A Sankofian appraisal on how to maximize translatability of rodent space radiation/CNS studies to astronauts"

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Abstract

There is an ever-growing body of evidence from ground-based rodent studies that space radiation (SR) exposure impairs performance in multiple cognitive processes, ranging from relatively fundamental processes to complex analogs/homologs of human cognitive tasks. The overall consensus is that SR exposure impacts performance in multiple cognitive tasks, utilizing multiple cognitive processes governed by multiple brain regions. The mechanistic basis for the observed SR-induced cognitive impairments, and occasional enhancements, is increasingly being established. Translating the results from ground-based rodent studies into tangible risk estimates for astronauts is an enormous challenge, but NASA has a long history of choosing to do things like "go to the Moon in this decade and do the other things, not because they are easy, but because they are hard".

The rapidly approaching start of NASA's second phase of space exploration, that will see the first woman land of the Moon and then the challenging deep space voyage to Mars, necessitates that during the remaining time research be conducted that maximizes the translatability of ground-based studies to astronauts. Such studies cannot be extended versions of the historical approaches but must embrace new neuroscience concepts.

The Akan people of Ghana have developed the Sankofa philosophical approach, that is symbolically summarized by the mythical Sankofa bird, which while advancing, constantly and appropriately looks to its past to (re)conceptualize and (re)negotiate its future. This review has embraced the Sankofa philosophy and provided a comprehensive "historical" review of the important findings from previous ground-based studies and outlines how this accrued knowledge can be used to guide future investigations. Several new areas/concepts have been identified that if studied more thoroughly could maximize our insight into how SR impacts CNS functionality before astronauts start Artemis missions.

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Introduction:

NASA is on the verge of its second and most challenging phase of space exploration, returning to the Moon and then on to Mars. The challenges associated with long duration, deep space missions are many and will require some new operational approaches from those used on previous missions, including astronauts acting more autonomously. Astronauts will have to endure prolonged exposure to multiple flight stressors that could seriously impact their neurological and physical health. Due to inherent limitations of the spacecraft design and uplift capacity, space radiation (SR) exposure will ~~also~~ be an unavoidable flight stressor on a mission to Mars, and microgravity stressors for all space flight. In addition, there may be intermittent exposures to flight stressors such as inadequate sleep, psychological stress. Thus, the impact that Galactic Cosmic Radiation (GCR) exposure has on central nervous system (CNS) function may be markedly different on the return journey than on the outward journey.

The deep space radiation spectrum is composed of highly energetic protons, He and higher mass (Z>2) charged ions. Current estimates suggest that astronauts will be exposed to ~ 13 cGy of SR during each year of a mission to Mars (1), the majority of which will be incurred en route. The structure of the spacecraft will modulate the SR dose and alter the ion spectrum from that seen in free space (2). NASA has devoted considerable resources to establish the impact that SR exposure has on the functionality of the central nervous system (CNS), since any deterioration in the ability of the astronauts to perceive or respond to changes in their situation, or changes in their mental health, could have disastrous consequences for the mission.

There is an ever-growing body of evidence from ground-based rodent studies that SR exposure impairs performance in multiple cognitive processes, ranging from relatively fundamental processes to complex analogs/homologs of human cognitive tasks (Section 1). The overall consensus is that SR exposure impacts performance in multiple cognitive tasks, utilizing multiple cognitive processes governed by multiple brain regions. The mechanistic basis for the observed SR-induced cognitive impairments is increasingly being established, with several studies identifying physiological and structural changes within the brain, primarily in the hippocampus, but also in the striatum and prefrontal cortex (Section 2). In some instances, SR-induced neurophysiological changes have been associated with concomitant gliosis (Section 3). Collectively these physiological and structural changes are highly likely to damage neural network functionality/cohesiveness which would have detrimental impacts on performance in complex cognitive tasks (Section 4). Whether such changes are the cause or the consequence of SR-induced "omics" (e.g., proteomics, transcriptomics, metabolomics/lipidomics, etc.) alterations in the brain (Section 5) remains to be determined.

Translating the results from ground-based rodent studies into tangible risk estimates for astronauts is an enormous challenge, even when homologs or close analogs of human cognitive tests are employed. This is even more the case when dealing with multiple flight stressors. During the remaining time before astronauts start deep space explorations that research is conducted that will maximize translatability of ground-based studies to astronauts.

Section 1. Space radiation effects on cognition.

Any deterioration in the ability of the astronauts to perceive or respond to changes in their situation could have disastrous consequences. Not surprisingly, NASA has devoted considerable efforts to establishing the impact of flight stressors on CNS functionality using both in-flight and ground-based analogs. However, by default, virtually all the studies that have assessed the impact that SR exposure on CNS functionality have used ground-based rodent models.

1.1. Looking back: There is an ever-growing body of evidence from such studies that SR exposure impairs performance in many cognitive processes, ranging from relatively fundamental processes to complex analogs/homologs of human cognitive tasks. There are several excellent "historical" reviews on this body of data e.g., (3)(4) (5)(6). The majority of these studies have used well-established, highly characterized cognitive tasks that assess the functionality of individual brain regions.

The widely used Novel Object Recognition (NOR) task is a powerful investigative tool to interrogate the ability of the hippocampus to encode and recall a memory of a specific object. When presented with a new object, the dentate gyrus (DG) aids the CA3 region of the hippocampus to distinguish (perform "pattern separation") the new from the familiar object by providing a representation (memory) of the old object to contrast with the newly encountered (7). While the NOR task has provided considerable insight into the impact of SR on hippocampal functionality, there are now several tasks that can provide insight into more complex hippocampal dependent pattern separation skills of rodents. The rodent Updating task, incorporates many of the features of the human Mnemonic Similarity Task (MST). The MST is a behavioral task that was designed to tax pattern separation skills in patients with mild cognitive impairments (8), requiring the participants to respond to "Old", "Similar", or "New" on each trial. Preliminary data suggest that <30 cGy SR impairs performance of mice in the updating task (Limoli, HRP-IWS 2021). However, it should be noted that SR-exposed male and female mice show enhanced pattern separation performance in the Location Discrimination (LD) pattern separation task (6)(9). Interestingly, these studies used a battery of multi-domain touchscreen tasks and found that while there was an improvement in hippocampal dependent LD performance after SR exposure, in the same animals there was a loss of performance in the striatum-dependent Visuomotor Conditional Learning task (9). This is not an isolated incidence of SR exposure differentially impacting performance in cognitive tasks regulated by different regions on the brain (*vide infra*).

The majority of studies to date, report a loss of performance in hippocampus-dependent tasks following SR exposure (3)(4)(5)(6); however, there are several studies that show no change in performance, or indeed an enhanced (6)(9)(10)(11) level of performance in hippocampal dependent tasks. While the enhanced level of performance in the SR-exposed animals may seem contrary to the other studies, the enhanced pattern separation (10)(6)(9), may be indicative of a SR-induced loss of memory encoding. However, the enhanced level of cognitive flexibility (active avoidance) would appear to be associated with a higher level of forgetting the previous memory of the adverse encounter, which would be consistent with the observed elevation in neurogenesis (11). Thus, even within the realm of hippocampus dependent behavioral tasks, the effects of SR exposure may be highly variable, and care must be taken in making conclusions without

thoroughly understanding the fundamental aspects of the behavioral task (e.g., sparse encoding/forgetting).

Astronauts are routinely screened during space flight for performance in a 10-test battery of cognitive tasks that NASA deemed necessary for mission success (12)(13). Seven of the tasks in the "fit-for-duty" performance battery assess some aspect of executive function. Executive function, in lay terms, can be summarized as the "Triple A": the ability to Assess, Adapt and Achieve. More technically, executive functions are a set of higher order cognitive abilities that animals utilize to keep information "in mind," attend to appropriate cues (e.g., nonverbal and verbal working memory stimuli), update information as contingencies change and invoke alternative, more appropriate responses to new situations.

The rodent version of the psychomotor vigilance test (rPVT) is virtually identical to the PVT test that is part of NASA's "fit-for-duty" performance battery on the International Space Station (ISS) (12)(13). PVT performance is sensitive to fatigue, drug use, and age (14)(15), and exposure to mission-relevant (25 cGy) doses of protons results in deficits in accuracy, impulsivity, and lapses in attention, all of which are indicative of deficits in sustained attention (16). Such lapses in attention account for 80% of flight accidents in the Navy and Marine Corps (17).

A key process that allows humans to adapt to different situations rapidly and efficiently is task- or set-shifting. An attentional set is formed when complex stimuli have been discriminated and classified as relevant or irrelevant to a particular task/situation. Set-shifting can be simplistically thought of as the ability to relearn what the most important discriminating stimulus (for a particular endpoint) is in a changing environment. Attentional set shifting (ATSET) is thus a highly important skill that are required to deal with a sudden emergency.

The rodent ATSET task is a 7-stage progressive test, where the rat has to form an association between the presence of the food reward and a physical cue (either the digging medium or odor) (18). By altering the combination of odors and digging media, progressively more complex cognitive processes can be tested. Performance in the ATSET task requires associative recognition memory, sequential rule learning ability, utilizing information gained in a previous stage to solve the subsequent tasks, and incorporates many of the essential elements of the Wisconsin Card Sorting Test that is widely used to assess task switching in humans (19), with task switching deficits being increased in patients with Parkinson's disease (20)(21) and autism (22).

Using rats that have been selected for high levels of ATSET performance prior to irradiation, performance in a structurally identical ATSET task to the one used to prescreen the rats, but with different associative cues, is significantly impaired after exposure to $\leq$ 15 cGy of 600 MeV/n ^{56}Fe (23), 1 GeV/n ^{48}Ti (24), 600 MeV/n ^{28}Si (25) and protracted low dose rate neutrons (26). The main impact of SR on ATSET performance is primarily confined to the first two discrimination stages [Simple (SD) and Compound (CD) discrimination]. The SD stage of the ATSET test assesses the decision-making ability of the rats, i.e., their ability to form an attentional set (associative memory formation) on the correct associative cue for a food reward. The CD stage tests the ability of the rats to maintain the attentional set formed during the SD stage when the irrelevant dimensional cue is altered. Should similar effects occur in humans, astronauts would exhibit a decreased ability to

identify and maintain focus on relevant aspects of the task being conducted. Thus, for tasks that are time and/or event sensitive, i.e., a problem has to be solved quickly and on the first attempt, SR-exposed individuals may not be able to perform well. However, SR-exposed rats were able to perform very well in an ATSET task that was identical to the one used for prescreening. These data are consistent with the rats retaining (for at least 100-120 days) a working memory of the rules for the food reward, even after being exposed to SR. SR exposed mice exhibit similar issues in learning the initial rules in a touchscreen-based Pairwise Discrimination task (6)(9). However, similar to the rats in the ATSET task, once the SR-exposed mice learnt the rule for the reward in the task they had no problems completing the more complex pattern separation stages (6)(9). Should similar effects occur in humans after SR exposure, astronauts would most likely be able to perform the tasks that they had been previously trained to do. Thus, from an operational perspective, SR-induced performance deficits may only be manifested in scenarios where the astronauts have to transitively apply their knowledge to solve problems that they have not previously encountered (23).

The studies that have established that SR exposure impacts various aspects of attention have also demonstrated that there is a dichotomous response to SR (Z=1 to Z=28) (16)(26)(25)(23). Some irradiated rats have performance metrics comparable to that of the shams, while others struggle to complete the task, even when given a second opportunity. In these studies, the rats had been preselected for high performance prior to SR exposure, and thus there is a high degree of confidence that this dichotomous response is not due to inherent inter-individual performance variability. These data thus suggest that some individuals are able to ameliorate the deleterious effects of the SR, while others are unable to do so.

Traditionally ground-based studies have determined if the cohort average value of the metric being investigated differed significantly between the sham and SR exposed subjects. Typically, some form of Gaussian-based statistical analysis is conducted on the generated data. Such analyses are problematical in many regards. Firstly, there are few instances when a “perturbing” agent leads to a constant level of performance decrements across the entire population, resulting in treatment-related skewness (gamma distributed) in the performance metric, which has to be analyzed using non-parametric statistical methods. Secondly, cohort analyses do not quantify what proportion of SR-exposed subjects have impaired performance, nor the severity of impairment. A 20% decrease in a cohort’s performance metric could reflect 100% of individuals performing at 80% of the sham’s performance level or could reflect 50% of individuals performing at 60% performance level with the rest having comparable performance to the shams. Thus, a more comprehensive understanding of how SR exposure impacts cognitive performance, specifically the incidence and severity of cognitive impairment, can be derived if individualized performance data are generated and analyzed. Clearly, it is only possible to calculate the probability of an adverse event occurring if there is some definition of what loss of performance constitutes an adverse event, which will be highly context specific. One frequently used metric to define significantly different from “normal” is the Z-score, with a Z-score of +2 reflecting the 95th percentile of the population’s metrics, while a Z-score of -2 represents the 5^{th} percentile. However, Z-scores are only valid when there is a normal Gaussian distribution of data.

In statistics, kernel density estimation (KDE) is a non-parametric way to estimate the probability density function of a random variable and is frequently used to make inferences about a population based on a finite data sample. KDE is thus a useful tool to assign a level of performance that can be considered to be sub-optimal for the task under consideration. That metric can then be used to quantify the numbers of individuals within the test population that display a severe impairment. In the few studies that have utilized KDE analysis (25)(26)(27), the 5th percentile of the sham cohort performance profile (conceptually analogous to a Z-score of -2) was considered to be the threshold for severe impairment. KDE analyses revealed that relative to the 5^{th} percentile of sham rat performance, 41% of neutron- (26), and ~44% of ^{56}Fe- (23) exposed rats had severely compromised performance in the SD stage of the ATSET test. Interestingly, this is almost identical to the conclusions in a parallel study on Object In Place (OIP) performance in mice exposed to protracted neutrons (27). In the event that a task requires a higher or lower level of performance to perform that task safely/effectively, the same data set could be reanalyzed using other percentiles (e.g., the 1st or 99th) as the cut-off value.

The Numbers Needed to Harm (NNH) algorithm is a measure that is increasingly used to express the potential harmful effects of an intervention in clinical trials. A similar approach can be used to generate the probability of SR-induced cognitive impairment, where SR would serve as the intervention. Substituting the derived values for severely impaired individuals into the NNH algorithm provides estimates of absolute risk and the frequency that severely impaired cognitive performance will occur following SR exposure. Should similar effects be induced in humans, the NNH value of 2.8-3.4 from the rodent studies (26)(27) would mean that in a crew of five astronauts traveling to Mars, two would display severe deficits in one executive function (ATSET) by the time they return to Earth.

1.2. Looking forward: It could be argued that currently available data warrants that a cross-species validation of the SR-induced cognitive performance deficits be initiated now. However, such studies will be costly, time consuming and politically sensitive as they will have to be conducted in animal models that are more reflective of humans. When such studies are started, it is important to determine whether performance in operationally significant tasks is impaired.

Astronauts on deep space missions will have to act more autonomously than on previous missions, especially when rapid responses to unexpected problems are required, thus creative problem-solving skills will be of significant importance to astronauts on a mission to Mars. Creative problem solving requires the integrative use of several mental capacities, including executive functions involved in planning, organization, decision making, judgment, task monitoring, attention, hypothesis generation, abstract thinking, and cognitive flexibility (28)(29)(30)(31). Recent studies have shown that low (<20 cGy) doses of SR impact creative problem solving in rats (25)(26)(32). However at the individual level, poor creative problem-solving performance in the irradiated rats was not necessarily associated with poor ATSET performance, and vice versa (25)(32). SR exposure impairs both spatial memory and ATSET performance, however, when the relative performance of individual rats in each task was compared there was no correlation between SR-induced loss of performance in each task (33). Recently, SR exposure has been reported to have a differential effect on performance (at a cohort level) in striatum versus hippocampus-

dependent tasks (9). Such data strongly suggest that risk assessments for SR-induced neurocognitive impairment derived from a single cognitive domain may greatly underestimate the severity of the problem.

The switch task provides a direct measure of an individual's ability to maintain and schedule two mental task sets, as well as working memory. Such critical multitasking skills are necessary for dealing with complex situations such as landing an aircraft. The rodent switch task is a homolog of the human global switch task (34) where the rat has to repeatedly switch its attention to a new reward cue, after completing a block of trials where the opposite cue was rewarded. Such tasks can easily be made progressively harder by reducing the time rats have to respond to the sudden change in circumstances.

Traditionally, non-human primates (NHPs) have been considered to be the only animal model to have higher order thought processes comparable to humans. The ability to conceptualize, categorize with numerosity, to generate hypotheses, and to perform abstraction were not considered to be inherent cognitive processes for rodents. However, this is an outdated and spurious approach since several "primate-specific" cognitive tasks can be directly interrogated using rats, viz: metacognition (35), counting- or timing-like processes for encoding serial position, rule abstraction and hypothesis generation (36) and prospective timing (37).

Confidence regarding a choice/decision has often been thought of as an instance of "metacognition" (thinking about thinking), which was thought to require advanced neural architecture available only in primate brains (38)(39)(40). However, in a seminal study, Kepecs and colleagues (35) demonstrated that rats are capable of making confidence estimates on decisions they have made. In that study, rats had to first make an estimate of the probability of getting a reward based upon their perception of the relative balance of rewarded and non-rewarded odors within a test mixture, the composition of which was randomly varied. As time progressed without the food reward being delivered the rat had the chance to assess its confidence in getting the food reward and had the option to reset the task. The conclusions from these experiments demonstrated for the first time that rats were capable of generating confidence estimates based upon incomplete data, a process that is believed to involve metacognition and conscious awareness.

Utilizing a series of complicated, partially overlapping odor recognition tasks, it was shown that rats use at least three cognitive processes concurrently in serial pattern learning tasks, namely, item memory involving external discriminative cues, counting- or timing-like processes for encoding serial position, and rule abstraction for encoding an internal representation of pattern structure (36).

Such complicated tasks are far more difficult to conduct, much more labor intensive and time consuming than simple tasks like the NOR. From a traditional behaviorist point of view such tasks are "messy" since multiple brain regions (often operating in close harmony) regulate performance in such task. However, NASA is more interested in getting astronauts back to Earth alive from the mission to Mars than doing "purist" academic research. As a result, from an operational point of view, the impact that SR exposure has on complex tasks that could have a substantial impact on

mission success needs to be determined. It is also vital that such testing is conducted under realistic conditions. Under routine circumstances, most people with adequate levels of skill and competency can usually perform well in tasks, however it is the ability to perform under unpredictable or stressful circumstances that characterizes individuals who are considered to be expert performers. In many ways, astronauts are amongst the most elite of expert performers, having to perform in a wide range of complex tasks with little to no margin for error at all times. Consequently, future studies that assess SR effects on performance in more demanding tasks will have to do so under stress-loading conditions. Stress-loading is an integral approach in NASA's engineering projects, and in astronaut training in general. However, most rodent cognitive testing to date has been conducted under very conducive conditions. Thus, it would be far more valuable to push the performance limits of SR-exposed animals until there is a catastrophic failure in performance (within the permissible limits of animal welfare). Recently, there has been an increased emphasis of establishing the impact of exposure of multi-flight stressors on the CNS, since during flight astronauts will have to contend with exposure to multiple stressors, often in close proximity to each other.

For multiple reasons, astronauts sleep less while in space than they normally do on Earth (41)(42)(43)(44), and also the quality and structure of sleep during space missions gets altered. During spaceflight, there are quantitative changes in various sleep metrics including reduced latency to rapid eye movement sleep (REM), shorter REM episode duration, and a redistribution of slow-wave sleep (SWS) between the first and the second sleep cycles (45). There is also 27-50% reduction in REM and SWS sleep time in space compared to that on Earth (46)(47)(48), and astronauts on the ISS exhibit increased sleep pressure (greater theta activity in the EEG and more local sleep events during waking) (49). Astronauts extensively use sleep-promoting drugs during spaceflight to mitigate some of these effects (41), but such agents tend to not promote restorative sleep.

Both sleep deprivation and sleep fragmentation (SF) have been linked to reduced neurocognitive functioning in humans and animals (50)(51)(52). Sleep deprivation has a major impact upon performance in multiple cognitive domains (53), including the PVT (54), ATSET (51) and spatial learning (55) tasks. Even a single night of very mild sleep restriction (2 h reduction) can negatively impact vigilance and impact cortical indices of motor preparation and execution (56). Therefore, it is likely that neurocognitive functioning will be reduced in astronauts who have disturbed sleep.

Two recent studies have established how inadequate/disturbed sleep alters the severity of SR-induced impairment of executive function. A single session of fragmented sleep uncovered latent ATSET performance deficits in rats exposed to both protracted neutron (26) and Si (57) irradiation that had no obvious defects in performance under rested wakefulness conditions. SF selectively impaired performance in the more complex IDR, EDS and EDR stages of the ATSET test in both neutron and Si irradiated rats. Set shifting performance has rarely been impacted by SR exposure in studies conducted with rats tested under rested wakefulness conditions. Thus, SR-induced cognitive impairment may not be fully evident in normally rested rats, substantially underestimating the level of impairment that may occur when astronauts are on mission. Cognitive

testing may thus have to be conducted under both rested wakefulness and SF conditions to get a more accurate assessment of SR-induced neurocognitive impairment.

Removal of toxic metabolites, such as lactate, from the brain is generally considered to be facilitated by the glymphatic system during sleep (58). It is believed that amyloid precursors are similarly removed during sleep via the glymphatic system. In the case of lactate levels, the post sleep lactate levels within the brain are <33% of the pre-sleep values. Lactate plays a key role in many aspects of neurophysiology, and thus lactate metabolism is finely tuned. While lactate can be a key energy resource for neurons, when lactate levels become too high, there can be (1) disturbance of cortical gamma rhythms by neural bursting or attenuation, (2) increased oxygen consumption and decrease neuronal network activity and (3) attenuation of synaptic transmission by reduced neurotransmitter release (59). Such changes in the functionality of neurons could be the basis for the changes in cognitive performance in individuals subjected to sleep loss and could also underpin the observed unmasking of latent SR-induced cognitive deficits. Recent conference presentations by Dr. O'Banion (at ICRR 2019 and HRP-IWS 2021) strongly suggest that blood-brain barrier (BBB) transport of amyloid-β (Aβ) mediated by low-density lipoprotein (LDL) receptor-related protein-1 (LRP1) is reduced following SR exposure. Previously, the same group reported increased levels of amyloid precursor protein in SR-exposed rats, which may be accounted for by the SR-induced changes in the LRP1 transport rates (60). Thus, a combination of SR exposure and sleep loss may result in significantly altered removal of toxic metabolites from the brain.

In addition to SR and sleep issues, astronauts on deep space missions will have to contend with microgravity and social isolation issues. Microgravity has well-documented effects on the skeletal, microbiome, gut mucosa, sensorimotor and ocular systems (e.g., (61)(62)(63)(64)(65)(66)(67)). It also produces significant effects on the brain, particularly in cerebellar, sensorimotor, and vestibular brain regions (Reviewed in (68)). Brain activity may also change in response to the need for increased processing required for postural stabilization, and integration of conflicting vestibular information in the microgravity environment (69). At the cellular level both simulated and in-flight exposure to microgravity results in persistent changes in the mitochondrial function and lipid metabolism of human oligodendrocytes (70)(71). Oligodendrocytes are essential for providing metabolic support to neurons, rapidly transferring (through cytoplasmic "myelinic" channels and monocarboxylate transporters) short-carbon-chain energy metabolites like pyruvate and lactate to neurons (72). Such microgravity induced metabolic perturbations are likely to be deleterious to neuronal function in their own right but will likely exacerbate SR-induced changes in neuronal functionality.

Social isolation is likely to be a significant concern for astronauts on interplanetary missions. In general, social isolation in both humans and animals has deleterious effects on multiple systems (73). Social isolation increases the risk of depression and reduced cognitive functioning (74)(75)(76), alters neurotransmitter systems and neuronal morphology (77)(78)(79)(80)(81). Humans with poor social support have exaggerated blood pressure and heart rate responses to stressful situations with slower recovery times (82). Social isolation can also increase the risk for

cardiovascular disease (83), and impacts inflammatory responses and the ability to respond appropriately to other stressors (83).

In addition to their previously described role in cognitive process, executive functions also regulate response inhibition, impulse control, processing and regulating affect, motivation, and arousal (84). Should SR-exposure alter such executive functions as it does those regulating cognitive flexibility, there is the possibility that SR exposure may exacerbate aberrant behaviors evoked by psychological stress related to isolation, confinement and boredom, or induce additional aberrant behaviors. Data from the Mars 500 study suggest that some individuals socially withdrew from contact with fellow crew members (85). SR exposure does result in the loss of social recognition memory (using either "orphaned" odors or caged conspecifics) in male rats or mice (86)(87)(88)(89)(90)(32). Three-chambered sociability data indicates a dose dependent decrease in interaction time after exposure to 50 cGy (but not 15 cGy) of a mixed SR beam (87). A significant reduction in social interaction by test subjects is often interpreted as social withdrawal (91)(92)(93)(94). Consideration of these data suggest that SR exposure has the potential to alter the sociability of exposed rodents, manifested in part as social withdrawal/indifference. Some individuals in the Mars 500 confinement study (85) did socially withdraw, so there is a possibility that SR exposure could exacerbate such withdrawals. More detailed investigations of the impact of SR on social withdrawal are clearly needed. One of the tasks in NASA's "fit for duty" battery of tasks is the Balloon Analogue Risk Task (BART). The BART is a computerized measure of risk taking behavior that models real-world risk behavior through the conceptual frame of balancing the potential for reward versus loss. If NASA becomes increasingly concerned about BART results in humans, a possible animal model analogue could be the rat gambling task (95) which is a touchscreen based task, conceptually identical to the BART, that could be used to establish the impact of SR on impulse control/risk taking.

1.3. Summary: The information gained from rodent studies has already identified a number of possible cognitive processes that could be sensitive to SR exposure. On-going studies on how astronauts respond to the single, compound, and potentially synergistic effects of mission-related stressors will be crucial for understanding factors that can impair performance and damage health (and then mitigating those factors). A key aspect of any future studies is the use of cognitive tasks that have a low practice effect, thereby allowing a base line value to be determined in SR-exposed animals in the absence of any additional stressors, and a re-evaluation of performance.

NASA's Human Research Program (HRP) has already initiated an integrated research strategy to use the vast body of data from the rodent studies to both identify operationally relevant brain performance pathways along with a robust focus on developing appropriate countermeasures (to include nutritional, intelligent systems, etc.), in the expectation that some level of cognitive impairment could occur in astronauts. The prevailing approach to developing countermeasures is to employ pharmacological agents that are either "traditional" prophylactic radioprotective agents or biological response modifiers (e.g., anti-inflammatory agents), used to ameliorate SR-induced effects. The choice of these agents may be driven by mechanistic studies on the effect of SR on neurological function or may be "off-the shelf" compounds used to treat neurodegenerative disorders or nuclear weapons related radiation exposure.

Given that operationally significant task performance is going to be determined by the impact of SR on multiple brain regions, potentially impacting multiple cell types and processes, devising an effective countermeasure approach empirically will be problematic. Careful consideration of how SR impacts the various processes, cell types, and brain region (individually and collectively) will be needed to ensure that effective countermeasures will be in place before astronauts start the Artemis missions to the moon. In subsequent sections of this review, we will outline the current status of our knowledge of the impact of SR and other flight stressors on the functionality/metabolism of the brain.

Section 2. Space radiation effects on neurophysiology.

2.1. Looking back: Performance in most cognitive tasks requires some form of contingency learning that determines what combination of events leads to a reward during the "training" stage and take appropriate action if those criteria are met or not (96). Rats, like many rodents, have highly developed contingency learning and causal reasoning skills. Causal reasoning is the process whereby the consequence of an action is remembered, and that information is used to predict future events (predictive behavior). In nature, it is clear that animals can represent hypothetical future experiences not only quickly but also constantly over time, as external events continually unfold. This is accomplished through constant sub-second cycling between representations of possible futures in the hippocampus (97). Underlying all such skills is the establishment and recall of memory.

Memory is stored as alterations in the strength of synaptic connections between neurons where alterations in the synaptic connections contributes to synaptic plasticity (98). The seminal work of Bliss, Lomo and Gardner-Medwin was the first to describe "long-term potentiation" (LTP) in context with strengthening of the synaptic transmission and the basis of memory formation at the cellular level (99). The ability to functionally measure changes in electrical conductivity became key to studying the physicochemical changes induced at the synapses associated with memory formation and expression at the cellular level. Induction of tetanus-induced forms of LTP in the CA1 region of the hippocampus that can be blocked by preventing the activation of N-methyl-D-Aspartate (NMDA) subtype of glutamate receptors (100)(101)(102) being the most frequently used technique to date.

The NMDA receptor is both a glutamate-gated and voltage-dependent channel (103), and the simultaneous presence of glutamate and a depolarized membrane is necessary (when the co-agonist glycine is present) to open the channel. The NMDA receptor regulates intracellular calcium concentration in the postsynaptic neurons, with increased calcium influx triggering LTP, especially in synapses that are both active at the pre-synaptic (glutamate release) and post-synaptic (depolarized membrane) compartments (104). In many cases, NMDA is responsible for activating "silent" synapses (which lack ionotropic glutamate receptors) in the post-synaptic cleft by triggering latent alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors by membrane insertion or post-translational activation of already-inserted receptors (105). AMPA receptor activation at "non-silent" synapses (where AMPA and NMDA receptors are both present),

leads directly to greater local membrane depolarization, unblock and glutamate activation of NMDA receptors, leading to long-term changes (both LTP and LTD) of synaptic strength. Increasing AMPA receptor ionic conductance and regulating the steady-state levels of AMPA receptors at the synapse may be the predominant mechanisms for early LTP in adult synapses, while activation of silent synapses may be more important in the context of developmental synaptic plasticity (106). Changes in AMPA receptor synthesis is another mechanism whereby synaptic strength can be altered (107).

Glutamatergic signaling that recruits metabotropic glutamate receptors (mGluRs) and downstream signaling elements play a key role on the formation/stabilization (and/or removal of spines) and changes in dendritic complexities associated with the formation and maintenance of long-term memories (108). Binding of glutamate to the G-protein coupled receptors (GPCRs), activates a G-protein mediated signaling cascades that results in the amplification of the signal via multiple downstream events. Moreover, mGluR activation also opens divalent cation channels, e.g., Ca^{2+} which, in turn, leads to more amplification. Thus, mGluRs mediate a sustained and strong response to glutamate neurotransmission from the presynaptic terminals, which in turn leads to long-lasting changes in structure and function of the synapse, including LTP that contribute to memory formation.

There is an increasing body of evidence that SR exposure leads to alterations in synaptic functioning (109)(110)(111)(112)(113)(114). At the cellular level, SR exposure leads to altered intrinsic membrane properties (114) and excitatory and inhibitory neurotransmission (112). Such changes in neurotransmission may lead to alterations in the ability to regulate synaptic plasticity, and also the functional connectivity between different brain regions (115).

Exposure to SR leads to significant alterations in glutamatergic signaling in the hippocampus. These changes include a reduction in the presynaptic readily releasable vesicular pool (RRP) of glutamate (111), changes in NMDA subunit expression (111)(116)(117), and also post-synaptic protein expression (116). It should be noted that changes in neurotransmission after SR exposure are not limited to the hippocampus or glutamate signaling. SR-induced (20 cGy ^{56}Fe) reductions in the choline RRP has been observed in the nerve terminals of the basal forebrain (110). SR-induced loss of spatial memory performance is associated with changes in the abundance of proteins involved in the regulation of short-term neuronal plasticity, regulation of neurotransmitter transport and G-protein coupled glutamate receptor signaling pathway (118) and neuronal synaptic or structural plasticity (119). Dopamine mediated neurotransmission is altered in the striatum of rats exposed to low (10 cGy) SR doses (120). Deficits in sustained attention induced by exposure to mission-relevant (25 cGy) doses of protons were associated with changes in dopaminergic proteins within the prefrontal cortex (PFC) (16)(121).

In the context of network level “learning” responses, both LTP and long-term depression (LTD) work in unison to allow repeated “consolidation” of memory, by appropriately regulating synaptic strength so that reinforcement is possible (122). This process is referred to as homeostatic synaptic plasticity (HSP), which is a negative feedback mechanism that neurons use to offset excessive excitation or inhibition by adjusting their synaptic strengths. Thus, great care is needed when interpreting studies that look at LTP and LTD in isolation. Thus while exposure to SR results in

LTP decrements in hippocampal (27)(123)(124), and medial prefrontal cortex (mPFC) (27) synapses, and reduces LTD in the PFC (25), the exact functional consequences of such changes is hard to determine. Disruptions to each of these processes can certainly adversely impact both excitatory and inhibitory neurotransmission (113), but without a detailed knowledge of the interplay between LTD and LTP the importance of the observed SR-changes in a single metric (e.g., LTP) may be largely speculative. Similarly, without a detailed knowledge of the relative stage of "experience-dependent plasticity" at the time LTP/LTD assessment are made, ascribing any significance to the observed changes in these metrics may be problematic. The level of experience that mice had with cognitive testing prior to SR exposure had a significant impact of the nature and magnitude of the changes in LTP that occur within the CA1 region of the hippocampus after SR exposure (124). While enhanced cognitive flexibility performance was observed in SR-exposed mice that exhibited elevated levels of LTP (11), it would thus be incorrect to suggest that it was the SR-induced elevation of LTP that was the underlying cause of the enhanced performance. A SR-induced reduction in LTD may have necessitated the enhance LTP for memory consolidation (reinforcement) to occur. While such a statement may seem pedantic, if countermeasures are to be employed to counteract the deleterious impact of SR exposure on the CNS, then it's important that they target the correct component, especially when a system if regulated by a homeostatic feedback mechanism.

Changes in neurotransmission properties within the SR-exposed brain have typically been established at relatively late time points post SR exposure and are thus a snapshot of HSP. Proteomic profiling has revealed that the neurotransmission phenotype is likely to be the net product of multiple changes in the biochemical/molecular composition of the synapses (118)(119). While there have been some attempts to correlate these "static" assessment of synaptic strength and/or neuroproteomic changes in the hippocampus and mPFC to the observed SR-induced decrements in cognitive performance (25)(119)(118), it would appear that while being significantly impacted by SR, changes in synaptic plasticity are not the sole determinant of SR-induced loss of cognitive performance. This may not be that surprising given that both the composition of the synapse, as well as the dendritic architecture (where the synapses are located) are extremely dynamic and responsive to changes in synaptic functionality, and subject to epigenetic regulation (125). SR exposure leads to significant increases in DNA methylation in hippocampus (126)(127)(128). The biological significance of these methylation changes is quite pronounced since SR-induced loss of hippocampal-dependent memory updating and LTP within the hippocampus were reversed by the use of histone deacetylase 3 inhibitors (129).

Dendritic structures/gliosis: Changes in dendritic morphology and numbers are indicative of the changes in synaptic function and can correlate with behavioral outcomes (130)(131)(132)(133)(134). For example, the volume of the spine head (which is directionally proportional to the postsynaptic density area) is indicative of the number of synaptic vesicles that are docked and/or available for neurotransmission. Thin spines orchestrate receiving mnemonic information (135)(136)(137) and their numbers reflect the dendritic reserve available to "learn" a memory. In contrast, mushroom spines provide an idea of stable memory resident in the associated synaptic area (135)(138)(139)(140), and thus is an index of synaptic plasticity in the system. Mushroom spines with their significantly enlarged postsynaptic density, greater number and

reserves of ionotropic glutamate receptors (particularly the AMPA receptors involved in early LTP) play a key role in regulating postsynaptic cell excitability, rapid modulation of synaptic plasticity and thus rapid changes in synaptic strength. The number of mushroom spines, length of the neck, and diameter of the mushroom head, are indices that can be effective in assessment of memory maintenance via pharmacological measures in altered neurological states (141). The greater the preservation of the mushroom spine characteristics, the better the memory reserve.

Within 1 week of SR exposure (50 cGy ^{56}Fe), there are marked changes in overall spine density in the dentate gyrus, with the density of thin spines and stubby spines being decreased and a concomitant increase in the proportion of mushroom spines. Moreover, there were marked reductions in the number of mushroom spines in the basal spines of the CA1 region. SR exposure ($\geq$5 cGy ^{16}O and ^{48}Ti) also results in reduced dendritic complexity and spine density in the mPFC (142)(24)(143). While these studies suggest some correlation between changes in dendritic architecture and OIP performance after SR exposure, it is clear that additional factors determine cognitive performance.

2.2. Looking forward: While it is clear that SR exposure elicits multiple changes in the neurotransmission properties of neurons within multiple brain regions, the studies to date have been "static" in nature. Typically, an assessment of the neuronal properties at single time points in rodents that have not recently been challenged cognitively. SR exposure alters the magnitude and timing of ARC activation in a fear-conditioning model (144). These data strongly suggest that there may be many other SR-induced alterations in neurotransmission properties in a brain under cognitive loading. There is clearly the need to establish the activity of brain circuits in real time while rodents are under cognitive loading using calcium imaging, implanted electrodes or some form of external monitoring (145)(146)(147).

There are important structural and functional enhancements in the human brain function and capacity that cannot be accurately assessed using the preclinical rodent nervous system. SR-induced changes in neurotransmission properties of human neurons could be investigated in organoids derived from human inducible pluripotent stem cells, but such systems can address neural network functionality. Ultimately NHPs will most likely have to be used to validate some aspects, but as mentioned earlier there remains a considerable amount of work that can be done in rodents and pigs, before such an undertaking should be started.

2.3. Summary: SR appears to impact neurotransmission, learning and memory via multitude pathways, ranging from changes in the intrinsic membrane properties of neurons, to pre- and post-synaptic neurotransmission and signaling, to HSP and dendritic morphology and architecture. By inference, the SR exposed brain may have a markedly reduced capacity to store and recall memories, which would explain some of the SR-induced changes in performance in tasks that require some aspect of memory formation and recall and utilization of such memories. However, most studies to date have been very neuron centric. While neurons are obviously a central part of the CNS, neurons don't exist in isolation, and neuronal functionality is highly dependent upon the associated "helper" cells. The impact of SR on the functionality of non-neuronal cells has largely been overlooked to date and needs to be addressed rapidly.

Section 3. Space radiation effects on multi-cellular neurophysiology.

The brain, which is the most functionally complex organ in our body, contains two broad categories of cell types: neuronal and non-neuronal cells. The non-neuronal cells can be sub-categorized based on their morphology and functions, e.g., pericytes, endothelia, glial cells etc. Glial cells, traditionally called "helper cells," are estimated to outnumber of neurons by ~ 10-fold (the exact number varies in different brain regions) and are broadly classified into three diverse types: microglia, astrocytes, and oligodendrocytes.

Historically, neurons have been the principal cell type investigated since they execute and control almost all the brain functions such as recognition, memory, depression, anxiety, etc. Recently glial cells have been found to constituently regulate the excitability of neurons under both physiological and pathological conditions. Microglial activation (neuroinflammation) depending on polarization state is now accepted to be a major driving force that accelerates the pathogenesis of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis (148)(149)(150)(151)(152).

Microglia are the principal resident immune cells of the brain, involved in homeostasis and performing host defense against pathogens and neurological disorders. Following stimulation by external/internal stimuli, microglia undergo morphological changes and/or proliferation, resulting in the production and secretion of a plethora of cytokines, chemokines, and neurotoxic factors. These factors in turn, play critical roles in regulating neuronal activity and function. The functional status of microglia plays decisive roles in determining neuroinflammation levels. Historically, microglia were classified into two different functional states: quiescent and activated. Activated microglia were further divided into M1 (pro-inflammatory) and M2 (anti-inflammatory) types based on the pro- or anti- inflammatory mediators they secrete in response to stimuli. However, recent advances have demonstrated that there is no quiescent state for microglia, even in healthy brains. Microglia are always active, not only screening for neuronal damage, but also actively communicating with both neuronal and non-neuronal cells. In adult brains, microglia closely interact with neurons to modulate neuronal excitability through secreted cytokines and chemokines. Generally, microglia perform three essential functions in the brain: 1) act as environmental sentinels, 2) conduct physiological housekeeping, and 3) protect against various injuries as warriors. Specific pathways mediate the interaction between microglia and neurons including the complement C1, CD200-CD200 receptor, and CX3CR-CX3CL1 axis (153)(154)(155).

3.1. Looking back: 3Several studies have demonstrated that SR-induced behavioral and memory deficits are associated with altered microglial activation. Male B6D2F1 (C57BL/6J × DBA2/J F1) mice irradiated with 50 cGy SR exhibit high activity levels, increased depressive behavior and decreased object recognition compared to sham-irradiated mice (156). In females, but not males, there were increased CD68 levels following irradiation. In males, but not females, there were reduced BDNF levels following irradiation. Other studies have also suggested that female rodents are more resilient to the long-term effects of exposure to SR (157) and simulated GCR (87)(88). Male mice exhibit reduced social interaction and impaired recognition memory after exposure to 50 cGy of SR (87)(88). The behavioral deficits in males were associated with microglia activation

and synaptic alterations in the hippocampus (87)(88). By comparison, no significant behavioral deficits or changes in hippocampus microglial activation were observed in females. A recent study suggests that "radiation resilience" of female mice is highly context specific (158)(9), which is supported by an increasing number of meeting reports that suggest this is not a universal finding. Nevertheless, there are clearly differences in the impact that SR has on the CNS functionality of male and female rodents. This is not surprising given that there is an increasing body of literature that there are intrinsic neurological differences at the cellular and molecular level in men and women (141).

In male mice exposed to SR, changes in microglia activation has been shown to be linked with concomitant loss of spatial, episodic and recognition memory and significant reductions in dendritic complexity, spine density and altered spine morphology in mPFC neurons (24). Animals receiving PLX5622 exhibited no radiation-induced cognitive deficits and near complete loss of IBA-1 and CD68 positive microglia in the mPFC and hippocampus. PLX5622 treatment preserved hippocampal-dependent cognition in mice exposed to He ions (159)(160). However, there is a persistent SR-related loss of phagocytic activity in the microglia that repopulated the brain after the PLX5622 treatment has ceased (159). A similar radiation-related change in microglial function was observed after X-ray exposure, where there was a reduced immune responsiveness to subsequent stimuli (161). It is possible that there may be a similar reduction in immunoresponsiveness of the microglial after SR exposure. These studies have clearly demonstrated that activated glial cells play a critical role in determining the severity of radiation-induced cognitive impairment and raise the possibility of targeting microglia as a countermeasure. However, such a strategy may be limited by the essential role that other glial cells play in maintaining neuronal health and function.

Astrocytes are another type of glia cell that contiguously tile the entire central nervous system and perform many essential complex functions to keep the brain healthy. Under stimulus conditions, astrocytes can be activated to secrete a plethora of cytokines, chemokines (astrogliosis) to contribute to neuroinflammation levels. Generally, microglia are believed to be the initiators of neuroinflammation while astrocytes act as amplifiers to exaggerate the existing immune response. In addition to interacting with microglia, astrocytes inherently regulate neuronal functions by direct contact (162)(163). Recent understanding of the reciprocal interactions between neurons and astrocytes have led to the concept of "tripartite synapse" (two neurons and one astrocyte acting as a functional unit). Within this kind of synapse, astrocytes secrete various transmitters including glutamate that target both pre- and post- synaptic sites, thereby modulating the structure and function of both excitatory and inhibitory synapses. On the other hand, the neurotransmitters released from neurons also bind receptors on the adjacent astrocyte process, activating signaling pathways in the astrocytes which modulate synaptic behavior. Astrocytes play a critical role in regulating glucose metabolism and energy supply to neurons (164)(165)(166). Exposure to carbon and iron ions reduces glutamate transporter activity in astrocytes within 7 days (167).

Oligodendrocytes (OLs) are myelinating glia cells, which are fundamental in maintaining connectivity and function in the adult brain. OLs predominantly, but not exclusively, locate in brain white matter which is comprised of axons. OLs enwrap a large subset of axons with myelin,

a highly organized multi- lamellar membrane structure that allows for long-distance fast excitatory impulse propagation, axonal growth and long-term axon integrity. In the CNS, OLs are derived from oligodendrocyte progenitor cells (OPCs) which are present in mature brains, constituting ~5% of total neural cells, and retain the capacity for self-proliferation throughout life. The main functions of OPCs and OL includes trophic support of ensheathed axons, formation of myelin, ionic homeostasis, synaptic transmission, brain energy metabolism, and learning and memory. Oligodendrocytes are essential for providing metabolic support to neurons, rapidly transferring (through cytoplasmic "myelinic" channels and monocarboxylate transporters) short-carbon-chain energy metabolites like pyruvate and lactate to neurons (72). SR exposure leads to significant changes in the percentage of myelinated axons, suggesting that OL function is significantly impacted by SR exposure (168). It seems unlikely that SR would only impact the myelination functions of the OLs and not the important neuronal homeostatic functions. From an operational perspective, as noted earlier, microgravity (both simulated and in-flight) result in persistent changes in the mitochondrial function and lipid metabolism of human oligodendrocytes (70)(71). While such microgravity induced metabolic perturbations are likely to be deleterious to neuronal function in their own right but will likely exacerbate SR-induced changes in neuronal functionality.

Neurogenesis and neurological stem cell niche: Adult neurogenesis is critical for learning and memory, with the subventricular zone and DG being the two main areas of persistent neurogenesis in the adult brain (169)(170)(171)(172). Exposure to a variety of SR particles led to significant changes in the neurogenesis (6)(173)(174)(175)(176)(177)(10)(178). In some instances where both behavior/cognition and neurogenesis were studied following mission-relevant SR doses, in some instances there was a concomitant SR-induced changes in both parameters (6)(176)(175)(10), while in others there was not (6)(174). This may be very dependent upon the nature of the cognitive task studied, and the relative importance of new neurons to consolidate performance memory in the task. As Whoolery and colleagues (6) recently pointed out "decreased hippocampal neurogenesis is proposed to diminish sensitivity to memory interference and thus improve performance in certain memory tasks (179)(180). Computational models support that decreased neurogenesis may enhance sparse encoding (181)(182)".

3.2. Looking forward: Clearly, the importance of neurogenesis as a determinant of cognitive performance may be very complex and highly context dependent. Ongoing research efforts will ultimately provide a better indication of the exact importance of SR-induced changes in neurogenesis in tasks that are considered to be of operational significance by NASA. However, it may now be judicial to assess the importance of neurogenesis from an alternate perspective, i.e., how SR impacts brain stem cells. Adult neural stem cells are sensitive to SR, with quiescent stem cells paradoxically having increased sensitivity compared to their actively proliferating counterparts (183). SR exposure leads to persistent (5-8 weeks) oxidative stress in neural stem cells (184). X-ray exposure results in neural precursor-cell dysfunction (185), reducing neurogenesis, but not the intrinsic capacity of stem cells to differentiate into mature neurons. These data suggest that the microenvironment surrounding the neural stem cells has become non-permissive for neurogenesis. Moreover, while the differentiation capacity of the irradiated precursor did not differ from that seen in shams, the differentiation of the irradiated precursors are not normal, suggesting an aberrant dysregulation of the differentiation process (185). At present,

it is unclear if SR exposure results in a similar non-permissive environment. The exact basis for the non-permissive environment for precursor proliferation/differentiation in the irradiated brain is unknown, but the multiple proteomic/metabolic changes noted earlier in this review (and expanded upon in Section 5) in multiple neuronal cell types would almost certainly play some role. Targeting such "micro-environmental" targets may result in an increased amelioration of the SR-induced cognitive deficits, either by increasing proliferation of the neural stem cells, increasing the functionality of the various cell types in the recovering brain, or by improving the functionality of neural networks.

3.3. Summary: The brain is a very complex organ whose functionality is regulated by multiple processes interacting in a highly coordinated fashion. Up until now, CNS cognitive defects arising from SR exposure have primarily been conceptualized as arising due to SR impacting neuronal cell performance. However, there is an increasing body of evidence that SR has profound and deleterious effects on the functions of microglia, astrocytes, and OLs, key cell types that regulate and maintain neuronal function. It is thus imperative that future studies aimed at resolving the mechanistic basis for SR-induced neurocognitive impairment cease to be solely neuron-centric and conceptualize that the "target" cell could be in fact be any one of the component cells within the brain, and most likely more than one cell type. It also seems likely that SR-induced cognitive impairments arise (in part) through a disruption of the reciprocal interactions between neuronal and non-neuronal cells. Considering the brain as a system has advanced neuroscience knowledge considerably and adopting a similar approach to investigating SR effects seems to be the next logical step.

Section 4. Neural network functionality may be the dominant factor in determining the severity of SR-induced cognitive impairment

4.1. Looking back: The historical approach to understanding cognition focused on individual brain regions and their roles in specific cognitive functions. This approach has been used in much of the work seeking to determine the effects of SR on the brain and its relevance for cognitive performance. In many cases studies have been conducted using highly defined cognitive tasks that have been designed to interrogate the functionality of a specific brain region. As alluded to earlier in this review while such defined tasks are very useful to interrogate the impact of SR exposure on specific brain regions, the complex tasks that are likely to have a higher operational significance require the effective integration of outputs from multiple brain regions.

SR-induced deficits in advanced executive functions could arise due to SR impacting: 1. loss of neurons; 2.) loss or reduced functionality of individual neurons; 3. loss or compromised coordination within neural networks in the brain that regulate specific tasks; or 4. a combination of any or all three. Previous portions of this review have highlighted the impact that SR exposure has on neurochemical/neurotransmitter systems at the single neuron or brain region level. Neuroimaging studies have demonstrated that spatially distributed brain regions are interconnected into functionally linked neural networks (186)(187)(188)(189)(190). These neural networks can be quite specific to cognitive domains and even psychological status (191)(192). Fully understanding the impact of SR, and its impact on astronaut performance, will require directly assessing its effect on neural network integrity and functioning. To date there has been little to no

research conducted on SR-induced physical changes on the functioning of large-scale neural networks which work in concert to support cognitive functions (188). SR (He) exposure results in a decrease in the functional connectivity between the hippocampus and perirhinal cortex (115), and activity within hippocampal neural networks (193). There seems little reason to suspect that this specific connection would be the only one impacted by SR.

While a variety of distinct functional networks have been proposed and described with different terminology (194), the major networks conceptually important for understanding the effects of SR on astronaut cognitive performance include a central executive network (CEN), a social brain/default mode network (DMN), and a salience/emotion processing network (SEN).

The CEN is comprised primarily of prefrontal cortex, especially the dorsolateral prefrontal cortex and lateral posterior parietal cortex (195). It is important for many higher order executive functions that require consciously directed and focused attention (196) including working memory, planning, and decision-making (197)(198). The DMN includes the medial prefrontal cortex and posterior cingulate cortex and is considered a "task-negative" network because it becomes deactivated during most cognitively demanding tasks (188)(199)(200). However, it is activated during tasks that require theory of mind and/or self-referential processing (188)(199)(200). The SEN includes subcortical structures, such as the amygdala and thalamus, as well as the insula and anterior cingulate cortex and acts to detect, and direct attention to, salient external stimuli (188)(195). It also plays a role in switching between "task-positive" networks like the CEN and "task-negative" networks like the DMN in order to mediate attention between external and internal events (197). Regions within neural networks, known as connector hubs, are widely connected to several other regions within and/or outside the network and are believed to be crucial for information transfer within networks and for communication between networks (194)(201).

4.2. Looking forward: Activity within and between networks offers a way of conceptualizing and understanding cognitive dysfunction. For example, the triple network model posits that the disordered coupling among the DMN, SEN and CEN is responsible for the cognitive impairment in many brain disorders (189). Interconnectivity between networks mediate monitoring and reciprocal influences of the internal mental environment (DMN), relevant interoceptive, autonomic, and emotional information (SEN), and higher-order cognitive function and attention control (CEN) (189). Disruption of intrinsic connectivity within and between these networks could be a core mechanism of cognitive impairment (189). Understanding the effects of SR on connector hubs may be particularly important as dysfunction in these hubs has been implicated in behavioral and cognitive impairments in several neurological and psychiatric disorders and they show greater abnormalities than non-hub regions in most brain disorders (201).

Coordinated communication amongst distributed and functionally specialized brain regions is necessary to produce coherently guided behavior and cognition (202). This communication is linked to the relationships among specific frequencies of oscillatory activity (202). Oscillatory activity in EEG recordings can reflect communication across cortical regions (203) and recordings of local field potentials (LFPs) reflect the common action of multiple neurons within individual brain regions (202). Measuring these oscillations is considered a way to measure orchestrated communication within neural networks (204). These oscillations can be impacted by neuronal

connectivity patterns, cellular membrane properties, intrinsic circuitry, speed of axonal conduction and synaptic delays (196)(197)(205)(198), and thus are subject to disruption at several levels.

Specific frequency bandwidths (e.g., delta, 1-4 Hz; theta, 4-8 Hz; alpha, 8-15 Hz; beta, 16-31 Hz; gamma, > 32 Hz (typically 40Hz, but may be 20-80 Hz)) are associated with states of sleep, arousal and attention, and also have been linked to memory formation, cognitive performance, and transfer of information and perception within the brain. Of these, theta and gamma, and their interrelationships, have received particular interest regarding their roles in communication between neural regions.

The large-scale theta oscillation detected in the EEG is primarily produced in the hippocampus (206) and theta frequency power increases in conjunction with a wide range of behaviors (reviewed in (207)). Theta frequency oscillations have been proposed to support large-scale coordination of subsystems, including the hippocampus and prefrontal cortex, serving in the formation and recall of memories and synaptic plasticity within subsystems (208)(204)(207). Functionally, the hippocampal theta oscillation is associated with the induction of LTP, a phenomenon associated with learning and memory (209)(210). Computational modeling of the impact that proton irradiation would have on the CA1 microcircuit (based upon the known SR-induced changes in neuronal membrane properties) predicts that the power of theta oscillation in pyramidal cell firing was reduced to 50.6% of that seen in unirradiated brains (114).

Gamma synchronization was first implicated in perceptual binding in the visual cortex (211)(212). However, it has been found in many cortical and subcortical areas (195), is induced by different stimuli or tasks, and has been suggested to be generally important in sensory binding and even in multisensory integration (207). It also is related to several cognitive functions including focused attention and efficient cognitive processing and it has been suggested to be a fundamental process that underlies cortical computation (213). It appears to be important in the hippocampus for memory formation (214). In the hippocampus, gamma oscillations exhibit their largest amplitude when they are nested within slower theta oscillations (215)(216)(217) and occur in bursts at particular times within the theta cycle during active behaviors (215)(186)(217)(218)(219). Gamma oscillations have also been associated with other frequency bands. For example, during a hand movement, high frequency gamma oscillations in the frontal cortex are correlated with low frequency beta oscillations in the parietal cortex, supporting the hypothesis that communication among neural regions does not require synchronized activity at the same frequency (214).

Oscillations of different frequencies can coexist and may be synchronized to each other or nested within each other (220). Cross-frequency coupling may enable communication between regions, e.g., it has been proposed that the magnitude of gamma oscillation is modulated by slower rhythms which may act to couple active patches that are separated within cortical circuits (195). Together, individual oscillations and their interactions with other frequencies provide a way to assess the effects of SR on functional communication within neural circuits. They may be particularly useful for assessing SR-induced impairments in functional systems at the neurocircuit/neural network level.

Sleep as a mediator of network functionality: Sleep consists of two basic states: non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep, also known as slow wave sleep and paradoxical sleep, respectively. NREM sleep is characterized by high-amplitude, low-frequency (e.g., delta) waves recordings in the encephalogram (EEG). REM sleep is characterized by an EEG with low amplitude, higher frequency activity similar to that seen in wakefulness at the same time that there is a nearly complete loss of voluntary muscle tone.

Sleep has long been implicated in learning and memory and cognitive performance, including facilitating underlying neural activity and communication between brain regions. Support for a role for sleep has come from studies in humans and animals using a variety of learning tasks. Conclusions from these studies have often been based on correlations between sleep amounts and subsequent behavioral and performance indices of learning and memory formation. However, there also has been contradictory evidence across studies that may result from differences in learning paradigms, behavioral measures, as well as individual differences in learning and/or in the effects of training and testing. Conclusions can also be impacted by the fact that memories can alter sleep, indicating potential reciprocal influences between sleep and learning systems that have not often been considered. Thus, even after decades of work, questions remain as to whether sleep subserves fundamental learning and memory mechanisms across learning types or is limited to certain types of learning and memory.

Sleep disturbances have been linked to reduced neurocognitive functioning in humans and animals (50)(51)(52) as well as to a variety of physical health problems. Sleep deprivation has a major impact upon performance in multiple cognitive domains (53), including the psychomotor vigilance test (PVT) (54). Even a single night of very mild sleep restriction (2 h reduction) can negatively impact vigilance and impact cortical indices of motor preparation and execution (56). There can also be neural effects such that even mild sleep restriction may interfere with the increase in neurogenesis that normally occurs with hippocampus-dependent learning (221). Sleep fragmentation, which can arise from stress (222) and occur without a reduction in overall sleep time, produces deficits in ATSET performance (51) and spatial learning (55). It is thus likely that neurocognitive functioning will be reduced in astronauts who have disturbed sleep. Both NREM and REM have putative, and differing, roles important for cognitive function, and it will be important to understand how SR affects each state and their interactions.

NREM is thought to be important for mediating activity within phase-locked hippocampal–cortical loops involved with the consolidation and retrieval of memories (223). This mediation involves high-frequency hippocampal sharp waves/ripples acting in concert with thalamocortical spindles and slow waves to promote neural plasticity underlying memory formation. Specifically, during slow wave sleep, neural activity resembling that during learning is reactivated within hippocampal networks, resulting in sharp wave-ripple activity (224)(200). These sharp wave ripples are short duration (100–200 ms), high frequency (150–250 Hz), hippocampal LFPs that occur during slow-wave sleep or immobile waking, and they occur in conjunction with the simultaneous activation of large populations of hippocampus neurons and cortical ensembles (187)(194)). NREM spindle waves are oscillatory patterns in the ~11-15 Hz frequency range that last ~1–4 s and occur across thalamic and neocortical areas (225); they have been found to increase in density after learning

(202)(211)(212). The depolarizing "up-states" of the slow oscillations (<1 Hz) are hypothesized to synchronize ripple activity and thalamocortical spindles (199)(201). These NREM sleep oscillations are thought to support long-term memory by coordinating the gradual relocation of short-term memory traces from the hippocampus to longer-term storage in the neocortex (187)(226)(199)(227). Slow wave activity during sleep is also important for the "synaptic homeostasis hypothesis" (228) where it has been proposed to reflect a global synaptic downscaling process aimed at the desaturation of synapses, to allow new subsequent learning. In this hypothesis, strongly activated memory traces during wakefulness would be enhanced indirectly by being spared from the process of global synaptic depression.

In REM sleep, neurons in the hippocampus are thought to change from a firing pattern that supports long-term potentiation to one that supports depotentiation (229), a process that putatively "resets" the hippocampus after memories have been transferred to the frontal cortex and clears the way for the formation of future memories. Local recordings have also found theta oscillations in the basolateral nucleus of the amygdala (BLA) (230) and medial prefrontal cortex (mPFC) (231)(232) during REM (233)(234), and we have found coordinated activity in BLA, mPFC and hippocampus in studies of fear memory. Theta-gamma coupling in the hippocampus occurs during REM sleep as well as during behavioral and learning tasks (235), and regional variations of coupling during tonic and phasic REM sleep may be differentially important for memory processing and consolidation (236). Earlier collaborative work in the Britten and Sanford labs found that exposure to 20 cGy 1 GeV/n ^{56}Fe HZE irradiation led to a marked reduction of peak magnitude theta oscillations during REM sleep. Given evidence that theta oscillations are involved in coordination among neural systems, SR-induced reduction of theta oscillations during REM could be detrimental to the overall neurocognitive performance of astronauts.

It has been argued that the role of sleep in memory consolidation is important for the "discovery and clarification of complex rules within one's environment" and "forming and strengthening associations within memory networks" (237). If true, understanding the role of sleep will be critical for understanding alterations of communication within neural networks and their impact on SR mediated cognitive dysfunction. Targets for NREM could include SR-induced alterations in sharp wave-ripples, thalamocortical spindles and slow waves whereas targets in REM could include alterations in theta oscillations, and/or theta-gamma coupling. Such studies could provide critical data regarding how SR could potentially interfere with the processing of learning and formation of new memories resulting in SR-exposed astronauts having difficulties in learning new tasks or making refinements in existing cognitive skills.

Lastly, data from studies of network activity across sleep states lend themselves readily to modeling efforts, both at the level of communication between discrete brain regions and at the level of broader, interacting circuits within the brain in real world situations. For example, our current NASA funded work is focused on identifying stress, learning and cognition related changes in network communication (238) and the data obtained can be incorporated into sophisticated models that include multiple interacting systems and real world contexts (239). They thus would have the potential to be incorporated into models of mission related scenarios. Such efforts could inform the development of sleep and EEG indices that could be monitored to assess the

effectiveness of countermeasures and/or predict performance decrements of astronauts during missions.

4.3. Summary: Focused network-level questions regarding altered neural communication and cognitive performance would be useful for understanding and modeling SR induced physical changes in the brain and their associated functional deficits. Because successful cognitive performance requires integration of information from multiple interacting systems, functional studies need to assess periods when tasks are being acquired and performed and periods when memories that will facilitate future performance are being consolidated. This would include determining SR effects on sleep states and related alterations in coordinated activity between brain regions critical for cognitive performance.

Section 5. Biochemical basis for SR-effects on CNS functionality.

5.1. Looking back: Over the last 30 years there has been a progressive increase in our knowledge of the impact that SR exposure has on the CNS, initially establishing phenotypic effects then gradually transitioning to investigations at the process (e.g., neurotransmission properties, neuroinflammatory responses) or at the network (e.g., neural or gut-brain axis) levels. As summarized in previous sections, there is an increasing body of evidence that SR exposure (at least in ground based rodent systems) results in a number of changes in CNS functionality that could impact astronaut performance on deep space missions. In addition, there are several studies that have demonstrated that other flight stressors such as microgravity, social isolation, inadequate sleep and stress also profoundly alter the functionality of the CNS.

While considerable effort is still required to determine the translatability of the ground-based studies to astronauts, it would be prudent to start considering developing strategies to avoid, reduce or ameliorate SR-induced changes in CNS functionality. The development of effective countermeasures will require a solid understanding of the underlying molecular mechanisms and systemic effects that are responsible for the neurocognitive loss. However, currently there are some major gaps in our knowledge of the mechanistic basis for these observed effects that could severely hamper the development of effective countermeasures.

At the simplest level, the critical target cell for the SR-induced neurocognitive loss remains to be determined. For obvious reasons, the neurons have received the highest level of attention, but as outlined in previous chapters neuronal functionality is dependent upon a host of other cells. Whether DNA damage in any cell type in the brain plays a role in triggering these cognitive deficits remains unknown. Conceptually, rejection of a DNA-centric explanation for CNS defects is a marked deviation from the prevailing radiobiological principles that have been used to explain many clinical responses (tumor and normal tissue) responses invoked after radiation exposure. While this may be highly pertinent for SR-induced carcinogenesis, at present there is little data to support DNA damage as being the most critical factor in the development of CNS deficits. Indeed, when the LET dependency of SR-induced deficits in NOR performance was investigated, the best fit for the data was a combination of non-targeted effects as well as targeted effects (240). It is thus germane to consider the nature of such non-targeted (non-DNA) effects.

Ultimately the observed SR-induced changes in CNS functionality stem from SR-induced changes in some aspect of cellular physiology, which in turn most likely arises from transient or persistent changes in the biochemical composition of the cells within the CNS. Investigation of this apparently fundamental issue has surprisingly been limited in the past, primarily because until recently there was limited information on the extent that neurocognitive processes are impacted by SR. The identification of several cognitive and behavior processes that are impacted by SR exposure has at least given some indication on what regions of the brain should be investigated in detail.

As outlined in previous sections, there have been several studies that have reported SR-induced changes in multiple aspects of neurotransmission, primarily in the hippocampus but also in the cortex. The underlying reasons for these changes remain largely unknown, although there have been some recent studies that have started to identify some of the "biochemical" changes that occur in brain tissue that have been exposed to SR. It is important to note that most studies to date have been macroscopic in nature, i.e., assessing a homogenate of all cells within the brain region investigated.

The advent of high throughput quantitative transcriptomic (RNA transcript levels) and proteomic (protein levels) techniques capable of measuring thousands of "gene products" in the same biological sample allows for much more comprehensive evaluations of the SR-induced changes in cellular biochemistry. Omics technologies have now been expanded to include areas such as genomics, transcriptomics, proteomics, metabolomics, lipidomics, and epigenomics, among others. In terms of exogenous exposures such as SR they can be collectively classified as exposomics.

Proteomic profiling has revealed that SR-induced changes in neurotransmission phenotype maybe related to complex changes in the biochemical/molecular composition of the hippocampus (119)(118). An interesting feature of these hippocampus proteomic profiling studies is the marked loss (>25%) of proteins within the hippocampal proteome three months after SR exposure compared to that observed in sham rats (119)(118). This observation is likely to be related to the SR-induced changes in the DNA methylation status within the hippocampus (241)(128)(127)(242). Such changes in DNA methylation occur after exposure to SR ions ranging from protons to ^{56}Fe, and in some cases there are very similar changes identified in brain and the heart (242). The biological significance of these methylation changes is quite pronounced since SR-induced loss of hippocampal-dependent memory updating and LTP within the hippocampus were reversed by the use of histone deacetylase 3 inhibitors (129). However, SR-induced changes in the hippocampal proteome were found to be complex; concomitant with the SR-induced loss of proteins, there were significant up-regulation of proteins involved with neuronal homeostasis, axonogenesis, pre-synaptic membrane organization, G-protein coupled receptors oxidative damage response, calcium transport and signaling. Proteomic changes that had high importance to neurotransmission were a major focus in these studies, but the proteomic profiling was conducted on both neuronal and non-neuronal cells, and closer inspection of the data revels that SR-exposure is impacting non-neuronal cell types. Recently, a highly targeted proteomic screening study demonstrated that exposure to a mixture of gamma-rays and ^{12}C nuclei alters the Glutamate/GABA

balance in the cortex by altering several aspects of the metabolism of those neurotransmitters (243).

Protein homeostasis is maintained by several mechanisms in addition to regulating transcriptional activity, and SR exposure results in the upregulation of the proteasome/protein degradation pathway (119). and marked changes in ubiquitination (244). In addition to SR-induced changes in protein synthesis and degradation, SR exposure may also alter the composition of the neuroproteome by changing protein clearance from the brain. Recent conference presentations by Dr. O'Banion (at ICRR 2019 and HRP-IWS 2021) strongly suggest that blood-brain barrier (BBB) transport of amyloid-β (Aβ) mediated by low-density lipoprotein (LDL) receptor-related protein-1 (LRP1) is reduced following SR exposure. Irrespective of the exact mechanism whereby SR exposure alters the protein composition of the brain, it is clear that that there are significant SR-induced changes in multiple protein pathways.

SR exposure does not only alter the neuroproteome, exposure to ^{12}C ions results in marked changes in lipid metabolism in irradiated rat brains (156)(245). In a recent study, SR exposure was shown to induced changes in fatty acids as well as metabolome and mitochondrial dysfunction in the spleen (246) and liver (247)(248), showing common themes with spaceflight related perturbations (249). Interrogation of the SR-induced mitochondrial dysfunction changes in the liver (228)(229) has already led to the conceptual development of several unique approaches that could serve as countermeasure against SR-induced damage (248)(250). It is logical to conceive that SR induction of mitochondrial dysfunction will be prevalent in other organs that have high abundance of mitochondria (i.e., brain and heart). As more studies emerge investigating SR-induced changes in lipidomic and metabolic profiles of the brain, it is likely that the complexity and enormity of the SR-induced changes in the CNS function will be even greater than is presently known. The utilization of an integrated metabolomics-DNA methylation analysis of SR-exposed hippocampus (and heart) has already revealed many more pathways impacted by SR than was suggested by conventional metabolomic studies (242). The aminoacyl-tRNA biosynthesis pathway, which plays a key role in protein synthesis including the accuracy of translation was significantly altered by proton irradiation in the hippocampus and left ventricle (242). While metabolic changes can alter neuronal functionality at the cellular level by altering energy availability, neurotransmitter synthesis and breakdown, the metabolome also has a marked impact on the functionality of neural networks (at least in the cortex) (197).

SR is not the only flight stressor to impact the biochemical status of the CNS. Simulated microgravity results in persistent changes in the mitochondrial function and lipid metabolism of human oligodendrocytes (70), and marked changes in multiple chaperon proteins in neuronal SH-SY5Y cells (251). Similar changes were observed is astrocytes that have been were flown to ISS and back (71). Importantly, proteomic analysis of the mid- and hindbrain from mice flown in space has identified dysregulation of pathways involved with neurovascular integrity, mitochondria, neuronal structure, metabolism, protein/organelle transport, adhesion, and molecular remodeling (252).

Aside from the impact these changes may have on neuronal function (either directly or through the helper cells), these SR-induced changes in the biochemical composition of the CNS may be

responsible for the non-permissive environment for neural stem cell proliferation (167), or the reduced immunoresponsive [142] or phagocytic activity (148) of glia cells. Thus, the omics changes observed after exposure to a single space flight stressor may be a fraction of the changes that might occur when exposed to the combination of stressors in real space flight.

5.2. Looking forward:

In recent years, significant strides in systems biology approaches and computing power have provided integration of endpoints for the generation of networks and understanding of underlying changes. By linking together transcriptomic, proteomic, lipidomic and metabolomic data sets among others in a systems biology approach, biochemical pathways involved in specific biological processes (carcinogenesis, cognition, disease, etc.) can be readily identified (242). When a portion of the system is perturbed (e.g., changes in lipids), it causes a ripple effect that is reflected in the other linked subsystems. It is the linked nature of the entire biological system that helps to magnify a slight change in one subsystem that might be overlooked in a narrow study. The unique ability of the system biological approach to construct biological pathways from a perturbed system allows comparison of parallel pathways which often leads to identification of novel mechanistic targets. These novel targets often become therapeutic targets or biomarkers, but they can also be exploited for the intelligent design of subsequent experiments or may be used to focus further analysis of the existing data sets to identify low level components in the microenvironment to aid in determination of specific biological mechanisms involved.

Due to logistical constraints the majority of studies have assessed changes in the biochemistry of the CNS at a distant time from the SR exposure, typically after cognitive testing has been completed. While such “snapshot” assessments are critical in identifying biochemical changes associated with a phenotypic endpoint, these snapshots represent the final status quo of the metabolic changes. The hippocampal proteomic profiling studies suggest that there may be compensatory responses being invoked to counteract at least some of the impact of the SR-induced loss of proteins (118,119). Whether the loss of proteins precedes the compensatory response, or whether these changes occur contemporaneously is complete speculation at present, but it is vital to know the etiology of such changes if effective countermeasures are to be developed.

Conceptually, countermeasures are more likely to be effective if they are administered closer to the SR exposure, before the compounding adjustments (ripple effect) have taken place. There is thus a great need to do some detailed longitudinal studies to identify early biochemical changes that result in adverse CNS effects. The use of readily obtainable (blood or CSF) “biomarkers” would be vital for such a process. There have been some serum biomarkers of SR exposure already identified (245)(253), but clearly now that critically important cognitive tasks have been identified that are sensitive to SR exposure there needs to be a concerted effort to identify biomarkers related to performance in those tasks.

While animal studies are more informative as they allow for correlating specific changes to behavioral testing, they currently have limitations due to low animal numbers that may provide limited power analysis for -omics studies, assessment of select time points, and have historically not included both sexes. Considerable efforts should also be put in place to thoroughly archive

samples and/or derived omics datasets from the multiple investigators and be available upon request through a centralized comprehensive database to maximize the experimental data output. This will allow for the comparison across similar experiments, ensure reproducibility of results, and serve to validate which omics technologies are translatable across species or allow for the increased validity of usage of organs-on-a-chip models to perform experiments that are not feasible due to ethical concerns. Significant efforts are already in place to enhance GeneLab and the Ames Life Sciences Data Archive (ALSDA), however linking to human data remains and will remain a barrier due to anonymity concerns, as the astronaut cohort still remains small.

To date, most studies have generally focused on whole tissue assessment, e.g., crude extracts of whole brain, typically only from selected brain regions, most frequently the hippocampus. Clearly as discussed previously cognitive performance in complex executive functions requires input from multiple brain regions. Thus, future studies need to interrogate omics changes in multiple brain regions (hippocampus, amygdala, striatum, etc.,) within the same animal. In addition, given the multi-cellular basis of efficient CNS functionality, such studies may need to be conducted within individual cell types (e.g., astrocytes, oligodendrocytes, microglia). Techniques already exist that allow for reproducible quantitative transcriptomic analysis in individual cells, but the advent of molecular barcodes opens up the prospect of higher throughputs, and importantly the ability to micro-dissect specific cell types from tissue slices. While many companies are trying to develop similar techniques for single cell based proteomic analysis, at the time of writing this is unlikely to be readily available for some time. However, single cell metabolomic and lipidomic analyses however are showing potential in neurobiology (254) and should be considered for future SR research studies. Understanding the integrity and composition of individual organelles (mitochondria, endoplasmic reticulum, etc.,) after SR exposure may ultimately be necessary to fully determine the mechanistic bases of SR-induced cognitive impairment and design targeted countermeasures. Integrated analysis of the findings from other cellular components, such as lysosomes and peroxisomes, could also provide a phenotypic comprehensive understanding of the plasticity of CNS components to SR. Thus in the future study designs that focus on dissecting the responses in individual brain regions and furthermore in individual cell types with single cell analysis (249)(255)(256) may be a highly profitable approach.

The recent progress on development of brain-on-a-chip platforms should provide expanded opportunities to study SR-induced intracellular changes, cellular integrity, and measurement of secreted factors with signaling properties. As mentioned earlier, SR exposure appears to lead to changes in the integrity of the blood brain barrier (BBB). Clearly, such effects could have marked impacts upon CNS function and has yet to receive a lot of attention. The use of the blood-brain barrier engineered microfluidic chip that has been assessed in orbit provides another unique opportunity to determine the impact that multiple flights stressors have on BBB integrity. Another key area for future studies in the role that extracellular vesicles (257) may play in regulating the severity of SR-induced CNS impairments. Such vesicles contain components of cells including lipids, metabolites, mRNAs and miRNAs, among others. This exciting and not yet fully understood area could be used to expand on investigations in intercellular communication and potentially as a countermeasure method (258). Such an approach has already been shown to be effective in rodent

models using Extracellular Vesicle–Derived miR-124 to ameliorate gamma-ray induced loss of cognitive function (259).

Finally, systems biology approaches could be employed to better understand the gut-brain axis connection (260). Shifts in microbiomic composition have been documented after SR exposure (261)(262), however it remains unclear whether these changes will be persistent or what systemic effects will be a consequence of such changes. Peripheral intestinal functions and dysbiosis however have been linked to changes in emotional and cognitive centers of the brain (263), in part through generation of bacterial metabolites and production, expression, and turnover of neurotransmitters. This is an exciting area of research and should be investigated further in the future to provide targets for mitigation.

5.3. Summary: Establishing the underlying mechanistic basis for SR-induced cognitive impairment is in its relatively early stages. However, now that investigators have identified cognitive processes (and thus brain regions) that are impacted by SR exposure it will be possible to apply some of the emerging analytic and data mining techniques to rapidly make major advances in understanding the biochemical basis of SR effects on the CNS.

6. Overall conclusions: This review has summarized the outstanding research that has been conducted over the last 30 years assessing the impact of SR (and other flight stressors) on the functionality of the CNS. In addition, the authors have outlined some of the newer concepts regarding the highly complex mechanisms that underpin the efficient functionality of the CNS, and some of those factors that appear to be altered following exposure to SR (and other flight stressors). The authors hope that other investigators will utilize this material and, like the Sankofa bird, utilize the information to reconceptualize how best to address how the deleterious effects of space flight stressors can be avoided or at least ameliorated in the astronauts who will be venturing into the hostile deep space environment.

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