobiological pathways that are involved in operationally-relevant behavioral and cognitive function. Such information will considerably accelerate our ability to assess risks associated with exposure to combined spaceflight stressors as well as accelerate the development of effective mitigation strategies to successfully respond to CNS-related risk. We focused our efforts on 3 distinct yet overlapping questions:

1) Does the existing literature provide insights regarding the effects of spaceflight stressor alone that can be used meaningfully to forecast if/how they might interact to alter (additive, synergize, diminish) molecular, neurochemical, and neurobiological signatures related to behavioral and cognitive performance?

2) What additional experiments need to be performed to inform how these stressors alone or in combination impact brain molecular, neurochemical, and neurobiological pathways involved in CNS functionality?

3) What additional information is needed to properly identify and implement effective spaceflight countermeasures to minimize certain CNS (and overall health) complications associated with the long-term presence of humans beyond Earth's protective magnetosphere?

In this review, we document the existing evidence of how the four major spaceflight stressors, including radiation, microgravity, isolation/confinement, and sleep deprivation, alone or in combination alter molecular, neurochemical, and neurobiological signatures that may be

linked to operationally-relevant neurobehavioral and neurocognitive performance (see Fig. 1). Throughout, we consider a broad array of existing research from laboratory animals to humans on how acute and long-term exposure to spaceflight stressors alone or in combination impacts behavior and cognitive function and, whether such effects can be attributed to multimodal changes in the brain (see Fig. 1). Although, it is likely that exposure to combined spaceflight stressors will alter brain activity at multiple levels in animals and humans, ultimately, it is critical that changes in molecular, neurochemical, and neurobiological signatures are consistently and reliably linked with operationally-relevant behavior and neurocognitive performance. Where appropriate, we repurposed knowledge from other CNS-health studies to astronauts (e.g., aging, disorder, disease). In addition, we appreciate that plasma metabolite or lipid biomarkers represent a useful approach amenable for real-time in-flight biomarker assessment. These easily obtainable bio-samples can be quickly and longitudinally analyzed to assess developing health problems and to understand any impact on behavior and cognitive performance. Thus, we also summarize current literature on the impact of spaceflight stressors alone or in combination on critical peripheral biomarkers that potentially could be associated with behavioral and cognitive deficits. Finally, we documented open questions and identified research gaps in knowledge base that connects molecular, neurochemical, and neurobiological pathways to operational performance and provided recommendations for future multimodal research on spaceflight stressors.

2. Spaceflight Stressors

The possibility of acute and long-term CNS damage to humans induced by space radiation during deep space travel is one of the most poorly explored health risks in ground-based studies of space radiobiology. Human deep space missions will require travel beyond the Earth's protective magnetic field and involve different patterns of extended radiation exposure to galactic cosmic rays (GCRs) and solar particle events (SPEs), which consist of particles of high energy and charge (HZE) and protons. Mounting evidence suggest that the brain is sensitive to space radiation (National Research Council, 2008), raising concerns that exposure during extended deep space travel (e.g., a Mars mission) may lead to acute and long-term damage to CNS function, thereby jeopardizing mission success (Parihar et al., 2015a). Although astronauts will be exposed continuously to low doses of multiple HZE particles and protons during deep space travel, to date, studies in rodent models of neurobiology, behavior, and cognition have primarily focused on acute CNS effects following exposure to relatively high doses of HZEs or protons (Kiffer et al., 2019b). Results from these studies indicate that high doses of GCR/SPE lead to cellular and molecular damage to the CNS and produce short- and long-term decrements in behavioral and neurocognitive function. Moreover, recent studies show that exposure to low doses of different types of HZE's particles (e.g., ^{56}Fe, ^{48}Ti, ^{16}O) or protons also can produce profound and long-lasting changes in brain function, including neuroepigenetic, neurobiological, behavioral, and cognitive deficits (Parihar et al., 2016). These results indicate the urgent need for further research to systematically identify the acute and long-term CNS consequences of radiation exposure, with a focus on exposure that is representative of what will be encountered during deep space travel (e.g. mixed GCR exposure). In addition, exposure to other spaceflight

stressors including microgravity, isolation/confinement, and sleep deprivation also may significantly harm crew health and performance and may exacerbate space radiation-induced deficits in CNS function. Thus, identifying how exposure to spaceflight hazards alone or in combination alters key molecular, neurochemical, and neurobiological signatures that may provide an indication of the behavioral and neurocognitive performance consequences will permit realistic estimates of spaceflight hazard risk to the CNS associated with human space exploration (see Fig. 1).

2.1. Radiation

On interplanetary missions, astronauts will be exposed to a variety of particles of high energy and charge (HZE particles) that are not experienced in low earth orbit. The charged-particle flux that constantly irradiates the solar system originates from supernovas that occurred thousands of years ago within the Milky Way. These GCR are composed of approximately 86–91% protons, 8–13% helium nuclei, and 1% heavy (Z > 2) energetic (HZE) nuclei (Mewaldt, 1994; Nelson, 2016). Estimates are that initial manned missions to Mars will likely last 800–1100 days, of which approximately 500 days will be spent on the planet's surface, depending on final mission design (Drake, 2010). Mission dose estimates due to GCR are on the order of 25–50 cGy. Charged particles are qualitatively different than electromagnetic radiation, due to the different distribution of energy deposition in tissues and materials. The initial linear energy transfer (LET) of a given particle prior to tissue or material interaction can inform the number of ionization events the particle will induce. The LET of a given energetic charged particle will slowly lower as

the particle interacts with the target material, comprising the “plateau region” of the energy-absorption curve.

In attempting to predict risk for acute and delayed biological effects of such exposures, the assumption is made that the relative biological effectiveness of a particle is directly related to its LET: the higher the LET the greater the likelihood of significant effects on biological, behavioral, and cognitive endpoints. Much of the available data links the relative biological effectiveness of HZE particles in producing cytogenetic damage to a given particles LET. The effectiveness of HZE particles on disrupting neurobehavioral function is only partially dependent on particle LET. As particle LET increases, a lower dose is required to disrupt performance: exposing rats to ^{28}Si or ^{48}Ti particles disrupts neurobehavioral function at lower doses than are needed following exposure to the higher LET ^{56}FE particles (Rabin et al., 2007).

HZE particles are most effective at disrupting early performance decrements while neutron radiation is the least effective (Bogo et al., 1989). Increased dosage from heavy particle radiation has increasingly deleterious effects on behavior and neural function, while exposure to protons alone do not show any of these dramatic effects (Shukitt-Hale et al., 2004). HZE particle-induced changes in cognitive function do not require that the particles directly impact the brain but can develop as a consequence of irradiation of the body (Rabin et al., 2014), suggesting that astronauts may be at even greater risk for radiation-induced cognitive deficits. There are an array of neural and cognitive impairments following radiation exposure that may be a major factor in manned missions to Mars (Kiffer et al., 2019b). Over the course of a three year mission to Mars, brain neural cells will be subject to a direct hit by: a) an HZE particle ($z > 15$; 46% of cells exposed),

with 13% of the neurons traversed by an 56Fe ion (z = 26); and b) a proton, with all cells being traversed on the average of once every three days (Curtis et al., 2000, 1998). Even under conditions of low Earth orbit (90 days), an estimated 45% of brain hippocampal cells are likely to be hit by a high linear energy transfer (LET) particle and these numbers rise to >90% of the cells over a one-year mission (Yasuda et al., 2001). Based on such estimates, it is evident that exposure to GCRs and SPEs may both jeopardize mission success and cause short- and long-term CNS damage.

The limited number of humans that have travelled in space, their different missions, durations, and variable medical follow-up confound significantly, efforts to extrapolate how longer term, deep space radiation exposures and associated environmental stressors (microgravity, social isolation/confinement, and sleep deprivation) might interact to compromise mission success and CNS functionality. This fact has necessitated the use of animal models exposed to a variety of space relevant irradiation regimens to probe the multitude of effects on the brain. Notwithstanding, a now-rich literature points unequivocally to an elevated risk of manifesting mission critical neurocognitive performance decrements caused by space radiation exposure. A wealth of research using rodents subjected to carefully controlled behavioral tasks have uncovered that low dose level (≤ 50 cGy) and low dose rate (~ 1mGy/day) exposures using a range of lighter (^{1}H, ^{2}He) to heavier (^{16}O, ^{28}Si, ^{56}Fe) single and mixed charged particle beams of various energies (MeV-GeV) cause persistent deficits in learning and memory, executive functioning, attention, and disruptions in mood. While exposure to microgravity, isolation/confinement, or sleep deprivation have clearly been shown to have an impact on CNS

function individually, at present, it remains uncertain how each of these insults scales or interacts with other spaceflight stressors in a manner that may adversely affect the operationally-relevant activities of astronauts engaged in deep space travel.

2.2. Microgravity and Hypergravity

Microgravity refers to an environment where the pull of gravity is weak, resulting in an experience of weightlessness with a near 0 g-force (gravitational pull). In rodents, the effects of microgravity are often measured by partially simulating weightlessness through a procedure of tail-suspended hindleg unloading, wherein the tail and hindlegs are suspended with a downward head-tilt of approximately 30 degrees for some extended period of time. In humans, a similar procedure involves laying in a supine position with a downward head-tilt of approximately 6 degrees. In both cases, although the gravitational force is clearly not eliminated, the goal is to induce a fluid shift and relieve the brain and body of several of the effects of the downward force of gravity. These methods have been successful in revealing both physiological and neural changes as a result of the shift in gravitational pull, even though weightlessness itself is not achieved. Another methodological approach to studying microgravity is parabolic flight, where an aircraft increases in acceleration and decreases, resulting in about 20 seconds of 1.8G and ~0G exposure. This technique does not allow for exploring the long-term effects of microgravity but is used to expose humans to a 0G environment. The increase in acceleration is also referred to as hypergravity, where the force of gravity exceeds that on the surface of the earth. Hypergravity may also have an impact on physiology and neural function, which can be simulated by exposure to a centrifuge, generating high artificial gravity through rapid spinning. Finally, many studies

investigating the effects of hypergravity and microgravity have tested individuals (rodents, monkeys, and humans) before, during, and after spaceflight itself. While these studies are the most relevant for determining the effects of space travel on brain structure and function, they cannot isolate the various contributions of microgravity, hypergravity, stress, high workload, lack of sleep, and social isolation.

Space shuttle crewmembers have reported some degree of disorientation and perceptual illusions, accompanied by miscoordination while in flight, likely resulting from the microgravity environment (Bloomberg et al., 2016). The effect of microgravity results in loss of situational awareness, spatial disorientation, and sensorimotor problems, including difficulties with vision, head-hand-eye coordination, and an inability to judge distance and velocity that can contribute to in-flight errors.

2.3. Confinement and Social Isolation

Stress due to confinement and social isolation can affect emotions and cognitive performance, including adaptive and maladaptive coping styles and strategies. Space travel, particularly long duration space missions, results in a prolonged isolated and confined extreme (ICE) environment, with personal accounts of depression, insomnia, irritability, anxiety, fatigue, and decrements in cognitive performance (Palinkas, 2001). The effects of ICE environments have been studied in animal models and extended deployments to remote locations such as polar camps or hyperbaric chambers. In addition, social isolation can have significant negative effects on human mental health, particularly perceived social isolation (i.e., loneliness) (Cacioppo and Hawkley, 2009). Many of these effects follow a linear dose-response pattern with longer duration

of exposure to ICE environments resulting in greater behavioral and cognitive impacts. Among the range of neurobiological and behavioral effects of perceived isolation documented in human adults are increased anxiety, hostility, and social withdrawal; increased sleep fragmentation and daytime fatigue; increased vascular resistance and altered gene expression and immunity; decreased impulse control; and increased negativity and depressive symptomatology (Cacioppo et al., 2015). However, many of these deficits undergo an accommodation period, returning to normal levels following a period of time in space (Casler and Cook, 1999).

2.4. Sleep Deprivation

Sleep is not a homogenous state, but instead, the brain passes through multiple sleep stages, associated with dramatic alterations in neurochemistry across various regions in the brain. Establishing a normal light-dark cycle is important for establishing circadian rhythms related to healthy sleep cycles (Zulley, 2000). There is some evidence that cognitive impairments due to sleep deprivation start to be noticeable following periods greater than 16 hours of wakefulness (O'Hagan et al., 2018). One hallmark of sleep deprivation is the competing demands on neurobiological systems designed to keep the individual awake, while the other is exerting pressure to fall asleep, resulting in increased variability in alertness and motor coordination. Sleep deprivation increases the homeostatic drive to sleep, with resulting changes in proinflammatory cytokines and glycogen levels. The long-term consequences include hypertension, reduced parasympathetic tone, increased proinflammatory cytokines, increased oxidative stress, and increased cortisol and insulin (McEwen, 2006).

There are two distinct types of sleep: non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep, which are most easily distinguished on the basis of their patterns of brain activity (electroencephalogram, EEG) and muscle activity (electromyogram, EMG). The NREM–REM sleep cycle is repeated throughout the sleep phase, with an overall NREM to REM sleep ratio of about 4 to 1. Currently there is no consensus about the precise purpose of sleep. However, multiple theories advocate its involvement in neuronal recovery and plasticity processes, which are crucial for proper brain functioning including cognition and emotion. Learning and memory are particularly effected when restricted sleep becomes a chronic condition, causing a reduction of hippocampal cell proliferation and neurogenesis, a reduction in hippocampal volume, and impairments on hippocampal-dependent tasks and long-term memory consolidation.

3. Impact of Spaceflight Stressors on Behavior and Cognition

An important goal for evaluating the impact of spaceflight stressors on human behavior and cognition is to identify a series of behavioral tasks in laboratory rodents and nonhuman primates that translate reasonably well to humans. In particular, the behavioral tasks utilized to monitor an astronaut's in-flight performance ought to be based on their ability to uncover both overt and subtle deficits in neurobehavioral and cognitive processes that may impact mission success. For example, behavioral task that reveal a lack of attention, impulse/motor control, motivation, and/or anhedonia/depression to complete routine and new operations and/or task that highlight difficulty learning new spacecraft operations, solving problems, adapting to situations, and maintaining behavioral performance and cognitive control will be especially

valuable (see Fig. 1). Box 1 describes the different aspects of behavioral and cognitive performance that ought to be measured during spaceflight. While studies in humans have employed a variety of behavioral and cognitive tasks, NASA has defined a series of behavioral tasks that map reasonably well between rodents and humans. Radiation-induced decrements in spontaneous activity tasks, fear conditioning and extinction, psychomotor vigilance test, and attentional set shifting, have all been demonstrated and appear to map to some extent with some of the radiation studies in nonhuman primates (i.e., delayed match to sample test). We reviewed such studies and explored the use of touchscreen-based tasks to evaluate behavioral and cognitive function by primarily focusing on the following behavioral and cognitive domains (see Fig. 1): 1) learning (stimulus discrimination); 2) memory (object-location), 3) cognitive flexibility (stimulus-reversal); 4) cognitive control (flanker task); 5) working memory (delayed matching-to-sample or -position); 6) attention/vigilance (psychomotor vigilance task); and 7) depression/anhedonia (probabilistic reward task). These tasks were selected because recent advances in touch screen technology permit translational assessment of complex neurobehavioral performance in rodents using tasks that are well-established in non-human primate and human subjects (e.g., Kangas and Bergman, 2017). In addition, we reviewed studies that help provide information on how laboratory subjects respond to unexpected situation in these tasks, i.e., after radiation exposure in combination with other spaceflight stressors including social stress, metabolic stress, anxiety, and depression. Finally, we chose to focus on aspects of higher-cognition for this review, although some of these spaceflight stressors also have

negative impacts on perception and sensation, which would then impact performance in more complex cognitive domains.

3.1. Radiation

In early behavioral work, exposure to conventional γ radiation was reported to produce dose-related and reversible disruptions in motor function and operant behavior (Bogo et al., 1989; Mele et al., 1990, 1988; Mele and McDonough, 1995; Winsauer et al., 1995; Winsauer and Mele, 1993). In other studies, radiation exposure also was shown to decrease unconditioned activity, including aggressive, defensive, ambulatory, and rearing behaviors (Burghardt and Hunt, 1985; Chaput and Berardo, 1974; Chaput and Kovacic, 1970; Landauer et al., 1987; Maier et al., 1989). These early findings encouraged further research on the behavioral effects of space radiation, including ground-based studies using a wide range of endpoints, e.g., reactivity to stimuli, motivation, cognition, and mood (see below). Generally, these studies have shown that exposure to HZE, and to a certain extent, protons can produce profound deficits in simple and complex levels of motor and cognitive function and that these changes may be similar to those observed in age-related conditions such as Alzheimer's Disease. More recently, exposure to low doses of ^{56}Fe particle radiation has been reported to impair cognitive function as measured in contextual fear conditioning, novel object, attentional set-shifting, and spatial learning assays in rodents (Cherry et al., 2012; Lonart et al., 2012; Rola et al., 2005). Studies also have shown that exposure to low doses of different types of HZE's particles or protons can produce profound and long-lasting changes in brain function, involving epigenetic, neurobiological, behavioral, and cognitive deficits (Acharya et al., 2017; Britten et al., 2017a; Cherry et al., 2012; Davis et al., 2015,

2014; Hadley et al., 2016; Lee et al., 2016; Machida et al., 2010; Marty et al., 2014; Parihar et al., 2016, 2015b; Rabin et al., 2014; Sanchez et al., 2010). As noted above, effective doses of HZE particle radiation in those studies are comparable to those that can be expected during deep space travel. The below section highlights currently available information on how space radiation exposure impacts various aspects of behavior and cognition.

3.1.1. Learning and Memory

There is extensive evidence that exposure to the 1 GeV/u ^{56}Fe-Particle radiation dose (~20 cGy) that astronauts are likely to receive on a deep-space mission results in measurable impairment on various hippocampus-dependent spatial memory tasks when tested in animal models. For example, this dose has shown impaired performance on the Barnes maze (Britten et al., 2017a, 2017b, 2016a, 2012; Wyrobek and Britten, 2016), Y-maze (Kiffer et al., 2018), and novel-object recognition memory tasks (Krukowski et al., 2018a, 2018b). Irradiated mice and rats showed increased latencies in finding the hidden platform on the probe trials in the Morris Water Maze task (Manda et al., 2008; Shukitt-Hale et al., 2000) and increased errors on an 8-arm maze (Shukitt-Hale et al., 2003), consistent with radiation damage to the hippocampus and possibly striatum. Similarly, like aged animals, irradiated animals spent less time in the middle of the open field and less time exploring novel objects in an object-location task (Casadesus et al., 2004). Radiation-induced impairments in spatial, episodic and recognition memory were temporally coincident with deficits in executive function, reduced rates of fear extinction, and elevated anxiety (Parihar et al., 2016; Whoolery et al., 2017). Together, these results point to a clear and reliable disruption of the hippocampal, “declarative” memory system.

Moving out of hippocampal-based memory, the dose and frequency of radiation exposure had an impact in an operant conditioning task, ranging from 0.5 to 4.5 Gy, which was reversible until at much higher doses of 6.5, 7.5, and 9 Gy (Mele et al., 1988; Mele and McDonough, 1995). Frequent dosing of a much smaller radiation exposure is not as detrimental to performance as a single exposure to a very high level of radiation (Chaput and Kovacic, 1970; Mele et al., 1990). Similarly, dose-dependent increases (from 1 to 8 Gy) in the overall response rate on a repeated-acquisition task have been reported for 24-72 hours after exposure (Winsauer and Mele, 1993). This repeated-acquisition task suggests that the impairment lies more in new learning than in retrieval (Winsauer et al., 1995). Likewise, low doses of ionizing radiation exposure induce conditioned taste-aversion (Hunt et al., 1989; Rabin et al., 1989; Rabin and Hunt, 1986) and impairments on conditioned place preference (Rabin et al., 2003), although this effect can be mitigated by pre-exposure to radiation (Rabin et al., 1989). Finally, exposure to 5 Gy of 1GeV/u ^{56}FE radiation attenuated the disruption of the pre-pulse inhibition response and the acoustic startle response, consistent with a disruption to the dopaminergic system following HZE radiation (Haerich et al., 2005). Collectively, these observations point to the presence of impairments in "non-declarative" forms of memory that are not reliant upon the hippocampus. Thus, memory impairments are more widespread and complex. Attempts to mitigate these effects will need to address the wide range of brain structures and plasticity mechanisms used across these tasks.

3.1.2. Cognitive Flexibility & Control

Whether an individual rat exhibits radiation-induced impairment on a set-shifting task, measures of prefrontal cortex (PFC) function seem completely independent of whether it exhibits

radiation-induced spatial memory deficits and vice versa (Britten et al., 2016a, 2016b). These finding suggest that the PFC and hippocampus are differentially sensitivity to irradiation that varies between individuals and that neither brain region is consistently more sensitive than the other. Executive functions are also impaired following low-dose radiation exposure. Rats exposed to 1GeV/u ^{56}FE radiation showed impairments on a set-shifting task, both in the reversal of discrimination and in intra-dimensional set shifts (Britten et al., 2014; Lonart et al., 2012). These data are consistent with the evidence for a loss of functionality in several regions of cortex, including medial PFC (simple discrimination), anterior and posterior cingulate cortex (intra-dimensional shift), and basal forebrain (first time stimulus reward reversal). These regions are also important for planning, working memory, inhibition, mental flexibility and the initiation and monitoring of action. Further, these behavioral decrements appear to be associated with a reduction in the cholinergic expression within basal forebrain, which has been shown to play a major role in regulating the activity of the PFC. Similarly, the ability of the rats to conduct compound discrimination reversal (CDR) and compound discrimination (CD) was impaired (Hadley et al., 2016). Impaired compound discrimination performance results in a decreased ability to identify and focus on relevant aspects of a task being conducted, while impaired CDR performance reduces the ability to recognize when that factor changes from a positive to a negative factor for the successful completion of a task.

Pre-selecting rats that can perform a set-shifting task prior to radiation shows that radiation exposure had less impact on working memory but a greater impact on associative learning and memory (Jewell et al., 2018). Thus, radiation-induced performance deficits may

differentially manifest in a context-specific manner as scenarios change and where astronauts are required to transitively apply their knowledge to solve problems that they have not previously encountered. Nevertheless, potentially one-third of astronauts may not be able to perform event-critical tasks correctly. The implication of this data, from a probabilistic risk assessment perspective, is that cognitive performance studies that use naive rodents may overestimate the risk of radiation-induced cognitive deficits.

3.1.3. Attention/Vigilance

In rats, exposure to 5 Gy of 1 GeV/u ^{56}FE decreased discrimination accuracy and increased false alarms in an analogue to the human psychomotor assessment task, consistent with reduced inhibitory control and a shift towards anticipatory responses at the cost of decreased accuracy (Hienz et al., 2008). Similar findings have been shown following proton irradiation, with negative impacts on psychomotor vigilance in rats (Davis et al., 2015, 2014).

In nonhuman primates, the results are largely consistent, but may be more readily translated to the human. As noted by Desai and colleagues (Desai et al., 2021), a majority of the studies on behavioral and cognitive performance in nonhuman primates can be grouped into those that were conducted about 50-60 years ago (~1958-1968) and those conducted within the last 10 years (~2011-2016). A vast majority of these studies were conducted in rhesus macaques using whole body (e.g., x-ray, gamma, neutron) and fractionated whole-brain irradiation. Results from the NHP studies are generally consistent with numerous rodent studies examining simplified cognition-related behavior in that they both demonstrate radiation-induced deficits across several diverse cognitive endpoints. Moreover, the consistency of effects observed within

a particular NHP cognitive domain across studies (i.e., 60 years ago vs. last 10 years) is somewhat remarkable. For example, repeated acquisition and discrimination reversal tasks, which are thought to assay, respectively, basic features of learning and cognitive flexibility, show magnitude-related radiation impairment in both eras, despite relatively primitive apparatus and methods available in the 1950's versus the modern technology available today. Nevertheless, functional similarities in impairment are obvious. Crucially, one thread that connects several studies on irradiation and cognitive performance in monkeys across the decades relates to a recurring finding of the impact of cognitive load on observed impairments. For instance, a number of studies that varied the task difficulty in addition to magnitude of radiation (e.g., Davis, 1961; Hanbury et al., 2016; Robbins et al., 2011) revealed important effects of lower radiation magnitude insults on behavior and cognition that are likely translational and might not otherwise have been observed. This appears critical for the ability to examine spaceflight stressor effects on behavioral and cognitive performance that, although subtle, may have long-lasting and significant consequences on astronauts during and/or after long-duration deep space missions. More generally, this highlights the critical need for targeted studies in nonhuman primates, as rodents are considerably inferior subjects to use for examination of performance under tasks that vary in cognitive load that include tasks that assess multiple endpoints (e.g., learning, memory, attention) (Desai et al., 2021).

3.2. Microgravity

3.2.1. Learning & Memory

Long term exposure to microgravity has a clear impact on hippocampal function and spatial memory. For example, over the course of 7-28 days, microgravity led to dysfunction of the cholinergic system that increased with the amount of exposure and coincided with decreases in learning and memory performance on the Morris water maze task (Bellone et al., 2016; Temple, 2002; Wu et al., 2017; Zhang et al., 2018). Similarly, while place field selectivity of hippocampal neurons appears to be intact for three-dimensional spaces in microgravity, it appears to require a longer period of adaptation before the place fields fully stabilize (Knierim et al., 2000), contributing to imprecise memory for space. Further, impairments in vestibular input and locomotion under conditions of microgravity may contribute to performance on spatial memory tasks, in addition to reductions in hippocampal volume (Besnard et al., 2012; Brandt et al., 2005; Machado et al., 2014).

3.2.2. Attention/Vigilance

In normal gravity, visual perception obeys the principle of size constancy and distance to the observer but in microgravity, depth cues are distorted by the conflict between retinal, visual, and gravity-based cues to body orientation. Perceptions of orientation (Dyde et al., 2009), height, depth, and distance of objects (Clément et al., 2013) and visual illusions (Villard et al., 2005) are altered during microgravity periods. Exposure to a microgravity is likely to disrupt the objective visual cues involved in orientation, depth, and distance. The gravitational frame of reference seems to facilitate the mapping of body-part representation onto spatial coordinate positions, which can be altered in a microgravity environment (Grabherr and Mast, 2010), as are illusory body movements (Roll et al., 1993) and accuracy in pointing (Watt, 1997). Similarly, manual

tracking errors are higher following extended exposure to a microgravity environment, possibly because complex motor programming resources are altered during sensorimotor adaptation to the space environment (Bock et al., 2010; Eddy et al., 1998; Fowler et al., 2000; Heuer et al., 2003; Manzey, 2000; Manzey et al., 1993; Semjen et al., 1998; Wollseiffen et al., 2016). These microgravity-based issues are important because changes in depth perception could result in spatial disorientation episodes, errors in object (e.g., approaching vehicle) distance perception, and difficulties in navigating within or outside a given spacecraft. In addition to psychomotor and perceptual decrements as a result of exposure to microgravity, dual-task impairments suggest that there are attentional costs and cognitive resource limitations associated with spaceflight (Manzey et al., 1998, 1995; Seaton et al., 2007). In addition, increases in gravity have been associated with poor perception of time (Clément, 2018), resulting in underestimates of how much time a task would take in space.

3.2.3. Depression/Stress

In addition to cognitive impairments during spaceflight, emotional processing may be negatively affected, with decrements observed on an emotional variant of the Stroop task (Pattyn et al., 2005). In a study of a single astronaut during an extended space mission, subjective mood ratings were remarkably stable across the 2nd to 14th month in space (Manzey et al., 1998). Long-term space missions may be associated with disturbances of attentional processes during adaptation to living conditions in space and re-adaptation to Earth conditions after the flight and may reflect the conglomerate of stressors associated with these critical phases, which also are reflected in subjective feelings of reduced personal strength and elevated workload. Stress

triggers the hypothalamus-pituatary-adrenal (HPA) axis, producing glucocorticoids that contribute to the regulation of both neural and behavioral responses. Acute stress results in a U-shaped response curve, with small increases in glucocorticoids improving hippocampal-mediated memory but large increases impairing hippocampal function. Chronic stress can also result in morphological changes in the hippocampus, but in both chronic and acute adult stress, these effects are reversable after a few weeks of non-stress (Lupien et al., 2009).

3.3. Confinement and Social Isolation

3.3.1. Learning and Memory

In conditional visual discrimination, serial reversal learning (Jones et al., 1991), and probabilistic reversal learning (Amitai et al., 2014) tasks, rats raised in isolation were impaired relative to socially reared rats, possibly reflecting a greater focus on gaining reward during rule-learning in this group. While initial learning is typically normal, rats reared in isolation struggle with set-shifting or updating new reward-contingencies based on changing environmental demands. Indeed, isolation-reared rats had significantly increased levels of metabolites indexing the dopaminergic system, a neurotransmitter involved in the reward system (Jones et al., 1991). Likewise, social isolation negatively impacts social recognition memory, even following periods of social isolation as brief as 1-day (Kogan et al., 2000) and rats raised in isolation are impaired on novel object recognition, a task reliant on the hippocampus, and attentional set-shifting, largely dependent on the medial prefrontal lobe (McLean et al., 2010). Outside of learning and memory deficits, there is little evidence for a negative impact of isolation on decision making tasks (Hockey and Sauer, 1996; Hockey and Wiethoff, 1993).

In addition to performance deficits such as spatial memory impairments on the Morris water maze task, isolation-reared APP/PS1 transgenic mice (designed with a genetic predisposition to develop Alzheimer's disease pathology) show an acceleration in AD-like pathology with greater levels of beta-amyloid plaques and decreased NMDA receptors in the hippocampus (Huang et al., 2011). These data suggest that social isolation can contribute to the exacerbation of AD-related pathology and behavioral deficits.

3.3.2. Depression/Anxiety

Social isolation can result in symptoms of depression due to lack of social contact, social support, and integration within a larger community (Ge et al., 2017). In addition to signs and symptoms of depression, social isolation may increase signs of anxiety. Some reports suggest that anxiety levels were not higher following social isolation (Gorlova et al., 2018; Hockey and Sauer, 1996; Kogan et al., 2000). Consistent with this, cortisol levels have also been shown to be stable following social isolation (Ross et al., 2017). However, there have been conflicting results of reduced exploration time in an open-field test (Linge et al., 2013), suggesting a possible role for anxiety. In addition, increasing social isolation has been shown to increase aggressiveness (An et al., 2017). Further, returning animals to group housing, even for 1-day, can mitigate the impairment in social recognition memory (Shahar-Gold et al., 2013) and aggression (An et al., 2017), consistent with a change in mood.

There has been a plethora of research identifying a central role for oxytocin and vasopressin in the regulation of social behavior, both of which modulate activity in the amygdala and lateral septum that projects to the hippocampus (Leser and Wagner, 2015). Long-term social

recognition memory is mediated by oxytocin-dependent synaptic plasticity in the amygdala, which is abolished in rats that have been socially isolated for 1-week (Gur et al., 2014). The administration of arginine-vasopressin (AVP) following social isolation has also been shown to mitigate the long-term memory effects of social isolation on social recognition memory, suggesting a possible treatment avenue (Shahar-Gold et al., 2013). AVP is a hormone that increases the amount of soluble water that can be reabsorbed into circulation and constricts arterioles, resulting in an increase in arterial blood pressure. Thus, social isolation results in malfunctioning of the oxytocin-mediated neuromodulatory mechanism of the amygdala that may benefit from interventions such as external administration of oxytocin or vasopressin.

3.4. Sleep Deprivation

3.4.1. Learning & Memory

Learning and memory rely on sleep-dependent consolidation, or the changes in plasticity associated with the long-term retention of new material. Sleep deprivation disrupts these processes, negatively affecting learning and memory of new procedural skills, working memory (Pasula et al., 2018; Xie et al., 2015; Zhu et al., 2019) and some other forms of memory (Chee and Chuah, 2008; Walker and Stickgold, 2004). For example, sleep deprivation has been shown to negatively impact memory performance for both items and associations (Ratcliff and Van Dongen, 2018), suggesting that attentional processing underlying encoding may be more impacted than memory processes *per se*. Consistent with this finding, false recognition was elevated following total and partial memory deprivation, while veridical memory was intact, consistent with sleep-related impacts during encoding more than retrieval of information (Lo et

al., 2016). The negative impact on sleep deprivation extends to the intention for future actions as well, resulting in decreased prospective memory performance (Esposito et al., 2015; Grundgeiger et al., 2014). Finally, sleep plays an important role in memory consolidation, with increased performance on spatial navigation in maze tasks and nonspatial odor memory following a night's sleep (Chee and Chuah, 2008).

In animals, sleep deprivation has reduced freezing behavior following contextual fear conditioning, particularly following longer periods of sleep loss (Hagewoud et al., 2010). Likewise, sleep deprived rats were impaired on the acquisition, consolidation, and retrieval of a discriminative avoidance task following 96 hours of sleep loss, all of which were recovered following a 24-hour sleep period (Alvarenga et al., 2008). While performance decreased during 16-24 hours of wakefulness, this decline may diminish or recover up to 40 hours of wakefulness in a divided attention task (Chua et al., 2017) and psychomotor vigilance tasks (Jung et al., 2011), suggesting different time courses for cognitive decline following increased sleep deprivation depending on the task demands or circadian rhythms.

3.4.2. Cognitive Flexibility & Control

Sleep deprivation has a profound impact on attention and executive functions, both functions tied to the prefrontal cortex (PFC) and frontostriatal circuit. Attention can be divided into four categories: 1) selective (maintain focus despite distraction), 2) divided (ability to respond to multiple task demands simultaneously), 3) orienting or switching (mental flexibility to shift focus of attention across various tasks), and 4) sustained attention or vigilance (the ability to maintain focus during continuous and repetitive activity) (Ma et al., 2015). Attention allows us

to focus on a single percept while suppressing others and may be particularly vulnerable to sleep loss because it is highly dependent on precisely timed suppression mechanisms across the brain that dynamically select and suppress competing stimuli (Kirszenblat and van Swinderen, 2015).

Sleep deprivation also negatively affects the ability to update task-relevant information in response to changing circumstances, while the ability to maintain task-relevant information in the focus of attention is relatively spared (Whitney et al., 2019). For example, working memory performance (Gosselin et al., 2017; Raidy and Scharff, 2005), task-switching (Heuer et al., 2004) and set-shifting (McCoy et al., 2007) are impaired following sleep loss, with deficits in the Iowa Gambling Task (Whitney et al., 2019), n-back tasks (Choo et al., 2005), go/no go tasks (Chua et al., 2017; Satterfield et al., 2018), the Stroop color-word task (Gevers et al., 2015; O'Hagan et al., 2018), and the Stop Signal task (Kusztor et al., 2019; Slama et al., 2018; Zhao et al., 2019a). Further, more complex tasks are more sensitive to sleep-deprivation than simple ones (Leenaars et al., 2012). These tasks require the continual updating of information based on prior outcomes, requiring flexibility in attentional control for good performance and the reallocation of attention away from irrelevant to relevant information (Alfarra et al., 2015; Drummond et al., 2012; Honn et al., 2019). Also, sleep deprivation may cause a devaluing of feedback or outcome value, resulting in impairments in reversal learning and an over-reliance on habit-based control (Chen et al., 2017). Interestingly, the decrements in cognition following sleep deprivation are not always predicted by subjective reports of fatigue, suggesting that humans are not good judges of the impact of their sleep-deprived status (Slama et al., 2018).

3.4.3. Attention/Vigilance

Typical impairments in attention following sleep loss involve “lapses” or “microsleeps”, which involve response failures and errors of omission, and increases in reaction times with highly variable and erratic response profiles. The psychomotor vigilance task (PVT) is routinely used to measure reduced alertness because it is highly reliable and sensitive to sleep loss. It involves a simple reaction-time task that can be repeatedly administered with minimal impact from repeated testing. The number of lapses and increased reaction times provide a sensitive measure of instability in sustained attention. This task has proven to be very sensitive to performance deficits as a result of sleep deprivation (Arnal et al., 2015; O’Hagan et al., 2018; Slama et al., 2018). Additionally, this task can be administered in a 10-minute lab-based version, but a 3-minute tablet-based version has also proven to be a sensitive measure of sleep deprivation on cognitive performance (Grant et al., 2017).

Depression/Anxiety

Sleep loss results in altered emotional functioning, including a decline in mood and impairments in emotional perceptual, control, comprehension, and expression (Kilgore, 2010). For example, following 56 hours of wakefulness, individuals showed significant elevations on clinical scales of depression, anxiety, and paranoia. Similarly, sleep disturbance is a significant risk factor for subsequent clinical depression (Riemann et al., 2001; Tsuno and Ritchie, 2005). Task-switching deficits following sleep loss may prevent appropriate updating of emotional states when evaluating affective stimuli (Alfarra et al., 2015). Further, functional imaging suggests that sleep deprivation weakens top-down inhibitory control over the amygdala by the PFC, leading to

dysregulation of emotional processing (Kilgore, 2010). Sleep loss also affects risk-taking, altering the expectations and valuation of gains and losses by affecting activation within reward regions of the brain. Similarly, sleep deprivation can reduce empathy and negatively impact emotionally guided moral judgments, resulting in a significant slowing of decision making when the dilemma was high emotionally charged (Kilgore, 2010).

4. Molecular Basis for Spaceflight Stressor-Induced Behavioral and Cognitive Deficits.

Studies in rodents suggest that space radiation exposure elicits changes in inflammation but does not elicit the acute radiation syndromes. Relatively small changes in hematopoietic function and cell numbers caused by radiation exposure are not likely to have a significant functional impact. However, whether systemic factors (derived from whole body exposures) impact CNS inflammatory responses is unknown. Persistent inflammation is likely a major mechanism contributing to the lasting signature of radiation injury in the brain. In addition to radiation, the impact of other spaceflight stressors on molecular and cellular brain function remains unclear. We reviewed studies that use immunohistochemical and Western blot measurements to assess changes in brain microglia, astrocytes, and key receptors (e.g., puringergic receptors), as well as studies that utilized ELISA to determine blood and brain levels of pro- and anti-inflammatory cytokines (e.g., IL-1beta, IL-6, IL-10, TNFalpha) and chemokines. In addition, DNA microarray and RT-PCR of changes in gene expression in key brain areas (e.g., PFC, nucleus accumbens, amygdala, hippocampus) to align with analyses of neurochemical, neurobiological, and behavioral/cognitive function measurements were documented.

4.1. Radiation

One of the most fundamental biochemical changes observed in the irradiated CNS is a persistent oxidative stress involving elevations in reactive oxygen (ROS) and nitrogen (RNS) species (Giedzinski et al., 2005; Limoli et al., 2007; Tseng et al., 2014). The persistence of such changes is believed to be the result of dysregulated mitochondrial oxidative phosphorylation (OXYPHOS) rather than the a long-lived reactive species (Leach et al., 2001). Following irradiation, electrons have increased residence times at each mitochondrial complex (in particular complex I and III), effectively increasing the likelihood that electrons back react with oxygen forming superoxide. Superoxide can then react at diffusion-controlled rates with nitric oxide to form peroxynitrite, the second most reactive free radical cells must deal with after irradiation. Superoxide can also be eliminated by three geographically distinct isoforms of superoxide dismutase (SOD), namely cytoplasmic SOD1, mitochondrial SOD2 and extracellular SOD3, forming hydrogen peroxide and molecular oxygen (Wang et al., 2018). Hydrogen peroxide can then participate in Fenton chemistry to generate the most reactive free radical cells ever encountered, namely, the hydroxyl radical, which is also a direct product of water radiolysis (Hall and Giaccia, 2012). The importance of radiation-induced oxidative stress cannot be overemphasized, as it established the prerequisite conditions for altering cellular signaling that interferes with neurotransmitter release and neurotransmission in the irradiated brain.

The importance of oxidative stress in the space environment has been reviewed (Steller et al., 2018) and is particularly critical in regulating the response of the brain to charged particle irradiation. Evidence of the foregoing can be found in a series of studies utilizing mice genetically

engineered to overexpress human catalase targeted to the mitochondria. These mice were originally developed to increase longevity (Schriner, 2005), exhibit improved cognition (Parihar et al., 2015b), enhanced neurogenesis (Liao et al., 2013) and have a preservation of hippocampal CA1 neuronal morphology after proton irradiation (0.5, 2 Gy) when compared to wild type mice (Parihar et al., 2015b). The protective effects of catalase overexpression are presumed to mitigate the radiation-induced increase in mitochondrial derived hydrogen peroxide resulting from perturbed OXYPHOS.

Neurogenesis has been the endpoint that has brought together the fields of radiation biology and neuroscience, and has demonstrated the exquisite sensitivity of newly born neurons to ionizing radiation (Mizumatsu et al., 2003). Earlier work has demonstrated clear dose-response curves for reductions of proliferating cells in the neurogenic regions of the rodent brain following x-irradiation (Tada et al., 2000, 1999). Recent hippocampal neurogenesis studies suggest that doses of ionizing radiation may have damaging biological and molecular effects on brain mitotic and post-mitotic cells (Norbury and Zhivotovsky, 2004). Studies have demonstrated that the cellular response to ionizing radiation is complex and varies across cell types and forms of radiation (Pouget and Mather, 2001). Radiation-induced damage to DNA has been shown to activate the apoptotic process in neurons, leading to the emergence of peripheral neuropathies, neurodegeneration, and neuropathological conditions (Chen et al., 1997; Enokido et al., 1996; Nakajima et al., 1994). This cascade may involve molecular events, including the regulation of tumor suppressor protein p53, or its target components downstream (Enokido et al., 1996; Morrison et al., 2003; Wood and Youle, 1995). Exposure to ionizing radiation alters the molecular

and cellular structures that impact critical activities within the irradiated CNS. Changes include the structural deterioration of neurons, electrophysiological disruptions at both the cellular and network levels, and reductions in synapse density and myelination that all interact to impair neurotransmission.

Recent data has now confirmed just how deleterious charged particle exposures are to the structural integrity of neurons and supporting structures. In a recent study (Dickstein et al., 2018), low dose space-relevant charged particle exposures (30 cGy) were found to reduce synapse density and myelination within select regions of the irradiated hippocampus. Such data suggests that many of these structural alterations portend the types of behavioral decrements observed after low dose charged particle exposures. These findings, along with data demonstrating elevated inflammation persisting 1 year following space relevant exposures, point to logical biomarkers that can be pursued, where the challenge clearly lies in determining if/how such dramatic structural and inflammatory responses can be longitudinally assayed in a convenient and non-invasive format during actual deep space travel.

It is currently unknown precisely how space radiation-induced molecular and cellular changes coupled with inflammatory responses in the CNS translate to functional deficits in CNS-related activity in the whole organism. A critical evaluation of the most promising candidate biomarkers for assessing resultant changes in operationally-relevant behavioral and cognitive performance is crucial. Interestingly, recent studies have shown that exposure to low doses of different types of HZE's particles (e.g., ^{56}Fe, ^{48}Ti, ^{16}O) or protons causes cognitive dysfunction and increase amyloid plaque pathology in an APP/PS1 mouse model of Alzheimer's disease (Cherry

et al., 2012). Of note, effective doses in these studies are comparable to those that astronauts might encounter during a Mars mission (Cucinotta and Durante, 2006). Additionally, it is unclear whether a combination of spaceflight hazards (i.e., radiation, microgravity, isolation/ confinement, and sleep deprivation) will exacerbate molecular and cellular deficits as well as inflammatory responses to further impact crew health and behavioral performance during deep space missions, effects that will likely exhibit sexual dimorphisms.

4.2. Microgravity

At a cellular level, the effects of microgravity are moderated by factors such as hemodynamic and hydrostatic pressure, fluid shear stress, three-dimensional tissue stress, mass transport, and permeability. These effects influence cells in many ways, such as membrane-bound receptors and ion channels, primary cilia, cell shape, cytoskeletal and membrane structure changes. These changes are important because cell morphological polarity determines behavior. For instance, non-polarized cells are more likely to undergo apoptosis. An increase in cell apoptosis is a significant consequence of the changes in cell structure and function that occur in microgravity. Thus, microgravity has effects on both cell shape and cytoskeleton (Mann et al., 2019). Hindlimb unloading, as proxy for microgravity, results in altered gene expression, including changes in protein classes involved in learning and memory and synaptic transmission (Frigeri et al., 2008). The highest percentage of upregulated genes were found in the TIC class (transport of small molecules and ions into the cells) and the most down-regulated genes in the JAE class (cell junction, adhesion, extracellular matrix) (Frigeri et al., 2008). While many of these findings relate to all cell types, including neurons, there may be some specificity for gene expression alteration

in the hippocampus that relate the learning and memory functions (Chen et al., 2016; Xiang et al., 2019). Further, there is evidence that microgravity may inhibit the repair of DNA by radiation (Zhang et al., 2015).

4.3. Confinement and Social Isolation

Social isolation has been associated with upregulation of proinflammatory gene transcripts (e.g., mRNAs encoding proinflammatory cytokines and other inflammatory mediators), and a downregulation of anti-inflammatory markers (e.g. bioinformatic indications of reduced transcriptional activity of the glucocorticoid receptor), which may contribute to an increased risk of inflammatory disease over time (Cacioppo et al., 2015). Proinflammatory gene-regulation dynamics observed in mouse paradigms involving repeated social threat derive in part from catecholamine-mediated alterations in immune cell development within the bone marrow, which generates a population of glucocorticoid-resistant monocytes that are primed for hyperinflammatory responses as they subsequently circulate throughout the body.

Social isolation in rats has resulted in increased HDAC5 expression, decreased H3K9 and H4K12 acetylation, reduced brain-derived neurotrophic factor (BDNF) levels, and impaired long-term memory (Lander et al., 2017; Viana Borges et al., 2019; Zaletel et al., 2017). Among the epigenetic mechanisms activated in the hippocampus by chronic stress is the modulation of histone acetylation (such as HDAC5), which promotes gene transcription. Further, social isolation in rats has been shown to cause cognitive dysfunction and decreased synaptic protein (synaptophysin or PSD93) expression in the PFC, hippocampus, amygdala, and caudal putamen, and reduced the levels of BDNF, serine-473-phosphorylated Akt (active form), and serine-9-

phosphorylated GSK-3β (inactive form) in the hippocampus (Gong et al., 2017). In addition, BDNF is distributed throughout the brain, but highly concentrated in the hippocampus. It is involved in the growth and differentiation of new neurons and synapses. Even just 4-8 weeks of social isolation is enough to cause a decrease in BDNF in the hippocampus, along with decreases in learning and memory behavior (Zaletel et al., 2017). Consistent with the findings of reduced BDNF, social isolation has resulted in reduced neurogenesis in the dentate gyrus of the hippocampus (Cinini et al., 2014)

Social isolation also exacerbated the effects of stress with weight gain, induced anxiety-like behavior, and decreased AcK9H3 levels (Viana Borges et al., 2019). Similarly, social isolation results in anhedonia and depression-like behavior after 8 weeks, concomitant with decreases in spine density and levels of Synapsin1, PSD95, and GluR1 in the mPFC, changes that were reversed by a single injection of ketamine (5 mg/kg) (Sarkar and Kabbaj, 2016). Interestingly, antidepressant drugs have been shown to reverse the BDNF decrease induced by social isolation through enhancement of histone acetylation at BDNF promoters, suggesting a possible mechanism for offsetting this deficit (Gong et al., 2017).

4.4. Sleep Deprivation

Figure 2 provides an overview of the impact of sleep deprivation across various endpoints (Abel et al., 2013). Several molecular mechanisms modulate structural and synaptic plasticity such as the pathways that require cAMP, glutamatergic signaling, protein synthesis through mTOR, and gene transcription. Sleep deprivation negatively impacts these signaling events (Raven et al., 2018). Sleep deprivation may impair hippocampal neuronal plasticity and memory

processes by attenuating intracellular cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) signaling which may lead to alterations in cAMP response element binding protein (CREB)-mediated gene transcription, neurotrophic signaling, and glutamate receptor expression (Kreutzmann et al., 2015).

Sleep deprivation impairs cellular excitability necessary for inducing synaptic potentiation and accelerates the decay of long-lasting forms of synaptic plasticity (Abel et al., 2013). Furthermore, sleep promotes mRNA translation, while extended wakefulness caused by sleep deprivation negatively impacts clusters of genes critical for translational processes, including those known to be essential for memory encoding and consolidation. For example, sleep and sleep deprivation may specifically modulate the function of transcription factors such as CREB that bind to the cAMP-responsive element. Consistent with this view, CREB phosphorylation within the hippocampus is elevated during and reduced after 5-6 hours of total sleep deprivation or longer periods of REM sleep deprivation (Abel et al., 2013). Sleep deprivation has also been associated with alterations in the expression levels of the AMPA and NMDA receptors in the hippocampus, thus affecting synaptic strength and capacity for plasticity in this region (Xie et al., 2015). The 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and N-methyl-d-aspartate receptors (NMDARs) are the main ionotropic glutamate receptors and play a vital role in the synaptic plasticity and acquisition of spatial memory in the hippocampus.

Hippocampal long-term potentiation (LTP) is a cellular model for memory storage. Both LTP and memory processes that require the hippocampus are particularly susceptible to sleep loss. Slow wave activity (SWA) during NREM (non-rapid eye movement) sleep, which is at its

highest at sleep onset and decreases with time spent asleep and intensifies as a function of prior wake duration. This SWA is a marker for synaptic strength, contributing to synaptic renormalization, thus a disruption in SWA can lead to the occlusion of LTP in the hippocampus, critical for successful learning and memory performance (Wolf et al., 2016). Decreased BDNF is a marker of sleep-dependent reduced synaptic plasticity and neuronal atrophy, which can return to baseline levels following recovery sleep.

5. Neurochemical Basis for Spaceflight Stressor-Induced Behavioral and Cognitive Deficits.

Mounting evidence from *in vitro* studies in rodents suggests that exposure to radiation alters brain neurochemistry and the availability of key neurotransmitters that may be related to neurobehavioral and cognitive function. Research using *in vivo* microdialysis and other neurochemical assays that measure real-time changes in key neurotransmitters (dopamine (DA), glutamate (GLU), γ-aminobutyric acid (GABA), serotonin (5-HT) etc.) and other neurochemicals including metabolites (e.g., DOPAC, HVA etc.) in different brain regions (e.g., PFC, nucleus accumbens etc.) involved in complex neurobehavioral and cognitive function was reviewed. The identification of persistent changes in brain neurochemical signatures in irradiated laboratory animals that have also been exposed to other spaceflight stressors is likely to be a major factor in behavioral and neurocognitive abnormalities. Moreover, identifying changes in specific neurochemical brain systems that may be associated with exposure to spaceflight hazards will accelerate the future development of targeted novel treatment strategies to counter the effects of spaceflight hazards.

5.1. Radiation

While a vast majority of studies surveyed, indicate some alterations in distinct neurochemical signatures in specific brain regions, it is unclear whether such radiation-induced changes is the cause of behavioral and neurocognitive impairments. Investigators have attempted to relate behavioral and neurochemical sequelae of HZE particle and proton radiation exposure, focusing most often on the presumed relationship between DA neurochemistry and DA-mediated motoric and cognitive endpoints (Haerich et al., 2005; Hunt et al., 1989; Joseph et al., 1998, 1992; Rabin et al., 2004, 2003, 2001, 2000; Rice et al., 2009; Shukitt-Hale et al., 2007, 2004). However, previous studies, conducted exclusively with *in vitro* assays of DA function and DA-related behavioral studies in rodents, often have yielded mixed results. For example, Rabin and colleagues provide evidence for a relationship between radiation-induced damage to DA in selected regions (substantia nigra and striatum) and deficits in DA-mediated motoric and cognitive measures (Haerich et al., 2005; Hunt et al., 1989; Joseph et al., 1998, 1992; Rabin et al., 2004, 2003, 2001, 2000; Rice et al., 2009; Shukitt-Hale et al., 2007, 2004). In those studies, observed behavioral and neurochemical deficits were quickly evident and persisted following a threshold HZE (^{56}Fe) radiation dose (Joseph et al., 1992; Rabin et al., 2004). However, these investigators also report a puzzling lack of association between the LET (linear energy transfer) of HZE particles and their relative effectiveness in disrupting behavior or DA regulation (Rabin et al., 2004). Other investigators have reported somewhat different effects of exposure to ^{56}Fe particles, e.g., alterations in cocaine's locomotor stimulant effects but no change in its other behavioral effects or in the density of DA transporters in midbrain and forebrain regions (Rice et

al., 2009). The extent to which such different findings among studies reflect variations in behavioral and neurochemical procedures is unknown. Alterations in learning and memory and DA signaling in striatal slices also were found to be more apparent in older rats, suggesting that the aging brain may be more susceptible to the deleterious effects of space radiation (Carey et al., 2007).

It is noteworthy that, while there has been a focus on DA function in previous studies, a growing literature indicates the relevance of other neurochemical systems in neurobehavioral and cognitive processes (Altman et al., 1996; Bussey et al., 2012, 2008; Goodman, 2008; Kehagia et al., 2010; Kueh et al., 2008; Liu et al., 2008; Obulesu and Rao, 2010; Van Dam and De Deyn, 2011). To that end, more recent *in vitro* studies indicate that exposure to low doses of GCR/SPE produces persistent deleterious neurochemical changes in GLU transmission, including reductions in levels of NMDA receptors in the hippocampus (Machida et al., 2010; Marty et al., 2014; Sanchez et al., 2010), GABA (Lee et al., 2016; Marty et al., 2014) and DA (see above). Along these lines, Belov et al. (2019) conducted perhaps the most extensive neurochemical study thus far in which changes within the monoamine system in rat brain tissue from five key regions (e.g., PFC, hypothalamus, nucleus accumbens, hippocampus, and striatum) were determined at 1, 30, and 90 days after exposure to a single dose of 1Gy of protons or ^{12}C particles. Overall, results from this work showed significant changes in various aspects of the monoamine system, especially brain monoamine metabolism endpoints, in PFC, nucleus accumbens, hippocampus, and striatum 24 hr after exposure to protons. These changes appeared to persist in the nucleus accumbens, hippocampus, and striatum 30 days after exposure. At 90 days post-exposure,

changes were observed in the nucleus accumbens, hypothalamus, and hippocampus, but not other brain regions. These data suggest that pharmacological agents targeting the monoamine system may be useful in restoring normative neurochemical function to mitigate the impact of space radiation. However, the time-dependent changes in monoamine-based neurochemical signatures in distinct brain regions after radiation exposure renders this approach somewhat challenging. In the only study cited that examined a combination of stressors, both behavioral outcomes and neurochemical changes in brain tissue were determined in rats that were exposed to antiorthostatic suspension and γ-radiation (Kokhan et al., 2017). Unfortunately, data from these studies were generally mixed and effects appeared to be small both on behavioral and neurochemical endpoints. For example, the largest effects were observed in monoaminergic metabolism, i.e., 20-24 % increase in 5-HT, 5-HIAA and 5-HIAA/5-HT ratios in hippocampus, PFC, and hypothalamus and decrease in 5-HT (20%) and DOPAC (40%) in hippocampus and/or PFC, in the suspension alone or irradiated group of rats compared to controls. Interestingly, a change in acetylcholine levels was also observed in the hippocampus, in that, levels increased in both the irradiated group and the irradiated + antiorthostatic suspension groups by 59% and by 48%, respectively, compared to the suspension group alone (Kokhan et al., 2017).

There is little doubt that exposure to GCR/SPE produces a wide-range of deleterious *in vitro* neurochemical changes in multiple systems, including DA, GLU, GABA, and ACh, which play a key role in behavior and cognition. However, we do not know the predictive value of *in vitro* information regarding the deleterious effects of radiation alone or in combination with other spaceflight stressors, on neurochemical systems *in vivo*. The collection of *in vivo* functional data

for multiple neurochemical systems at different points in time after irradiation will permit a powerful and efficient means for determining the acute and long-term impact of exposure to spaceflight hazards on brain neurotransmission.

5.2. Microgravity

A simulated spaceflight environment (SSE), including microgravity and isolation, has been associated with alterations in numerous neurotransmitters, including norepinephrine (NE), DA, ACh, GLU and GABA, particularly in the hippocampus (Wu et al., 2017). Microgravity was also associated with the upregulation of CREB and BDNF, proteins involved in cell growth, proliferation, and survival that contribute to learning and memory performance (see section 4). An iTRAQ-based proteomic analysis found 75 proteins that were overexpressed and 72 that were under-expressed following 28 days of hindlimb unloading in rats, mimicking a microgravity environment (Wang et al., 2017). Many of these proteins are associated with synaptic transmission and the regulation of GLU and GABA, including GluR1 and GluR4. Together with neuronal apoptosis, these results suggest that 28 days of microgravity exposure might result in excitotoxicity in the hippocampus that may underlie cognitive deficits, particularly in learning and memory. Moreover, exposure to microgravity has also been associated with increases in ACh (Zhang et al., 2018) and DA (Kulikova et al., 2017), particularly in the hippocampus. Finally, weightlessness simulation has been reported to produce significant decreases in hormone levels, including plasma renin activity and atrial natriuretic factor (Maurice et al., 1990).

Hindlimb-unloading in ground-based animal models has been reported to develop depression- and anxiety-like behavior. For example, there are decreased levels of NR2A/2B

subunits of the N-methyl-D-aspartate receptor and GLU levels, which may be related to changes in neural oscillations (i.e., reduced theta-gamma phase synchronization in the hippocampal perforant path way and dentate gyrus - regions that play a crucial role in synaptic plasticity as well as memory function (Nday et al., 2019). Similarly, hypergravity is associated with increased levels of hippocampal corticosterone (CORT), which are known to be associated with increased fear conditioning, similar to that observed in patients with post-traumatic stress disorder (PTSD) (Porte and Morel, 2012). Further, hypergravity induces neuronal apoptosis in the cortex and hippocampus, with spatial memory impairments on Y-maze (Sun et al., 2009), Morris water maze tasks (Feng et al., 2010), and radial arm maze (Mitani et al., 2004). Interestingly, electroacupuncture is associated with the preservation of CA1 pyramidal neurons may be responsible for rescuing learning & memory performance (Feng et al., 2010) and could serve as a useful intervention for ameliorating the negative effects of hypergravity.

5.3. Confinement and Social Isolation

Acute and chronic social isolation stress results in a variety of endocrinological changes, including activation of the hypothalamic-pituitary-adrenal (HPA) axis, culminating in the release of glucocorticoids and catecholamines, and activation of the sympatho-adrenomedullary system, releasing Oxytocin and vasopressin (Cacioppo et al., 2015). In humans, the HPA can be probed through measures of salivary cortisol, which correlates with assessments of loneliness and social isolation (Cacioppo et al., 2015). Chronic social isolation not only elevates basal levels of glucocorticoids, but also enhances neuroendocrine responses to acute stressors (i.e., stress reactivity, Cacioppo et al., 2015; Kamal et al., 2014). Social isolation increases corticosterone

levels and chronic corticosterone infusion in socially housed phenocopied the long-term potentiation impairments observed in socially isolated mice. Infusion of the glucocorticoid antagonist RU38486 rescued the LTP-impairments following social isolation (Kamal et al., 2014).

In addition, social isolation alters levels of neurotransmitters such as DA, 5-HT, GABA, glutamate, NMDA, and the opioid system (Mumtaz et al., 2018). Moreover, social isolation has been reported to downregulate the expression levels of the phosphorylated forms of neuro-signaling proteins, calmodulin-dependent kinase II (p-CaMKII), cyclic AMP-responsive element binding protein (p-CREB), and early growth response protein-1 (Egr-1) in the hippocampus (Okada et al., 2015). In other work, social isolation has been shown to produce deficits in glutathione that is accompanied by elevated concentrations of N-acetylaspartate, alanine, and glutamine, and the ratio of glutamine-to-GLU, and by a reduction in levels of myo-inositol and choline-containing compounds in the frontal cortex of knockout animals with respect to wild-type littermates (Corcoba et al., 2017). Similarly in another study, Shao et al. (2015) found: a) decreased antioxidant enzymes catalase, glutathione peroxidase, superoxide dismutase, and total antioxidant capacity; b) increased levels of hydrogen peroxide in some brain regions with PFC and hippocampus being the most vulnerable; and c) decreased levels of GLU, glutamine, n-acetyl-L-asparate (NAA), and phosphocreatine in dorsal hippocampus but not cerebral cortex. Social isolation also results in increased interleukin IL-1β and corticosterone that may be dose-dependent, with the potential for adaptation over time (Gądek-Michalska et al., 2017) that may be exacerbated my multiple stressors including confinement and social crowding. Taken

together, these alterations contribute to the activation of symptoms associated with depression and anxiety, affecting neurobiological properties of the HPA axis.

5.4. Sleep Deprivation

Extracellular GLU levels in the cortex have been reported to steadily increase and remain elevated during the first few hours of total sleep deprivation, after which they start to decline, suggesting that loss of sleep perturbs glutamatergic signaling, which is attenuated by longer periods of sleep deprivation (Abel et al., 2013). Additionally, studies have examined how extended wakefulness and sleep also change extracellular adenosine, a degradation product of ATP whose levels increase with brain metabolism. Adenosine promotes sleep and there is evidence for up-regulation of adenosine A1 receptors in cortical brain regions following sleep deprivation (Wolf et al., 2016). Adenosine is a degradation product of ATP and cyclic adenosine monophosphate (cAMP). As a product of cerebral energy consumption, extracellular adenosine increases during wakefulness and declines during sleep, acting as a neurochemical signal for the homeostatic sleep drive, as well as a regulator of energy restoration in the brain (Kreutzmann et al., 2015). High adenosine turnover may contribute to the attenuation of hippocampal activity and subsequent learning and memory impairments. Finally, cortical 5-HT levels are high during wakefulness and reduced during sleep, with sleep deprivation producing an upregulation of 5-HT2A receptors, which may reduce the likelihood of synaptic down-scaling (Wolf et al., 2016).

Several studies in humans have used proton magnetic resonance spectroscopy to assess the effects of sleep deprivation on neurochemical changes in the brain. For example, Plante et. al (2014) found elevated levels of phosphocreatine and increases in electroencephalographic

slow wave activity in gray matter during recovery sleep; no significant changes were found in white matter or in β-nucleoside triphosphate. In other studies, small or no changes were observed in ratios of N-acetyl-aspartate, total creatine, and choline-containing compounds to water in the occipital cortex (Urrila et al., 2006). No significant changes in phospholipid metabolism, high energy phosphate metabolism, and intracellular pH after sleep deprivation in humans (Murashita et al., 1999). Similarly, Dorsey et al. (2003) found no significant chemical changes in the brain after sleep deprivation; though, after recovery, some increases in total and β-nucleoside triphosphate and decreases in phospholipid catabolism, measured by an increase in the concentration of glycerylphosphorylcholine, were observed. Finally, Murck et al. (2009), found that sleep deprivation did not change levels of GLU or related neurochemicals, slightly increased total creatine and choline signal in the dorsolateral prefrontal cortex; no change was observed in the parieto-occipital cortex.

With regard to studies in laboratory rodents, sleep deprivation-induced memory impairment does not appear to be associated with BDNF, magnesium, oxidant, or antioxidant balance in the hippocampus (Nabaee et al., 2018). Interestingly, in another study, analysis of amino acid-based neurotransmitters after paradoxical sleep deprivation revealed significant increases in cortical GLU, glycine and taurine levels while in the hippocampus, GLU, aspartate, glutamine and glycine levels increased significantly (Mohammed et al., 2011). These data suggest that paradoxical sleep deprivation induced neurochemical changes that may impact normal brain function. Similarly, in another study paradoxical sleep deprivation significantly increased GLU and glutamine in the brain cortex (Bettendorff et al., 1996). In this study, GABA levels did not change

during the instrumental sleep deprivation but increased during the rebound period. In other work, highest levels of 5-HT observed during wakefulness, whereas a progressive decrease of 5-HT going from non-rapid eye movement sleep to rapid eye movement sleep was found (Peñalva et al., 2003). During the whole sleep deprivation period, 5-HT levels were elevated substantially when compared to 5-HT levels during basal wakefulness. However, no changes in 5-HT levels and the relationship between hippocampal 5-HT and vigilance state were found during the subsequent recovery period. Sleep deprivation caused a marked rise in free corticosterone levels; however, this effect appears to be independent of this hormone, because adrenalectomy did not affect the rise in hippocampal 5-HT during sleep deprivation.

6. Neurobiological Basis for Spaceflight Stressor-Induced Behavioral and Cognitive Deficits.

There is evidence for radiation-induced structural alterations in multiple neuronal subtypes in rodents, along with major changes in myelination and synaptic density after cosmic radiation exposure. In particular, we explored how such effects can be examined across species using a variety of approaches. Notably, we appraised studies that use a multi-modal neuroimaging approach in humans, nonhuman primates, and rodents to non-invasively characterize changes in: a) white matter microstructure/region-specific grey matter volume (diffusion weighted imaging/morphometry); b) brain region-specific neurochemistry and metabolite levels (proton (^{1}H) and phosphorus (^{31}P) magnetic resonance spectroscopy); and c) functional brain connectivity/activation patterns (functional magnetic resonance imaging; task-based and resting state).

6.1. Radiation

Evidence thus far suggests that radiation exposure has a significant impact on the structural properties of several brain regions that play a key role in neurobehavioral and cognitive processes. In particular, research has primarily focused on:

6.1.1. Hippocampus

Within the hippocampus, a key structure for learning and memory, several neurobiological alterations have been noted following radiation exposure. Dendritic spines undergo changes in proportions of subtypes (Allen et al., 2015; Parihar et al., 2015c), with reductions in dendritic length and complexity in the dentate gyrus (DG), CA3, CA2, and CA1 hippocampal subfields following radiation exposure (Kiffer et al., 2019a). Similar changes have also been reported for subiculum neurons after exposure to 150 MeV protons (Chmielewski et al., 2016). A decrease in dendritic length, branch point, and spine density are apparent in the DG of 1H-irradiated mice that received 0.1 and 1 Gy doses at one-month post-irradiation (Parihar et al., 2015c). Alterations in the expression of GLU and synaptic density-associated proteins in the CA1 and dentate gyrus of the hippocampus have also been observed 3 months post-irradiation (Kiffer et al., 2018). Within the hippocampi recovered from rats exposed to the HZE radiation, there is persistent oxidative stress and altered adenosine metabolism, with altered brain plasticity and failure to invoke key proteins in those pathways (Britten et al., 2017a). Exposure to ^{28}Si radiation reduces mouse hippocampal DG proliferation and neurogenesis in the short term and decreases new neuron survival in the long-term (Sweet et al., 2016; Vlkolinský et al., 2008, 2007; Vlkolinsky et al., 2010; Whoolery et al., 2017). Exposure to 60 cGy 1 GeV/u ^{56}Fe particles

results in a persistent (for at least 180 days post-irradiation) perturbation of glutamatergic neurotransmission in rat hippocampal nerve termini and a reduction in the levels of the glutamatergic NMDA receptors NR1, NR2A and NR2B (Machida et al., 2010). Further, elevated anxiety and depression-like behaviors that coincided with a persistent decrease in the frequency and amplitude of the spontaneous excitatory postsynaptic currents in principal cells of the perirhinal cortex, as well as a reduction in the functional connectivity between the CA1 of the hippocampus and the perirhinal cortex (Parihar et al., 2018) have also been noted. The hippocampus appears to be more sensitive to the effects of radiation compared to associative cortex (Machida et al., 2010). It is also important to note that while high doses of irradiation can cause motor impairments (Landauer et al., 1987; Maier et al., 1989), low doses impact neural function in the absence of physical impairments in the ability to perform the tasks (Burghardt and Hunt, 1985).

6.1.2. Striatum

Radiation exposure also has negative effects on striatal neural function (Joseph et al., 1998). Alterations in striatal function have also been observed following whole-body radiation. Radiation doses ranging from 0.10 to 1 Gy were effective in decreasing responsiveness of muscarinic cholinergic heteroreceptors in the striatum to agonist stimulation, which corresponded to a decrement in motor performance (Joseph et al., 1992). The free-radicals produced during heavy-particle irradiation may induce neuronal membrane structure and functional alterations that may involve changes in lipid content, increases in membrane rigidity, or protein crosslinking (Joseph et al., 1993). However, immunohistochemical studies have also

indicated no differences in staining for tyrosine hydroxylase, the key enzyme in DA synthesis, after 12 months of irradiation (Rice et al., 2009). Differences between studies could be a due to several factors, including dose and type of radiation, when testing occurs, which breed or strain of animal is being tested, among others.

6.1.3. Prefrontal – Perirhinal Cortex

HZE radiation has been shown to modify dendritic morphology within the prefrontal cortex (PFC) and hippocampus. HZE particles appear to modulate spine density, dendritic length and bifurcations. For examples, charged particle exposure elicits persistent and significant alterations in the prevalence of certain synaptic proteins, in addition to substantial reductions in dendritic complexity and spine density in the PFC (Parihar et al., 2016, 2015c). Interestingly, such structural changes may explain the loss of long distance (multi-synapse) and functional connectivity between the hippocampus and perirhinal cortex, where exposure to helium ions (5 and 30 cGy) caused near complete loss in evoked firing in regular- and late-spiking principal cells in the perirhinal cortex measured 1-year after exposure (i.e., following stimulation in the hippocampal CA1; Parihar et al., 2018). These structural changes were temporally coincident with behavioral deficits in novel object recognition, object in place memory, temporal order memory, increased anxiety and depression and impaired extinction memory (Parihar et al., 2018, 2016, 2015a), all of which can be attributed in part, to alterations in the integrity of the PFC.

6.1.4. Dopaminergic reward-based system

Cellular membranes and transport mechanisms that can alter membrane structure and function have been observed following irradiation exposure in rodents, inducing deficits in

muscarinic-receptor mediated signal transduction, with impacts on the striatum that parallel those seen with respect to aging. In addition, radiation exposure involves profound loss of dopaminergic cells in the substantia nigra, which induces motor behavioral deficits similar to those seen in Parkinson's disease. The effects of aging and irradiation on signal transduction and decreases in sensitivity in muscarinic receptors may originate from similar oxidative stress induced alterations in membrane microenvironments. DA metabolism in the caudate nucleus (Hunt et al., 1989; Rabin et al., 1994) and striatum (Rabin et al., 2002, 2000, 1998) was significantly altered after exposure to high-energy iron particles. In vitro studies showed that reduction of potassium-enhanced striatal DA release 12 hours following exposure to 0.5 or 1 Gy of 600 MeV/n ^{56}Fe particles is observed 180 days following radiation, revealing a possible permanent alteration to the DA system resulting from radiation exposure (Rabin et al., 2004). Striatal signaling molecules are also negatively correlated with reference memory errors. Similarly, proton irradiation can disrupt psychomotor vigilance performance, accompanied by decreases in TOH (tyrosine hydroxylase), a rate-limiting enzyme for DA synthesis (Davis et al., 2015). Together, these findings suggest that radiation-induced pre-synaptic facilitation may contribute to some previously reported radiation-induced decrease in striatal DA release, disruption of the central dopaminergic system integrity, and DA-mediated behavior.

6.2. Microgravity

6.2.1. Gross cortical and hippocampal alterations

The hippocampus itself may be particularly impacted by microgravity, as hippocampal CA1 neurons appear to be more sensitive to the effects of microgravity than other rough-surfaced

neurons, showing decreases in area, perimeter, and synaptic cleft and increases in the number of nodes, spines, and spine density (Ranjan et al., 2014). However, investigations of neural changes following microgravity exposure have revealed significant impacts on gross brain structure and function, indicating this is far from selective to the hippocampus. Investigations of astronauts using magnetic resonance imaging (MRI) before and after spaceflight missions have revealed lateral ventricular enlargement and narrowing of cerebral spinal fluid spaces (Lee et al., 2019; Roberts et al., 2017) and decreases in thalamic volume and thinning of the occipital cortex (Riascos et al., 2019). Following 30 days of head-down bed rest, there was gray matter volume reduction in the frontal lobes, temporal poles and medial temporal regions (including the hippocampus), with concomitant increases in the vermis, precuneus, precentral, and postcentral gyri (Koppelmans et al., 2016; Li et al., 2015), regions involved in learning, memory, and coordination. Gray matter volume decrease in microgravity may result from decreased neurons impulse and suppressed synaptogenesis or changes of cerebral vascular flow and increased vasoconstriction or redistribution of cerebral spinal fluid. Interestingly, there is little evidence for gray matter volume differences associated with the length of time in space (Koppelmans et al., 2016), with results following spaceflight remarkably consistent with those following head-down bed rest. Most of the loss in the gray-matter volume seen immediately postflight recovered to preflight levels, while CSF volume continued to increase in the subarachnoid compartment (Van Ombergen et al., 2018). The expansion of CSF spaces in light of postflight decreases in the gray-matter volume and a reduction in the white-matter volume at follow-up suggests a persistent disturbance in CSF circulation even many months after a return to Earth.

6.2.2. White matter integrity

Moving beyond volume, white matter integrity in the brain can be assessed using diffusion-weighted imaging (DWI), a form of MRI scanning. Following spaceflight, DWI has identified reduced diffusion in the fusiform gyrus and occipital cortex (Riascos et al., 2019), frontal, temporal, and parietal regions (Li et al., 2015), possibly reflecting decreases in axonal integrity, myelination, axonal loss or unpacking of white matter fibers. The involvement of the occipital cortex, thalamus, and changes in the optic radiation point to gray and white matter changes related to visual function, which, as noted above, is negatively affected following spaceflight and exposure to microgravity.

6.2.3. Whole-brain connectivity

There is a large body of human imaging research dedicated to measuring alterations in very low-frequency temporal correlations in brain activity, typically measured using functional MRI during rest. These functional connectivity analyses identify set of brain regions that covary in their activity (at very slow timescales), resulting in several sets of brain maps, including the default-mode network (DMN), which is preferentially active during rest when no cognitive task is required. Following 70 days of downward head-tilt (HDT), simulating microgravity, functional connectivity was altered in the thalamus (Liao et al., 2012), default mode network (Zeng et al., 2016), motor and somatosensory networks (Demertzi et al., 2016; Koppelmans et al., 2017), with the greatest increases in connectivity in these regions associated with the least deterioration in postural equilibrium following HDT (Cassady et al., 2016). Similarly, following an HDT intervention, alterations were reported in anterior, posterior and middle cingulate cortices,

regions involved in cognitive flexibility, attentional shifting, and arousal (Liao et al., 2015; Zhou et al., 2014). Note, however, that a clear limitation of these studies is that the HDT simulation of microgravity is compared with normal, unconstrained activity and not with a similar duration of bed rest without the HDT manipulation, which clouds the interpretation of these effects. In addition, microgravity could result in these physiological changes through the physical effects of body fluid changes induced by the absence of gravity or through the psychological stress resulting from varying gravity. Neural activity measured with EEG during parabolic flight identified alterations in the right frontal lobe, consistent with alterations associated with negative emotions, such as uncertainty and fear that may be experienced during weightlessness (Schneider et al., 2008). Certainly, microgravity is associated with body fluid changes that result in reduced blood pressure, cardiac output and cerebrovascular conductance (Klein et al., 2019), resulting in a redistribution of fluids and intercranial pressure (Koppelmans et al., 2017).

6.3. Confinement and Social Isolation

6.3.1. Hippocampus

There is some evidence that social isolation results in hippocampal dysfunction, including a reduction in volume of the CA1 subfield and a reduction in contextual fear conditioning, tied to a reduction in markers of synaptic plasticity in this region (Pereda-Pérez et al., 2013). Further, decreases in DG volume have been observed following prolonged physical and social isolation, coupled with changes in key neurotrophic factors, such as BDNF (Stahn et al., 2019).

6.3.2. Whole-brain connectivity

Perceived social isolation has been associated with altered functional connectivity in humans within brain networks underlying attention, such as the right central operculum and right supramarginal gyrus, that are not mediated by symptoms of depression (Layden et al., 2017). These structures are part of the cingulo-opercular network, which has been implicated in executive processing functions and emotional processing. Whole-brain structural MRI analysis using voxel-based morphometry revealed significant decreases in gray matter volume of the right dorsolateral prefrontal cortex (DLPFC) and left orbitofrontal cortex (OFC) after a 14-month expedition (Stahn et al., 2019). The DLPFC and the OFC are pivotal for executive control such as response inhibition, working memory and cognitive flexibility, but also the generation of and regulation of emotion. Projections between the hippocampus and orbitofrontal cortex (OFC), and to some extent also the DLPFC can foster cross-structural communication and might interact to influence behavior. Preliminary data from the MARS500 project also suggest decreases in white matter integrity of the right temporoparietal junction (TPJ) after 520 days of isolation and confinement (Brem et al., 2020). The right TPJ integrates multisensory information and has been suggested to play a critical role for reorienting of attention, i.e., being able to respond quickly to unexpected events in the surroundings, and social processes. These effects are likely to be attributed to sensory deprivation as well as lack of diverse social interactions associated with the prolonged isolation and confinement.

6.4. Sleep Deprivation

6.4.1. Hippocampus

The hippocampus, critical for the encoding and retrieval of memories, is sensitive to the effects of sleep loss, with reduced connectivity to frontal and parietal regions that can predict the degree of memory impairment following sleep deprivation (Kaufmann et al., 2016; Krause et al., 2017). Disruptions in functional connectivity between the hippocampus and other regions, such as the default mode network and thalamus, may account for deficits in episodic memory performance following sleep deprivation (Zhao et al., 2019b). Both hippocampal-neocortical dependent spatial memory associated with maze navigation and striatum-based circuits show alterations following sleep loss, reflecting a widespread network of regions required for long-term memory consolidation (Chee and Chuah, 2008). Finally, while the presence of effects of sleep deprivation on memory performance are universal, there is significant individual variability. The morphology, or shape of the DG/CA3 subfield of the hippocampus can account for some amount of this variance (Saletin et al., 2016). This same hippocampal structural measure was correlated with the quality of NREM sleep across individuals as well, suggesting a potential mechanism for the effect on hippocampal-based memory.

6.4.2. Striatum

Both the caudate and cerebellum are involved in the regulation of movement and control of motor vigilance. Stronger functional connectivity was observed between these regions during functional neuroimaging of the psychomotor vigilance task following 36 hours of sleep deprivation (Zhang et al., 2019), which was positively correlated with reaction times on the PVT. Likewise, altered functional connectivity between the cerebellum and superior temporal sulcus during a pursuit rotor task (Maquet et al., 2003) or rest (Zhou et al., 2017) has been reported

following sleep deprivation. The enhancement of connectivity between these regions may be to compensate for the decrease in motor alertness following sleep deprivation. Similarly, reduced cerebral activation in the "stopping network" (inferior frontal gyrus, supplemental motor area, subthalamic nucleus, and insular) and in visual-related regions (occipital cortex, fusiform gyrus) has been reported following 24 hours of sleep deprivation during the stop-signal reaction time task (Zhao et al., 2019a). Thus, sleep deprivation has a negative impact on neural circuits involved in inhibitory control, which may provide a common mechanism underlying sleep-loss related deficits in both attention and working memory.

6.4.3. Prefrontal Cortex

Sleep deprivation has been associated with reductions in task-based functional MRI activity during attention tasks in the dorsolateral prefrontal cortex (DLPFC) and intraparietal sulcus, related to deficits in attending to a specific stimulus while ignoring distractors or orienting attention to a location where a stimulus is supposed to appear (Krause et al., 2017). Similarly, a cortical "sustained-attention network", involving prefrontal, motor and parietal cortical regions, and subcortical structures such as the basal ganglia, and "default-mode network", involving a network of medial prefrontal and medial cortical regions, have both shown alterations in fMRI activity following sleep deprivation (Chee et al., 2006; L. Chen et al., 2018; W.-H. Chen et al., 2018; Kaufmann et al., 2016; Strangman et al., 2005; Wang et al., 2015). Alterations in task-based functional MRI activity have been also observed in the anterior cingulate, middle occipital gyrus, inferior frontal gyrus, medial frontal cortex, parietal cortex, and thalamus (Chee et al., 2006; Choo et al., 2005; Kilgore, 2010; Saletin et al., 2019). Anterior cingulate activation following sleep

deprivation is consistent with findings studying divided attention and working memory, implicated in detecting situations where errors are likely. Medial frontal regions are engaged during goal-directed processing and activity that can be modulated by task difficulty. Sleep deprivation has also been associated with higher theta/beta ratios and lower alpha frequencies in frontal areas, suggesting reduced frontal cortical regulation of subcortical drive after sleep deprivation and compromised emotional regulatory processing (Verweij et al., 2014; Zhang et al., 2019).

Interestingly, there have been reports of increased activity (Ma et al., 2015; Saletin et al., 2019) and decreased volume (Liu et al., 2014) in the thalamus following sleep deprivation, which plays an important role in maintaining alertness and vigilant attention. In addition, there is altered thalamocortical connectivity (Shen et al., 2017; Xu et al., 2018) and greater global signal variability (Nilsonne et al., 2017) following sleep deprivation. Indeed, during working memory tasks, functional activity was reduced in medial parietal, anterior frontal, and posterior cingulate regions, with greater activation in dorsolateral prefrontal cortex and bilateral thalamus when task demands increased, suggesting a mechanism for compensatory adaptations during some more complex tasks (Chee and Choo, 2004).

6.4.4. White-matter integrity

DWI measures of white matter integrity in humans have shown sleep-loss related changes largely restricted to frontal and temporal lobes, consistent with cognitive skills affected by sleep loss (Khalsa et al., 2017). There is also evidence that integrity of the superior longitudinal fasciculus and splenium of the corpus callosum accounted for nearly 37.5% of the individual

variability in lapses following 24 hours of sleep deprivation (Zhu et al., 2017). Similarly, sleep restriction (5.5 hours of sleep per night for one month assessed via self-report) has been associated with reduced brain connectivity via DWI between frontal regions, fusiform, supplemental motor area, and cingulate gyrus (Lee et al., 2016). Decreases in white-matter integrity resulting from sleep loss has been shown to be predictive of subjective reports of sleepiness (Elvsåshagen et al., 2015).

6.4.5. Whole-brain connectivity

Alterations in functional connectivity within the DMN have been observed following sleep loss (Dai et al., 2015; De Havas et al., 2012; Kilgore et al., 2010; Wang et al., 2015; Zhu et al., 2019), which is engaged during self-referential activity, episodic memory, anxiety and emotion, and mind-wandering. Further, decreases in default mode network connectivity is related to worsening self-reported emotional state following sleep deprivation (Wang et al., 2015).

6.4.6. Dopaminergic reward-based system

The mesolimbic reward system includes the midbrain ventral tegmental area, striatum, and regions of PFC, and has been shown to be sensitive to sleep deprivation, leading to alterations in motivated behaviors, risk taking, and impulsivity (Krause et al., 2017). This network is regulated by dopaminergic innervation from the ventral tegmental area to the striatum, which is negatively impacted by sleep loss. DA is associated with arousal: higher levels of DA predict lower sleep propensity, wake-promoting stimulants such as amphetamines block DA reuptake and stimulate DA release to increase wakefulness and depleting DA reduces vigilance and induces sleep. Given that DA is also involved in reward-driven behavior, sleep deprivation-induced

decreases in DA contributes to alterations reward processing. Furthermore, it may contribute to negative mood and averse emotional processing following sleep loss, in addition to alterations in noradrenaline that may lead to heightened and over-generalized responsivity within the affective salience network and poorer emotional discrimination (Krause et al., 2017). Similarly, sleep loss contributes to reductions in the functional connectivity between the amygdala and medial PFC that correlate with subjective assessments of mood, suggesting that decreased executive control function contributes to decrease emotional regulation following extended periods of wakefulness (Ben Simon et al., 2017; Lei et al., 2015; Motomura et al., 2017; Shao et al., 2014). Activation of the dopaminergic system occurs together with a blunted cortisol response, suggesting augmented motivational top down control and requiring increased involvement of prefrontal and limbic cortical area, particularly during emotional processing (Klumpers et al., 2015). Interestingly, individuals who score low on measures of narcissism are more resilient to the negative brain effects associated with sleep deprivation, contributing to individual differences due to sleep loss (Liu et al., 2014).

7. Other Outcomes: Impact of Spaceflight Stressors on Metabolomic and Lipidomic biomarkers of Behavioral and Cognitive Deficits

Metabolomic and lipidomic profiling of specific biologic samples, deemed a feasible approach for identifying potential biomarkers of interest to virtually any physiological outcome may be key to early indications of behavioral and cognitive deficits. This approach has successfully been used as a platform for biomarker discovery and biodosimetry following

radiation exposure (Altadill et al., 2017). Additionally, both approaches (lipidomics/ metabolomics) yield fundamentally similar data sets regarding how a given insult elicits changes in the lipidome, either in tissue specific or circulating markers, with the overarching goal of identifying predictive biomarkers of neurobehavioral outcome. In a first study, tissue specific metabolomics were used to identify endoplasmic reticulum stress in the irradiated hippocampus (Hinzman et al., 2018). More relevant to spaceflight, work analyzing plasma-derived extracellular vesicles (EV) that was able to yield predictive markers of cranial irradiation exposure in mice (Hinzman et al., 2019). Two days and two weeks post-exposure (9 Gy head only), plasma and plasma-derived EVs from these mice were collected. Using metabolomic and lipidomic profiling, several markers associated with inflammation that were up-regulated in EVs including triglycerides, platelet activating factor, carnitine, and C-16 sphinganine were detected. Moreover, significant decreases in palmitic amide were also identified, and importantly, none of these biomarkers were identified as significantly altered in plasma, indicating EV-cargo specificity. Additionally, whole-plasma profiling provided further evidence of systemic injury, including moderate dyslipidemia and a significant decrease in systemic β-hydroxybutyric acid, which serves as a neuroprotectant. These studies are the first to demonstrate that metabolomic and lipidomic profiling of plasma-derived EVs may be used to study clinically relevant markers of ionizing radiation toxicities to the brain that typically manifest as late effects. Obviously, it remains to be determined whether similar biomarkers would be identified following space relevant exposures. Here, we explored which potential EV cargo can be cross referenced between samples derived from different species (including humans), and whether specific biomarker

signatures of radiation injury track with adverse behavioral outcomes (either positively or negatively).

7.1. Radiation

Work over the years has sought to identify biomarker signatures specific for radiation injury and functional outcomes. Challenges include identifying causal relationships, dose-responsive and radiation quality biomarkers specific to environmental and/or clinical exposure paradigms. Unfortunately, many of such studies are confounded by disease, age, sex, and health status. While tissue specificity of biomarker approaches can be overcome by direct biopsies, such invasive approaches are not likely to be feasible for real time assessments during space travel. As such, the most attractive approaches ultimately revolve around the analysis of circulating biomarkers that can be obtained from bodily fluids (blood, urine, saliva), although such approaches routinely suffer by the inability to accurately pinpoint the biomarker source (cell, tissue, organ). In this section, we highlight certain potentially promising biomarkers, although to date, there remains no consensus biomarker/s profile that are responsive to dose, that can distinguish radiation quality or can be attributed to radiation- and/or combined spaceflight stressor-induced neurocognitive decline.

While several reviews and workshops related to radiation biomarkers and associated risks have been published over the years (Mu et al., 2018; Straume et al., 2008), few have dealt specifically with space radiation biomarkers, and fewer still have dealt with markers of CNS risk. Non-CNS biomarkers studies related to radiogenic cancers, cataracts and survival have been discussed (Straume et al., 2008) and other space radiation risks have typically focused on

chromosome aberrations in peripheral lymphocytes (Durante, 2005). Metabolomic studies investigating intestinal tissues have suggested that radiation can perturb nucleotide and amino acid metabolism, while heavy ion exposure seemed to preferentially impact dipeptide metabolism along with an upregulation of prostanoid biosynthesis and eicosanoid signaling (Cheema et al., 2014); the latter can affect inflammatory processes in the gut that could interact with CNS function. Other work finding reduced levels of plasma gelsolin after low dose (0.1-0.5 Gy) exposure to silicon ions point to a radioresponsive biomarker in the blood, but with uncertain significance to CNS functionality (Rithidech et al., 2018).

In other work, high-LET irradiation (^{16}O) caused changes in the microbiome of mice exposed over a range of doses (0.1-1.0 Gy) at 10 and 30 days post-irradiation (Casero et al., 2017). While a direct link between space radiation-induced changes in cognition and the microbiome remain difficult to establish, this does represent one potential area for future investigation. Although heavy ion exposure of cardiac tissue has been reported to alter one-carbon metabolism and epigenetics (DNA methylation) with long-term-elevations in cystathione (Miousse et al., 2019), it remains uncertain how such changes might interact with CNS function. Other metabolomic studies analyzing liver following higher doses of low LET radiation (gamma and proton) find changes that implicate multiple biological pathways, but again are difficult to pinpoint to the CNS (Xiao et al., 2017).

The first space radiation (proton) metabolomic study conducted from a biofluid (urine) that can be acquired non-invasively found that whole body doses (0.5, 2.0 Gy) led to significant changes in energy (fatty acid and TCA cycle) and amino acid metabolism (tryptophan, tyrosine

etc.) suggesting a possible impact on neurocognitive function – although this was not tested directly (Laiakis et al., 2015). Interestingly, whole-body exposures to low doses (0.1, 10 cGy) of oxygen ions caused deficits in social odor recognition memory in mice, that were associated with increased levels of the cytokine CXCL1, but only at the lower 1 cGy dose (Jones et al., 2019). The role of this cytokine in a range of important biological processes including angiogenesis, wound healing and inflammation suggest that circulating inflammatory cytokines might prove as useful radiation biomarkers but assigning and scaling these changes to functionally relevant cognitive decline remains a challenge.

Larger scale proteomic profiling of mice exposed to higher doses of (2-8 Gy) of gamma rays revealed several radioresponsive protein changes, many involved with metabolism, proteolysis and post-translational sugar modifications, but the early times of analyses (≤ 72h) make attributing such changes to CNS function difficult (Huang et al., 2019). Serum microRNAs (miRNA) represent another class of biomarkers that can be isolated from the blood and from circulating EV. These small strands of RNA can interact with multiple target mRNAs to modulate message half-life, intracellular trafficking and translation to impact multiple biological pathways (Leavitt et al., 2019). Whole body exposure to carbon ion, iron ion or x-irradiation (0.1-2 Gy) was used to identify miRNA species found to be sensitive to radiation dose and quality, but the early post-irradiation times of analysis (≤ 72h) confound efforts to establish correlations with cognitive dysfunction (Wei et al., 2017).

Extracellular vesicles are shed from cells in nearly all known tissues, with roles in many disease pathologies and are becoming important target for identifying circulating biomarkers. In

a recent study, plasma and plasma-derived EVs were isolated from mice subjected to head only x-irradiation using a dose (9 Gy) known to elicit persistent cognitive dysfunction (Hinzman et al., 2019). Metabolomic and lipidomic profiling performed two days and two weeks post-exposure identified significant changes associated with inflammation in EVs. In particular, both radiation-induced decreases in β-hydroxybutyrate (important in neuroprotection and anti-inflammatory processes; see Fu et al., 2015; Lee et al., 2018) and increases in triglycerides were able to discriminate irradiated from non-irradiated cohorts (Hinzman et al., 2019). Whole-plasma profiling in this study provided further evidence of systemic injury and represent the first studies able to demonstrate that profiling of plasma-derived EVs may be used to study clinically relevant markers of ionizing radiation toxicities to the brain. Many of the changes found in these circulating biomarkers were previously identified in a similar approach conducted on hippocampal tissue, where a similar dosing paradigm identified changes in hexosamine metabolism relevant to endoplasmic reticulum stress (Hinzman et al., 2018). Extending such an approach may provide a strategy for cross correlating biomarkers derived from different spaceflight stressors to functional CNS outcomes.

Finally, we should note that mechanisms mediating radiation-induced stress may be reflected in neuroimaging measures of the hippocampus, entorhinal cortex, and thalamus in rodents, suggesting that cutting-edge neuroimaging techniques (e.g., MRI's functional, diffusion or spectroscopy measures) may be an important non-invasive tool for measuring the effects of radiation exposure (Huang et al., 2009) on structural and functional changes in the hippocampus.

To this end, both diffusion and T2 relaxation times revealed radiation-induced changes in the hippocampus, entorhinal cortex, and thalamus over 18 months (Huang et al., 2010).

7.2. Microgravity

In a study examining astronaut urinary proteome, three proteins, glucosidase alpha acid (GAA), heparan sulfate proteoglycan (HSPG2), and alanyl aminopeptidase (ANPEP), did not return to baseline levels post-spaceflight, possibly correlating to changes in cytoskeletal reorganization, angiogenesis, extracellular matrix reorganization, and some features of hormone metabolism (Brzhozovskiy et al., 2017). Additional proteomic changes that have been detected include increased production of cytokines (and cortisol), changes in regulators of aerobic metabolism, and decrease in muscle and bone protein metabolism (Brzhozovskiy et al., 2017). Twin studies have identified metabolic changes, including altered amino acid metabolism, increased pro-inflammatory lipids, increased lactic acid production, and decreased mitochondrial respiration (Iosim et al., 2019).

Impairment in the physiological functions of mitochondria, such as interruption of the Kreb's cycle and impairments of the mitochondrion respiratory chain components, as well as impairment in osteoblast functionality, result from stress caused by exposure to microgravity (Michaletti et al., 2017). Overall, increased glycolysis and alterations in respiratory chain reactions, as well as changes in some metabolic pathways, are probably responsible for the subsequent microgravity dependent effects, such as pro-apoptotic state and cell de-differentiation. These effects may be more profound in the hippocampus, where models of microgravity resulted in the down-regulation of mitochondrial Complex I, III, and IV, isocitrate

dehydrogenase and malate dehydrogenase, and upregulation of DJ-1 and peroxiredoxin 6, which defend against oxidative damage (Wang et al., 2016).

7.3. Confinement and Social Isolation

A rodent model of chronic social isolation resulted in down-regulation of proteins involved in mitochondrial transport and energy processes, primarily tricarboxylic acid (TCA) cycle and oxidative phosphorylation, particularly in the hippocampus (Perić et al., 2018). These alterations can cause impaired function and structural integrity of mitochondria, consistent with patterns observed in models of depression and mood disorders. Strikingly, treatment with fluoxetine, used to treat depression, resulted in up-regulation of mitochondrial proteins involved in transporting processes, thereby reflecting a rescue of some of the stress induced by social isolation (Perić et al., 2018). These findings emphasize the importance of anti-depressants for potentially offsetting the effects of social isolation, which is often used a model for creating depression-like symptoms in rodents.

7.4. Sleep Deprivation.

Recent studies (Yoon et al., 2019) have examined systemic and local alterations of the metabolome and lipidome in the serum, hypothalamus, and hippocampus CA1 of rats subjected to chronic and acute sleep deprivation. They found no evidence for significant alteration of the metabolome and lipidome in the hippocampus CA1 region, but a considerable number of metabolites and lipids were found to be altered in the hypothalamus-enriched region. The discovery of metabolic and lipidomic dysfunction following sleep deprivation may reflect

dyslipidemia, energy malfunction, membrane structure, and oxidative stress. Functional enrichment and network analyses of the differential proteins revealed a close relationship between chronic sleep deprivation and several biological processes including energy metabolism, cardiovascular function and nervous function, with four proteins including pyruvate kinase M1 (PKM), clusterin (CLU), kininogen1 (KNG1) and profilin-1 (PFN1) were identified as potential biomarkers for chronic sleep deprivation (Ma et al., 2018). KNG1 has been associated with neuronal damage, blood-brain barrier leakage, and inflammation, which may make it particularly relevant for neural function.

8. Summary of Strengths and Limitations of Behavioral and Cognitive, Molecular, Neurochemical, Neurobiological, and Metabolomic/Lipidomic Studies.

Table 1 provides a summary of the strengths and limitations of each of the endpoints considered in this review.

8.1. Behavior and Cognition

A review of complex behavior and cognitive performance revealed deficits concentrated in the areas of learning and memory, cognitive flexibility and control, attention and vigilance, all of which were exacerbated by the role of depression and stress endured across these spaceflight stressors. A host of radiation studies have demonstrated a clear and reliable disruption to learning and memory and, particularly, the “declarative memory” reliant on medial temporal and prefrontal lobe structures, across a range of radiation types and doses. Similarly, cognitive flexibility is impaired following radiation exposure, measured by set-shifting tasks and implicating

damage to both the hippocampus and prefrontal cortex. Exposure to microgravity shows impacts on learning and memory, coinciding with dysfunction of the cholinergic system, which is critical for hippocampal function. Likewise, attention and vigilance are negatively affected following exposure to microgravity, influenced by deficits in perception and hand-eye coordination. Investigations of confinement and social isolation also implicate learning and memory systems, and are also exacerbated by stress and depression, with increased cortisol levels. Finally, sleep deprivation results in broad-ranging cognitive deficits: learning and memory, cognitive control and flexibility, attention and vigilance, and results in impacts on stress and depression.

8.2. Molecular

For tangible CNS risks relevant to the impact of individual and combined spaceflight stressors, few combined studies were identified that shed light on potential interactions between radiation and other spaceflight stressors, confounded by the generally invasive nature of the methodologies used to collect data. The majority of studies analyzing the impact of microgravity used rodent models subjected to hindlimb unloading, with sparse data sets from other cellular models and/or species. Changes in cellular proliferation, certain neurotrophins, neurotransmitter/receptor levels and perturbed metabolism pointed to the capability of simulated microgravity to alter cellular physiology, but robust data sets linking microgravity models of spaceflight stress to specific and/or altered neurocognitive outcomes was lacking. Several studies reviewed the impact of sleep deprivation on rodents and humans, and routinely found this stress to elicit adverse effects on cognitive outcomes. Impairment was likely caused by perturbations in a variety of factors controlling synaptic transmission, including changes in

neurotransmitter levels and circadian rhythms, but few studies save one following chronic neutron exposure linked sleep deprivation alongside radiation exposure. Social isolation was also identified to cause changes in rodent behaviors resembling changes in mood (anxiety, depression) linked to changes in neurotrophins and neurotransmitters, but the nuances of the testing made firm conclusions relevant to human behavior difficult to reach. The majority of studies with direct relevance to CNS functionality, were those in which rodent models were subjected to space relevant irradiation paradigms. While the varied nature of the experimental paradigms made certain generalizations difficult, there was little doubt that this spaceflight stressor caused the most marked changes in behavior that could be linked to a variety of radiation-induced pathologies. The majority of the cellular (inhibition of neurogenesis, increased inflammation and oxidative stress), structural (plasticity of various neuronal and glial subtypes) and electrophysiological (paired cell and network level recordings) results revealed several significant radiation effects, but as with the foregoing spaceflight stressors, these rodent studies were not conducted in combination with other stressors.

8.3. Neurochemical

A wealth of potentially valuable neurochemical changes, involving the dopaminergic, cholinergic, glutamatergic, neuroendocrine (HPA axis) systems along with a number of metabolic adaptations involving monoamines and glycogen were found. Here stress-dependent changes were most evident in specific brain regions, many of which could be expected based on the neurocognitive outcomes recorded. Unfortunately, common trends were more difficult to reconcile based in part on the somewhat disparate data sets obtained between species (rodent

and human). While changes in neurochemical signatures constitute perhaps the most direct biomarker link to behavioral outcomes, it was also difficult to establish the relative importance of many changes due to their dynamic, regional, and/or transient nature. Microgravity studies in mice found elevated mood disorders linked to changes in neural oscillations and excitatory signaling, effects that were not measured in human studies. Studies in rodents found that social isolation caused stress changes involving glucocorticoids and hippocampal LTP, while other studies found changes in glutathione metabolism and antioxidant enzymes, along with changes in monoamine levels that impacted signaling and conditioned fear memories, anxiety, and impaired working memory. Interestingly, crosstalk between the CNS and the peripheral immune system was linked though changes in the HPA axis (glucocorticoids) providing a clear mechanistic link at the neurochemical level of potential relevance to spaceflight risk. Sleep deprivation was also found to cause major (albeit expected) changes in brain chemistry, found in several studies in both rodents and humans. For the majority of rat studies, paradoxical sleep deprivation had a modest impact on mood, and was found to alter hippocampal neurotransmitter and antioxidant levels as the activation of excitatory signaling triggered compensatory metabolic responses. In general, rat/mouse data found a higher turnover of glycogen metabolism caused by stress inducing neurotransmitter release that resulted in reduced levels of glycogen in multiple brain regions.

For humans, sleep deprivation was interrogated by MR spectroscopy, psychosocial stress tests (cortisol stress reactivity), and biomarkers of HPA axis perturbation. In select studies, increased high-energy phosphates were identified in the grey matter, changes in glutamatergic

signaling (DLPC), and decreased levels of metabolites (N-acetyl aspartate, Choline). Certain changes were not found until recovery from sleep deprivation, where increased β-nucleoside triphosphates and decreased phospholipid catabolism were found but were not region-specific. Other studies found little to no change high-energy phosphate/lipid metabolism. Neurochemical biomarkers measured after radiation exposure were restricted to rat models at various protracted post-exposure times. Changes in neurotransmitter and respective metabolite levels were found, suggestive of altered (lower) glutamatergic signaling and monoamine turnover, especially within the 5-HT system. Brain region specific changes were not consistent over extended times. A combined study of radiation and simulated microgravity had mixed results with mild changes in working memory, reduced 5-HT and increased ACh in the hippocampus.

8.4. Neurobiological

For microgravity, several human studies were found incorporating largely bed-rest/head-down tilt models with some parabolic flight experiments aimed at determining if/how cephalic fluid shifts might cause CNS alterations. The majority of these pre- and post-follow up studies relied on resting state MRI and EEG to assess functional connectivity and overt changes in volume of discrete regions of the brain. Collectively, work showed changes in the thalamus, a variety of cortical regions (somatosensory, cingulate, motor), and the frontal and temporal lobes that were principally linked to disruptive fluid shifts, believed responsible for certain changes in grey matter volume, functional connectivity and altered blood pressure. Few of these studies were able to incorporate other spaceflight stressors. Similar findings were noted following MRI assessments of humans engaged in select forms of spaceflight, where volume reductions in select brain

regions were noted, but without extensive follow-up neurocognitive testing. A major limitation of these studies, however, is their inability to distinguish effects of fluid shifts from effects of confinement and long-term bedrest. For sleep deprivation, there were a large number of human studies that found significant and adverse effects on neurocognitive outcomes, changes that were largely investigated through resting state/functional MRI and cognitive assessments. Major changes in a variety of learning and memory, risk/reward behaviors and sensory impairments were noted that were temporally coincident with reductions in grey and white matter and functional connectivity in multiple brain regions. As with microgravity noted above, few of these studies were conducted alongside other spaceflight stressors. Findings of structural changes in rodents subjected to social isolation were similar to those noted above. For radiation exposure, structural and functional biomarker studies were again largely limited to rodent models, and the outcomes exhibited considerable overlap with brain structure and functional changes. Radiation exposure was found to elicit a range of deficits in learning and memory and mood-related disorders that could be linked to increased oxidative stress, inflammation and reduced neurogenesis related in part to the structural deterioration of newly born and mature neurons. These deficits were identified throughout a number of brain regions, and were found to be remarkably persistent (i.e., 1 year post exposure). For those rodent studies implementing MRI, radiation-induced changes in glia/ morphology were noted.

8.5. Metabolomic and Lipidomic

These efforts focused largely on radiation studies in rodent models. Although other spaceflight stressors were used, few were conducted in the context of neurobehavioral outcomes. Simulated microgravity reduced metabolic turnover, and increased monoamine turnover in one instance triggered a compensatory upregulation of hippocampal antioxidants. Interestingly, when combined with radiation exposure offsetting effects were observed. Sleep deprivation resulted in certain changes in energy and cardiovascular function, with more significant changes in the lipidome and metabolome in the hypothalamus-versus hippocampal-enriched regions. For radiation studies, whole body exposures to space relevant doses caused a variety of metabolic changes in rodents, but direct significance to CNS functionality was difficult to establish since many of the outcomes (behavioral) were not part of the experimental design. Metabolomic analyses of urine following charged particle exposures identified changes in energy and amino acid metabolism, while reduced levels of a circulating chemokines were found to correlate with deficits in social odor recognition. Larger scale responses to higher dose, low LET radiation triggered global protein changes involved with metabolic, proteolytic, and post-translational modifications, as well as certain miRNA signatures, but the early times of analysis (≤ 72h) confounded efforts to link these changes to behavioral outcomes, especially in the absence of direct cognitive testing. Perhaps the most promising approach involved a series of metabolomic studies analyzing the irradiated brain and circulating EV for biomarkers under conditions known to elicit neurocognitive decline. Markers of inflammation, elevated triglycerides and elevated endoplasmic reticulum stress point again to the importance of

inflammation in the localized and systemic response to irradiation and suggest the lipid compartment of the CNS as a possible target for further investigation.

9. Summary, Conclusions, and Future Considerations

It is noteworthy, that most studies that contribute to the combined effects of spaceflight stressors result from human spaceflight itself. A review of these studies found no consistent decrements in cognitive performance in the areas of emotion and social processing, attention, memory, learning, and executive functioning (Strangman et al., 2014). However, most of these spaceflights have been of short duration with limited radiation exposure. None of these stressors have been titrated during spaceflight to determine the relative impact of any one of them or how it may interact with others. These data are of limited value when assessing the prolonged exposure to these spaceflight stressors during the unprecedented, planned mission to Mars and other forms of deep space explorations. Therefore, these conclusions are limited by 1) extremely small Ns, 2) experimental designs that suffer from confounds, repeat testing effects, and lack of controls, 3) tasks that are not comparable across studies, 4) individual variability in the effects of these environments, and 5) limited use of social stimuli on cognitive processing (Strangman et al., 2014). Careful modulation and combination of these stressors in animal models and experimental human paradigms are needed to examine the interactions among the spaceflight stressors and their impacts on mission performance. Below we highlight several crucial issues that ought to be considered when designing future CNS-related spaceflight research.

9.1. Cognitive Variables

We identified seven cognitive domains of interest at the outset, listed in Table 2, along with example tasks designed to probe them and associated brain regions supporting them. Based on our review of the literature, we identified which cognitive domains were negatively impacted by each spaceflight stressor, supported by existing evidence (marked by X). For those with weak evidence of a negative impact (marked by ---), there is either limited data or small effects of the spaceflight stressor on that cognitive domain. From this summary, radiation exposure and sleep deprivation both have grave negative consequences for many of the cognitive domains considered here. Learning and memory are impacted by all of the spaceflight stressors, followed by cognitive flexibility and cognitive control, attention/vigilance, and depression/stress.

A breakdown of the operational tasks expected to be required for a mission to Mars identified 1,125 tasks to be performed across the 12 phases of the mission (Stuster et al., 2018). These tasks were then categorized into cognitive abilities based on ratings by subject matter experts (Stuster et al., 2019) and then categorized by crew member role (e.g., leader, pilot, mechanic, etc). This exercise emphasized the importance of cognitive control and flexibility for tasks required for seven of the eight crew member roles: Inductive Reasoning, Deductive Reasoning, Problem Solving, and Originality.

Studies of the effects of the spaceflight stressors on behavior and cognitive function should be careful to employ tasks that are demanding enough (i.e., increasing cognitive load) to be sensitive and care should be taken to minimize practice effects during repeated testing (Hockey and Sauer, 1996; Vaernes et al., 1993).

9.2. Role of Mood Disorders: Anxiety and Depression

While each of these spaceflight stressors have a negative impact on CNS structure and function, these effects can be exacerbated by anxiety and depression. Although NASA strives to select crew members who display emotional stability, mood disorders remain a significant issue, particularly during longer space missions. Data from the International Space Station, where missions are of a longer duration, have revealed that symptoms of mood disorders are much more prevalent than previously reported (Slack et al., 2016). For example, the Integrated Medical Model developed by NASA determined that the incidence rate for depression is .0036 per person-year for females and .0029 per person-year for males, while the incidence rate for anxiety is .0071 per person-year for females and .0019 per-person year for males. Importantly, based on extrapolated rates, there is an 85.2% chance for females and 22.8% chance for males to meet the criteria for anxiety, and a 43.2% chance for females and 34.8% chance for males to meet the criteria for depression over the duration of a mission to Mars. Sleep disturbances are a common comorbidity with depression, resulting in a greater risk of one with the presence of the other.

There are two approaches to countermeasures regarding mood disorders: 1) prevent the occurrence of the risk or mitigate the potential severity of the risk, and 2) monitor and treat the risk as it occurs. Countermeasures to reduce the risk include: 1) reducing environmental stressors by modifying the environment, 2) increasing the capacity of the crew to cope with and respond to stressors, and 3) provide crew with mechanisms and strategies for coping with and recovering from environmental stressors (Kearney, 2013). These countermeasures could be implemented in a variety of ways, including a computer-based system designed to assist astronauts in detecting,

preventing, and treating depression, stress, management, and even interpersonal conflict resolution. The effectiveness of such a system, particularly for long-duration space missions, remains to be determined, but the management of depression and anxiety-like symptoms is critical for both neural and behavioral health.

9.3. Interactions among spaceflight stressors

The interactions among the spaceflight stressors are likely to be varied and complex. Some of the underlying cellular, molecular, and neurochemical signatures may be compounded due to multiple spaceflight stressors, while other stressors may trigger each other (e.g. CNS damage resulting from radiation exposure may trigger sleep risk) or secondary outcomes (e.g. anxiety and/or depressive etiology) that can exacerbate behavioral and neural effects, operations performance, and team dynamics. Figure 3 (Slack et al., 2016) provides one of many examples of how these stressors might interact. While the full extent of mapping these interactions would require an enormous amount of time and effort, a cross-species approach targeting specific spaceflight stressors, like those covered here, within a confined dose limit that approximates those encountered during a long-exposure space mission, could fill important gaps in knowledge regarding the additive or interactive nature of these different stressors.

We have detailed the CNS systems and resulting cognitive domains that are impacted by these four spaceflight stressors, which are compounded by an increased risk of neurobiological reactions associated with depression and anxiety. It is likely that the negative consequences of these stressors could interact or even simply compound during prolonged spaceflight missions,

which requires an integrated approach to understand their scope and extent. Among other issues, the following questions should be addressed:

1. How can we determine if an astronaut is capable of performing a given task when it is required?
2. How do we determine what course of action is required to rescue these deficits through the application of countermeasures?

NASA currently plans to test up to 30 astronauts on International Space Station (ISS) missions lasting 2, 6, or 12 months, and a number of volunteers in Earth-based spaceflight analogs for 4, 8, or 12 months. These events provide several opportunities to compare these spaceflight stressors across both spaceflight and spaceflight analogs to explore the interactions and dose-response changes to anatomy, behavior, cognition, mood, and operational performance. For example, batteries of behavioral testing could be employed before, during, and after flight to assess performance following missions of varying durations and spaceflight stressors (e.g., no radiation exposure during the spaceflight analogs).

9.4 Integrated plan for improved risk estimation for CNS complication resulting from spaceflight stressors:

It should be emphasized, that the results of this integrated approaches review *did not* reveal any brain signature (or combination thereof) that was uniformly responsive across different regions of the brain to a single or given combination of spaceflight stressors. Based on the foregoing, we assimilate the current state of knowledge for providing a framework that will be useful for additional experimentation required to minimize the risks to CNS functionality

caused by long-term exposure to the spaceflight environment. The following considerations provide the backdrop for future investigations that prioritizes those areas deemed essential to address current gaps in knowledge and to make meaningful progress in addressing CNS relevant risks resulting from spaceflight stressors alone and in combination (Figure 4).

Considerations relevant to Stages 1&2 involving assessment of various behavioral and cognitive domains in pre-trained laboratory animals (Figure 4):

1) Astronauts undergo an extensive series of pre-mission training and activities designed to optimize their physical, mental, and emotional preparedness for upcoming spaceflight.
2) Thus, rodents destined for any spaceflight stressor and cross-species relevant cognitive testing, should undergo some level of pre-training, where “better” performers can be selected from the overall cohort based on pre-established criteria and outcomes.
3) Cross-species tasks have been prioritized and were selected to be relevant to human and rodent based testing paradigms. No “perfect” test exists that purely isolates a specific cognitive function and no “perfect” test exists that translates directly between animal models and humans (much less from animal models to performance in spaceflight). None of these will be developed in the near term, and extended discussions over the merits of one test over another becomes arduous and a pointless exercise. We can, however, leverage decades worth of behavioral and cognitive neuroscience research to select tasks that are robust, and cross-species relevant that implement increasing task rigor and/or cognitive load. It should be emphasized that combinations of the selected tasks offer

many advantageous over performing a single task, which runs the risk of missing more subtle decrements that could be relevant to mission critical task performance.

Considerations relevant to Stage 3 highlighting which spaceflight stressor ought to be studied (Figure 4):

1) While there was a wealth of information regarding the impact of individual spaceflight stressors between species, virtually no combinatorial data was found that could provide a meaningful molecular, neurochemical, neurobiological, or circulatory signatures amenable to predictive and/or real-time assessments portending adverse behavioral and cognitive outcomes, a fact that confounded efforts to identify common biomarker/s that were consistently altered by individual spaceflight stressors across.
2) After careful consideration, the CNS risks derived from radiation and sleep deprivation were deemed most relevant, due to the validity of the models and what they represented in terms of CNS-relevant risk. Terrestrial simulations of microgravity were not found to be robust. Nuances in rodent-based social isolation testing (i.e. odor recognition) were simply not relevant to a human situation, which arguable could be dealt with beforehand by judicious crew selections criteria.
3) Thus, for Stage 3, the individual (4 listed) and combined (4 listed) spaceflight stressors are ranked by priority from top to bottom. Lastly, these considerations as shown in Figure 4 are NOT scaled.

Considerations relevant to Stage 4a studies conducting post-treatment behavioral and cognitive testing (Figure 4):

The rationale for studies in this stage is based on the necessary follow-up for the behavioral testing performed in stage 1. The following criteria are critical and should be carefully evaluated at this point.

1) After exposure to the selected spaceflight stressor regimen, follow up testing on the same task used to pre-screen "good" performers from the bulk cohort is a necessary activity. Additional tests can also be conducted to garner further behavioral/cognitive data relevant to specific objectives of a given study.
2) Post-exposure time of task administration is also critical and must focus on spaceflight relevant timeframes. For example, analyzing behavioral/cognitive data directly after exposure to a spaceflight stressor is not informative except perhaps at the mechanistic level. Follow up behavioral and cognitive studies adjusted to rodent lifespans should be scaled to "acute" mission relevant spaceflight risks and "chronic" post-spaceflight, terrestrial CNS complications.

Considerations relevant to stages 4b-d assessing various non-invasive and invasive changes (Figure 4):

These stages represent the culmination of multiple research priorities with the greatest flexibility, largely driven by investigator expertise, and represents a relatively self-explanatory series of non-invasive (Stage 4b), invasive (Stage 4c), and terminal endpoints (Stage 4d). The

listed combinations of follow up testing are most easily conducted longitudinally but can be conducted in parallel or after termination of the activities detailed in Stage 4a. Depending on study focus, the majority of these endpoints were selected to be relevant in regard to the following criteria:

1) Can be done en-route or real-time (ie. non-invasive, longitudinal assessments) in rodent, NHP, and/or humans (Stage 4b).
2) Assessment designed to provide deeper mechanistic insight – non-amenable to human testing (Stage 4c).
3) Mechanistic based studies with a specific focus on evaluating pathways relevant to countermeasure identification, evaluation and administration (Stage 4d).

Completion of the subset of activities listed in stages 1-4 in part or in whole will provide a deeper data set that can inform on the CNS risks arising from the deep space travel. At this juncture, we expect that NASA will be prepared to more safely launce human gateway missions, select crews and countermeasures where appropriate validate in the countdown to the pending Mars mission.

10. References

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Box 1: Measuring Behavioral and Cognitive Performance in Spaceflight

Learning. Learning encompasses the acquisition of new information, rules, or procedures, sometimes reflected as the rate of learning over a session or number of trials required to reach a criterion. Stimulus discrimination paradigms often employ reward to encourage responding to a target stimulus but to withhold responding to distractor stimuli, making these paradigms relatively easy to employ across species. Examples of stimulus discrimination paradigms include conditional visual discrimination tasks, classical conditioning tasks, and the learning phase of maze tasks, such as the T-maze where one arm is rewarded and the other is not. These tasks often rely on the integrity of either the hippocampal or striatal memory systems, depending on the task structure (often referred to as "declarative" vs. "non-declarative" memory systems and "explicit" vs. "implicit" tasks). Note, while some task structures will encourage reliance on one or the other system, other task structures will lead to a situation in which either memory system can drive behavior.

Memory (Long-term). Memory tests reflect the retrieval of learned information following some delay (delays as short as 30 seconds can even be considered "long-term"). Like learning, performance on memory tasks can be driven by multiple different systems in the brain. One variant of a task might be highly reliant on the hippocampal memory system (hippocampus plus adjacent entorhinal, perirhinal and parahippocampal/postrhinal cortices) and less reliant on the striatal system. Another might begin by being reliant on this hippocampal system, but later independent of it and reliant on the striatal system. A third might never rely on the hippocampal system and always rely on the striatum. Thus, learning and memory are both heterogeneous terms as there are multiple complex memory systems in the brain. While one popular distinction separates this hippocampal, or "declarative" system from other, "non-declarative" systems, the hippocampal system is itself a complex one with multiple structures providing learning and memory functions. In addition, there is no one non-declarative system, as the term represents learning and memory functions that exist within a complex striatal system, cerebellar systems, and cortical systems.

In rodents, memory tests are often assessed through maze performance on a previously rewarded location (T or Y-maze) or submerged platform in a pool of water (Morris Water Maze). These types of maze tasks are more difficult to employ in humans and nonhuman primates, although virtual, computerized versions have been developed to attempt translation of findings between species. Tasks that employ memory for objects in locations have more translational ability and can assess both object-based memory and the association between the object and location. Associative memory (e.g., linking an object to a location or a context) typically relies on the hippocampus, while object-based memory or simple recognition can be supported by medial temporal regions outside of the hippocampus, such as the perirhinal cortex. Thus, even within the hippocampal system, there are clear divisions of labor and different memory tasks will differentially rely on these structures.

Conti…….

Cognitive Flexibility. Cognitive flexibility refers to the ability to switch between thinking about two different connects or to think about multiple concepts simultaneously. It can be reflected by reaction time costs or accuracy in responding when switching between different stimulus features. Stimulus-reversal tasks involve learning a rule that is rewarded and then adapting to a change in that rule. In humans, the Wisconsin Card Sorting Task is the best example of a stimulus-reversal task, where the rules of how to sort the cards (e.g. color) will switch to another feature (e.g. suit) without explicit direction, but instead based on feedback regarding the accuracy of the cards being sorted. In laboratory animals, a similar strategy is employed, although often in maze tasks where the rewarded arm of the maze undergoes reversal and animals must suppress the previous response strategy to employ the new one. Successful stimulus-reversal depends on the prefrontal cortex, particularly ventral regions, and the anterior cingulate. In many cases, cognitive flexibility tasks such as stimulus-reversal are also dependent upon the hippocampal memory system.

Cognitive Control. Cognitive control refers to the cognitive processes required for selecting and successfully monitoring behaviors to attain chosen goals, often requiring flexible and adaptive responses as conditions change. Cognitive control involves cognitive flexibility, but also involves repeated task-switching and monitoring based on evolving task conditions, more so than a simple rule-switch. To assess cognitive control, the flanker task presents a target that is flanked by two non-target stimuli that may be congruent or incongruent for the correct response. Inhibiting the incongruent stimuli requires cognitive control and can be measured by both reaction times and accuracy. Here, there is a coordination of brain regions required for successful performance: the prefrontal cortex (PFC) for sustaining attention across task-relevant pathways, the anterior cingulate cortex (ACC) for resolving conflict and detecting errors, and the ventral tegmental area (VTA) involved in responding to errors in predicting reward.

Working Memory. Working memory is a particular kind of memory that is worth isolating from the kind discussed above as it is critically reliant on different brain systems and computations. Working memory temporarily holds information in an “active” state, making it available for manipulation and for use in active cognitive tasks such as learning, reasoning, and comprehension. In its simplest form, working memory can be assessed using the delayed-match-to-sample (DMS) task, in which a stimulus (e.g., a visual image) is presented followed by a brief delay (2-3 seconds). After this, the sample stimulus is displayed along with several similar patterns. The subject can typically hold sample stimulus in working memory during this delay, so long as no other task or images intervene to replace it in the limited-capacity working memory. This DMS task has been successfully implemented in rodents, nonhuman primates, and humans, making it an ideal translational paradigm. Performance on this task relies on inferior temporal (IT) cortex in coordination with PFC.

Conti…….

Attention/Vigilance. Attention reflects the cognitive process of concentrating on a discrete aspect of information while ignoring other, distracting information, while vigilance refers to the sustained concentration of this attention. The psychomotor vigilance task (PVT) requires a button press whenever a light appears, every few seconds for 5-10 minutes and is designed to measure sustained attention, counting the number of lapses of attention. Sustained attention in this task engages several neural networks, including the default mode network and attention network, which includes the PFC, posterior cingulate, and inferior parietal cortex.

Depression/Anhedonia/Anxiety. Depression is a mood disorder that causes a persistent feeling of sadness, loss of interest in activities, and anhedonia (the inability to experience pleasure). While Major Depressive Disorder (MDD) is a diagnosable disorder, it is possible to experience depressive symptoms due to life experiences and living conditions. While symptoms of depression can be assessed with by self-report surveys, they can also be characterized by behavioral deficits in certain cognitive domains. For example, the probabilistic reward task involves positive or negative feedback during a learning task. Individuals with depressive symptoms benefit less from positive feedback, indicating dysfunction of the dopaminergic reward-based learning system in the midbrain.

Table 1: Strengths and limitations of behavioral/cognitive, molecular, neurochemical, neurobiological, and metabolomic/lipidomic studies for assessing impact of spaceflight stressors for humans

Strengths
Behavior & Cognitive Studies • Behavioral testing paradigms exist that can be administered easily and repeatedly in space to assess the impact of spaceflight environment on a variety of neural systems that are associated with cognitive domains. • Parallel behavioral testing paradigms exist that promote the translational value of rodent and nonhuman primate studies to human cognition. These tasks are affected by several stressors and there are clear parallels with in-flight cognitive requirements. • Negative cognitive effects of stress may have the potential to be mediated by the administration of oxytocin or vasopressin. **Molecular Studies** • Rodent work with radiation has identified major neurocognitive and other adverse functional outcomes, and likely defines the single biggest risk for human health during deep space travel. • Spaceflight stressors produced adverse behavioral outcomes, largely linked to synaptic proteins and neurotransmitter changes. • Inflammation and oxidative stress are key cellular mediators of spaceflight stress. **Neurochemical Studies** • Neurochemical biomarkers provide a direct readout of CNS functionality at multiple levels (behavioral, electrophysiological, endocrine, emotional and systemic stress). • Certain studies have begun to shed light on regional differences in sensitivity to specific spaceflight stressors. • Cross-species chemical correlates can be found between rodents and humans that could be translated to circulating biomarkers. **Neurobiological Studies** • Microgravity models and sleep deprivation studies in humans identified significant neuroimaging signatures suggesting that fluid shifts and metabolic changes underlie stress-induced disruptions in functional connectivity and reduced volumes of grey/white matter in the brain. • While complicated for real time, many of the outcomes rely on non-invasive imaging. • Cross-species imaging correlates are robust – with issues of size/sensitivity as the main limitation for smaller animal models.

- Unlike rodents, transgenic and knockout NHP models are being developed, but currently not as readily available.

Metabolomic & Lipidomic Studies

- Metabolomic and lipidomics likely represent the only approaches amenable for real-time (en-route) biomarker assessments. Blood, urine, and saliva samples represent a source of easily obtainable bio-samples that under the appropriate setting, may portend and inform NASA of developing health problems.
- Can be performed quickly and longitudinally.
- Omics approaches can be linked directly to changes in neurochemistry, deciding the temporal order of events under a chronic stress environment will be critical to avoid the "chicken and the egg paradox".

Limitations

Behavior & Cognitive Studies

- Limited combined spaceflight stressors studies–impact on behavior/cognition for short-/long-duration spaceflight is unknown.
- Hindlimb-unloading and downward head-tilt paradigms only mimic some of the physical effects of microgravity and induce negative consequences of stress and depression that confounds the results of these experiments.
- Rats that were pre-trained on a set-shifting task prior to radiation exposure showed ion- and dose-dependent deficits that varied across brain regions to differentially impact working and associative learning and memory. These data suggest that specific cognitive impairments may manifest under evolving mission scenarios. Such data also highlighted the clear need for assessment of the impact of spaceflight stressors on a wide-range of operationally-relevant neurobehavioral and cognitive tasks. Future investigations in this area should explore both novel and trained paradigms to assess level of impairment.

Molecular Studies

- Limited combined spaceflight stressors studies.
- Data derived from invasive assays and are not ideal for assessments in humans.
- Data regarding space relevant radiation exposures on neurocognitive outcomes in humans are virtually absent.
- No region or regions of the brain was identified as more or less resistant to the impact of spaceflight stressors, especially with regard to radiation, instead being completely reliant on the outcome measured.

Neurochemical Studies

- Limited combined spaceflight stressors studies.

- Data are not consistent across the disparate treatment groups across studies, arguing for more comprehensive and controlled studies with radiation in combination with other stressors to ascertain brain region sensitivities.
- Studies typically evaluated effects of spaceflight stressors on one neurotransmitter system (e.g., dopamine, glutamate, or serotonin). Few, if any, studies conducted a comprehensive and within-subject assessment of temporal and regional changes in multiple neurochemicals that were related to behavioral and cognitive outcomes.
- Data derived from human studies involved MRI spectroscopy, difficult to do in real-time (i.e., spaceflight), while data for rodents involved more invasive measures (microdialysis, direct tissue sampling etc.)
- Changes for sleep deprivation are difficult to interpret in the context of chronic spaceflight, the majority of measures/changes are transient and if/how that might interact with other stressors (radiation) remains uncertain over chronic exposure scenarios.

Neurobiological Studies

- Limited combined spaceflight stressors studies and radiation studies were limited to rodent models.
- Uncertain how persistent changes are in the brain in the context long-term space travel.
- While MRI was shown to be informative for certain stressors, it remains unlikely that this tool will be for real-time assessment given the equipment involved. It is currently unknown whether this modality is sensitive to space radiation doses and dose rates.

Metabolomic & Lipidomic Studies

- Combinations of spaceflight relevant studies were virtually absent.
- Number of studies conducted on CNS tissue or focused on CNS responses are limited, especially for the radiation exposure. Primarily due to the absence of cross-disciplinary expertise–few people with “omics” expertise are neurobehavioral scientists.
- Assigning metabolomic/lipidomic signatures to specific organ/tissue sites remains challenging.
- While the search for biomarkers specific for CNS spaceflight stressors remains appealing, it should be noted that despite years of investigation, this line of research has not identified robust, reproducible, and kinetically distinct signatures attributable to any radiation response. Thus, this approach may “never/not” yield the critical information NASA needs to assess cognitive risk.

Table 2: summarizes the findings from the animal and human studies regarding the 4 spaceflight stressors: radiation, microgravity, confinement/isolation, and sleep deprivation. Areas marked with an X denote areas where the negative consequences of that stressor on brain and behavior are significant, with ample supporting evidence. Areas marked with --- denote areas where there is limited evidence, or the negative impact is much less significant.

	Learning	**Memory**	**Cognitive Flexibility**	**Cognitive Control**	**Working Memory**	**Attention/ Vigilance**	**Depression/Stress**
Definition	Acquisition of new information	Retrieval of learned information	Switching between distinct concepts	Selecting and monitoring behaviors	Holding information for active processing	Sustained concentration while ignoring distractions	Feeling of sadness; lack of interest; apathy
Example tasks	Stimulus discrimination; classical conditioning	Object-location associations; mazes with rewarded locations	Stimulus-reversal tasks, such as Wisconsin Card Sorting Task	Repeated task-switching with distractors, such as the Flanker Task	Delayed-match-to-sample task with brief delay between items	Psychomotor vigilance task - pressing a button in response to a light	Self-report surveys of mood
Brain regions	Hippocampus; Striatum	Hippocampus; Medial temporal lobe	Prefrontal cortex (PFC); anterior cingulate	Prefrontal cortex (PFC); Ventral tegmental area (VTA)	Inferior temporal (IT) cortex; Prefrontal cortex (PFC)	Prefrontal cortex (PFC); posterior cingulate; inferior parietal cortex	Dopaminergic reward-based system in mid-brain
Radiation	**X**	**X**	**X**	**X**	----	**X**	----
Microgravity	**X**	**X**	----	----	----	**X**	**X**
Confinement /Isolation	**X**	**X**	----	----	----	----	**X**
Sleep deprivation	**X**	**X**	**X**	**X**	----	**X**	**X**

Figure Legends

Figure 1: Overview of how spaceflight stressors may alter brain molecular, neurochemical, and neurobiological endpoints to impact behavior and cognition relevant to understanding operational performance. The spaceflight stressors that impact CNS function include space radiation, microgravity/hypergravity, social isolation/confinement, and sleep deprivation. Here we highlight various molecular, neurotransmitter, and brain structure and function signatures that may be altered by spaceflight hazards to impact operationally relevant behavioral and cognitive performance. Also, evidence so far suggests that specific neurocognitive impairments may manifest under evolving mission scenarios (i.e., increased cognitive load) and therefore, assessment of impact on a wide-range of operationally relevant behavioral and neurocognitive tasks is critical. Thus, we highlight both novel and trained paradigms with increased difficulty to determine the level of impairment. Translation between animal models and humans is important and we highlight parallel behavioral and neurocognitive testing paradigms that exist between rodents ↔ NHPs ↔ humans.

Figure 2: Summary of the impact of sleep deprivation across multiple levels, including molecular, cellular, network, and whole brain. Adapted from Abel et al., 2013.

Figure 3: Primary and secondary risk from interactions between multiple spaceflight stressors on behavioral health and performance. These interactions among the spaceflight stressors are likely to be varied and complex. Some of the underlying cellular, molecular, and neurochemical

signatures may be compounded due to multiple spaceflight stressors, while other stressors may trigger each other or secondary outcomes that can exacerbate behavioral and neural effects, operations performance, and team dynamics. BHP: behavioral health and performance; BMed: behavioral medicine. Adapted from Slack et al. (2016).

Figure 4: Future investigations that prioritizes those areas deemed essential to address current gaps in knowledge and to make meaningful progress in addressing CNS relevant risks resulting from spaceflight stressors alone and in combination. Investigations are divided into 4 main stages: 1&2 involving assessment of various behavioral and cognitive domains in pre-trained laboratory animals; 3 highlighting spaceflight stressors to be studied; 4a emphasizing the need to conduct post-treatment behavioral and cognitive assessments; and 4b-d assessment of various non-invasive and invasive changes.

Figure 1

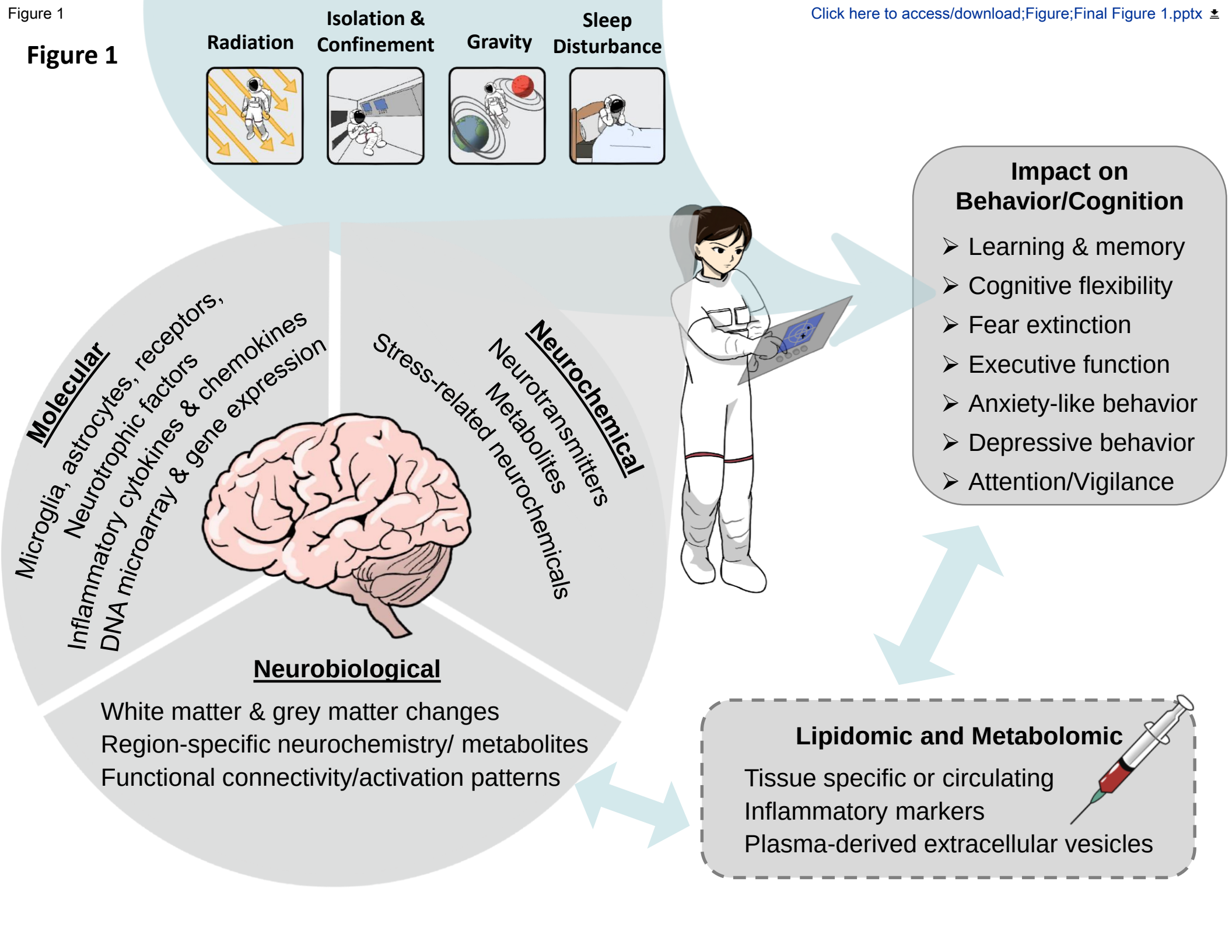

Figure 2

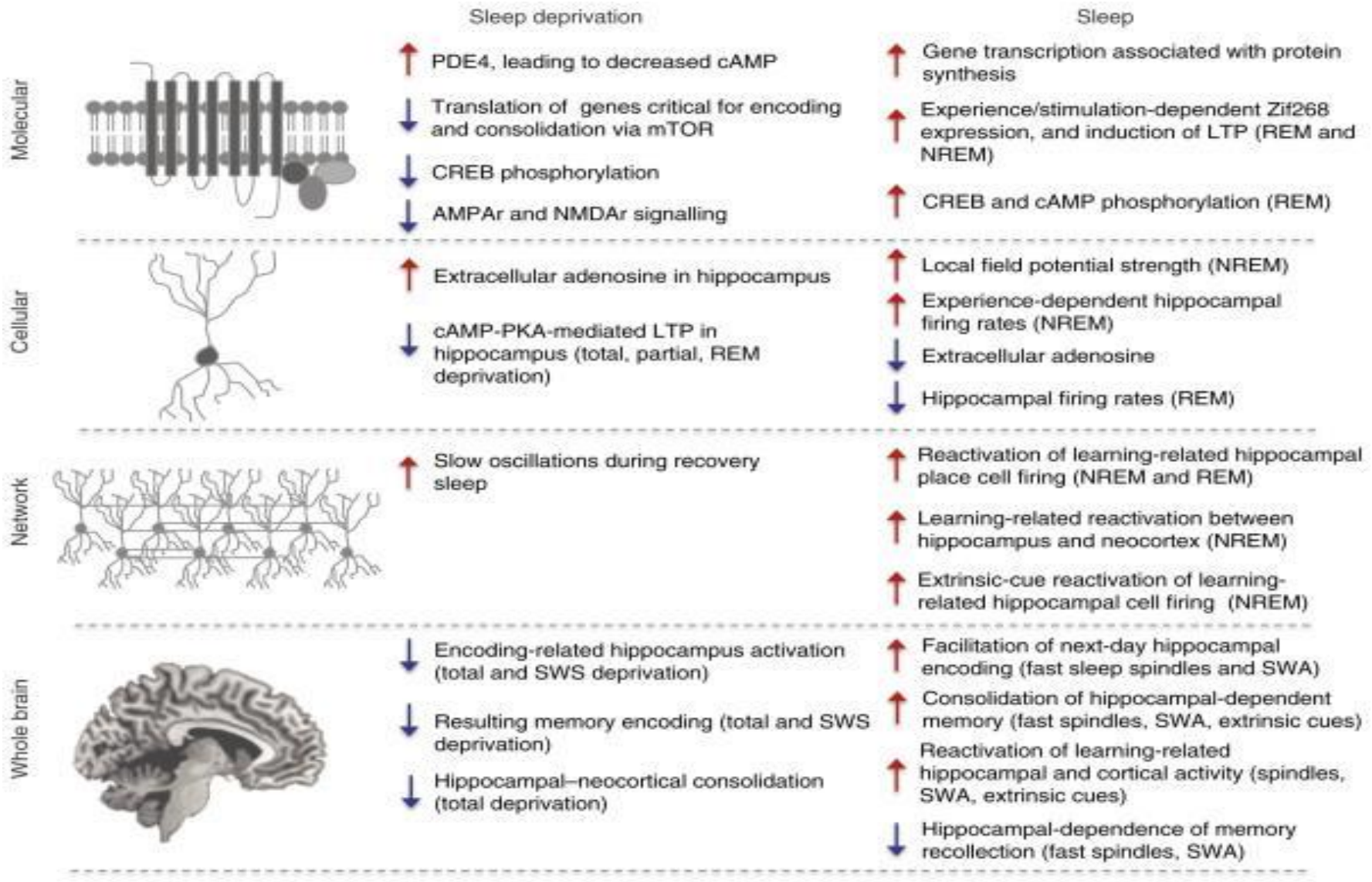

Figure 3

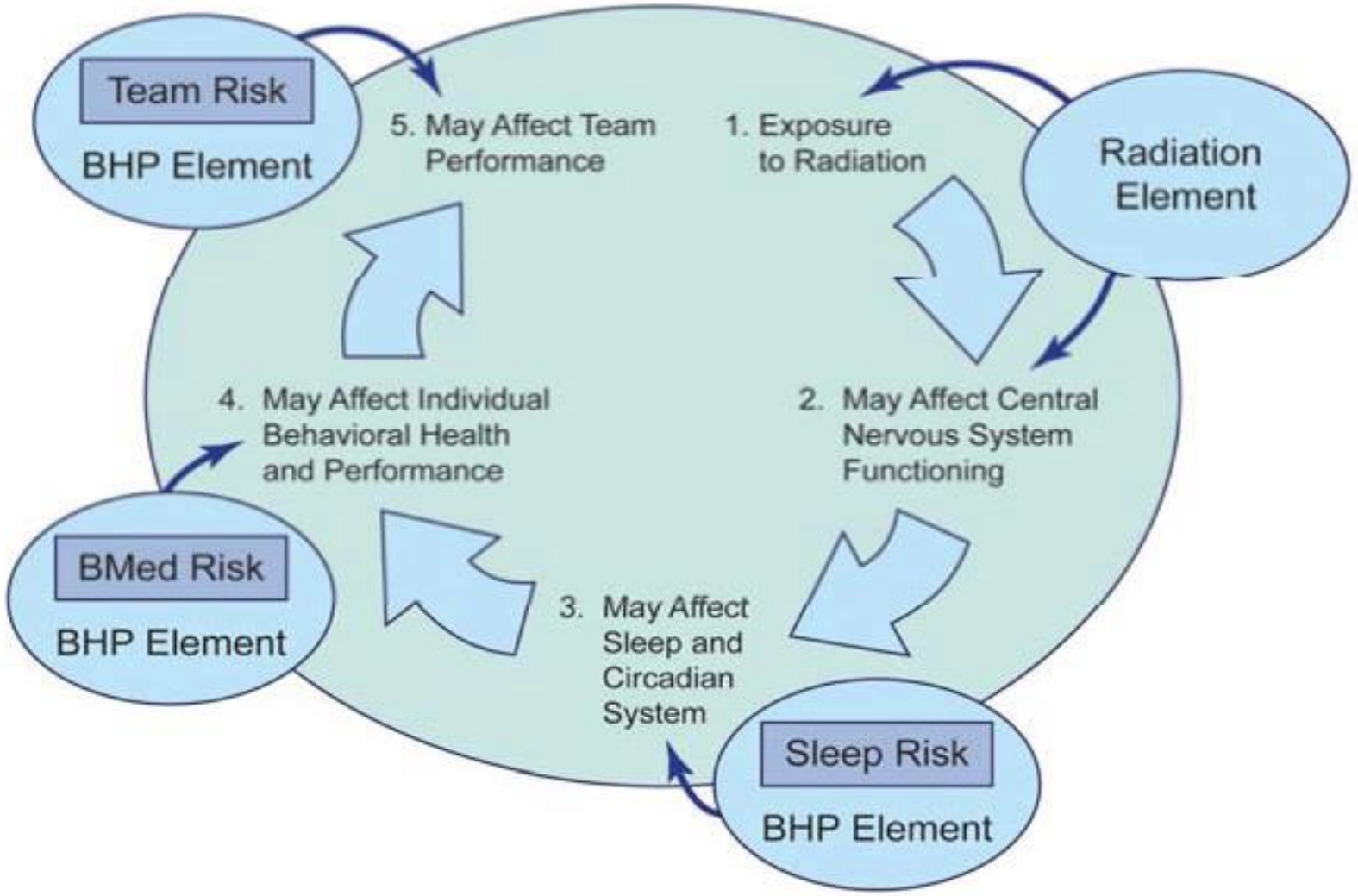

Figure 4