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Review article

Nonhuman primate models in the study of spaceflight stressors: Past contributions and future directions

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ABSTRACT

Studies in rodents suggest that exposure to distinct spaceflight stressors (e.g., space radiation, isolation/confinement, microgravity) may have a profound impact on an astronaut's ability to perform both simple and complex tasks related to neurocognitive performance, central nervous system (CNS) and vestibular/sensorimotor function. However, limited information is currently available on how combined exposure to the spaceflight stressors will impact CNS-related neurocognitive and neurobiological function in-flight and, as well, terrestrial risk of manifesting neurodegenerative conditions when astronauts return to earth. This information gap has significantly hindered our ability to realistically estimate spaceflight hazard risk to the CNS associated with deep space exploration. Notwithstanding a significant body of work with rodents, there have been very few direct investigations of the impact of these spaceflight stressors in combination and, to our knowledge, no such investigations using nonhuman primate (NHP) animal models. In view of the widely-recognized translational value of NHP data in advancing biomedical discoveries, this research deficiency limits our understanding regarding the impact of individual and combined spaceflight stressors on CNS-related neurobiological function. In this review, we address this knowledge gap by conducting a systematic and comprehensive evaluation of existing research on the impact of exposure to spaceflight stressors on NHP CNS-related function. This review is structured to: a) provide an overarching view of the past contributions of NHPs to spaceflight research as well as the strengths, limitations, and translational value of NHP research in its own right and within the existing context of NASA-relevant rodent research; b) highlight specific conclusions based on the published literature and areas needed for future endeavors; c) describe critical research gaps and priorities in NHP research to facilitate NASA's efforts to bridge the key knowledge gaps that currently exist in translating rodent data to humans; and d) provide a roadmap of recommendations for NASA regarding the availability, validity, strengths, and limitations of various NHP models for future targeted research.

1. Introduction

Given the unique challenges encountered by astronauts during long-duration deep space exploration (i.e., a mission to Mars), NASA's Human Research Program (HRP) seeks to minimize risks to crew health and behavioral performance that may be impacted by combined exposure to multiple spaceflight stressors, for example, space radiation, microgravity, and isolation/confinement, among others. As part of the Integration Research Plan, NASA is deeply concerned about three main risks: 1) acute in-flight risk to the central nervous system (CNS) following exposure to space radiation; 2) risk of adverse cognitive and behavioral conditions and psychiatric disorders (BMed); and 3) risk of impaired control of spacecraft/associated systems and decreased mobility due to vestibular/sensorimotor alterations associated with spaceflight. Under this integrated plan, NASA is seeking to better understand, analyze, and quantify the impact of simultaneous exposure to multiple spaceflight stressors on crew health and performance.

Research in laboratory rodents has identified that space relevant radiation exposures can produce profound and long-lasting changes in brain function, including neuroepigenetic, neurobiological, behavioral, and cognitive deficits (e.g., Acharya et al., 2017; Britten et al., 2017; Cherry et al., 2012; Davis et al., 2014; 2015; Hadley et al., 2016; Lee et al., 2017; Machida et al., 2010; Marty et al., 2014; Parihar et al., 2015; 2016; Rabin et al., 2014; Sanchez et al., 2010). These data underscore

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the potential risks for astronauts manifesting performance decrements in-flight during deep space missions and neurodegenerative conditions upon return to the terrestrial environment. In addition to space radiation, evidence also suggests that other spaceflight environmental stressors such as microgravity and isolation/confinement may also harm crew health and performance (e.g., Morey-Holton and Globus, 2002; Morey-Holton et al., 2005; Yumoto et al., 2010). While this research has led to significant advances in our understanding of how spaceflight stressors independently impact astronaut's health and performance during long-duration deep space missions, extrapolating current data sets from rodents to humans is an imperfect science. Moreover, very little information is currently available on how a *combination* of these three major spaceflight stressors will impact crew health and behavioral performance. Thus, there is an urgent need for further research to systematically identify the acute and long-term CNS consequences of exposure to multiple spaceflight stressors that is representative of what will be encountered by astronauts during deep space travel, for example, a mixed Galactic Cosmic Radiation (GCR) exposure, microgravity, isolation/confinement, and other environmental stressors. This information gap has hindered NASA's ability to more accurately estimate spaceflight hazard risk to the CNS associated with human space exploration and, consequently, may slow the advancement of future missions.

Despite the progress to date garnered from space radiobiology studies of rodents with or without other spaceflight hazards, there have been few investigations designed to assess directly the impact of spaceflight stressors alone or in combination using nonhuman primate (NHP) animal models. Given the accepted translational value of NHP data, this research deficiency is surprising and defines a critical gap in knowledge and limits a deeper understanding of the impact of individual and/or combined spaceflight stressors on CNS-related neurobiological, neurochemical, neurobehavioral, and cognitive function. The overarching objective of this review was to address this knowledge gap and provide an overall picture of the available data concerning the impact of spaceflight stressors on CNS function in NHPs. In particular, our aim was to: a) conduct a comprehensive and systematic evaluation of existing research on the acute- and long-term impact of exposure to spaceflight stressors on NHP CNS-related function; b) identify applied and mechanistic research gaps on the acute- and long-term impact of exposure to spaceflight stressors on CNS-related function that could be conducted in a translationally-relevant manner in NHP; c) provide a roadmap of recommendations for NASA regarding the availability, validity, strengths, and limitations of NHP models for future targeted research, including points of contact between existing NASA-relevant rodent assays and functional NHP analogs; and d) describe critical research gaps and priorities in NHPs that may hasten NASA's goals of bridging rodent-based outcomes to those of humans engaged in space exploration. Such information may help to proactively mitigate unexpected spaceflight hazards and avoid unanticipated catastrophic events that could compromise mission metrics and crew welfare.

Here, we focused on a broad range of endpoints in NHPs that may be impacted by a combination of spaceflight stressors. Specifically, we assessed if/how combined exposure to spaceflight stressors might cause acute and long-term molecular, cellular, physiological, vestibular/sensorimotor, neurochemical, and neurobiological abnormalities that lead to CNS-related deterioration in motor, neurobehavioral, and complex cognitive processes that may be involved in neurodegenerative/neuropsychiatric conditions and sensorimotor and vestibular function. This review is organized as follows. First, we provide a brief history of NHPs in space and their contributions to the human space program. Second, we highlight the general strengths, limitations, ethical considerations, and translational value of NHP research and their contribution to scientific discoveries. Third, we describe the methods, including the search strategy we used to identify NHP-related research on the impact of spaceflight stressors, our inclusion criteria, and issues related to data sets and analytical strategy. Fourth, we summarize the available evidence on the impact of spaceflight stressors in NHPs. Finally, based on the copious available information from rodent studies and paucity in functionally similar studies in NHP, we describe critical research gaps and priorities in NHPs that should be considered by NASA in moving forward to bridge the key knowledge gaps that currently exist in translating rodent data to humans.

2. Historical antecedents of NHP spaceflight research

For over 70 years, a wide range of laboratory animals, including NHPs, have contributed to an enhanced appraisal of the risk spaceflight travel might pose to humans (see Gray, 1998; Thomas et al., 1997, for historical reviews). Indeed, during the years prior to sending humans into space, there was considerable debate among scientists as to how astronauts would respond physically to various spaceflight stressors. In order to provide some early clues relevant to risk assessment, American and Russian scientists sent animals, primarily monkeys, chimpanzees, and dogs, into space. These early studies, though rudimentary relative to more recent investigations, provided critical verification of the ability to both launch complex living organisms into space and return them to earth intact. Between June 1948 and December 1949, there were three missions, each carrying a rhesus monkey, launched into space. Although in-flight metrics indicated no ill-effects during the voyage, unfortunately, each of the three trips ended in impact-fatalities during landing. However, a subsequent mission in September 1951 had a successful conclusion when a rhesus monkey (along with 11 mice) returned to earth unharmed. This was followed by additional mission successes throughout the 1950s with rhesus and squirrel monkeys providing fundamental confirmation of the ability to survive rapid accelerations, high altitudes, weightlessness, microgravity, and cosmic radiation, among other spaceflight stressors. In January 1961, NASA sent the first chimpanzee into suborbital flight and despite technical problems that caused the mission to go higher (157 rather than 115 miles), faster (5857 rather than 4400 miles per hour), and end in an off-target landing into the Atlantic Ocean some 60 miles from the stationed recovery ship, the chimpanzee performed well during-flight and, notwithstanding some dehydration and fatigue, quickly and fully recovered. This feat, along with previous work in NHP, served as important precursors for the successful suborbital flight of America's first astronaut, Alan B. Shepard, Jr., less than 6 months later in May 1961. Another chimpanzee would be the first to successfully orbit in November 1961, which would serve as an essential and reassuring validation prior to John Glenn's first human orbital flight in February 1962. These and other early NHP contributions to the assessment of spaceflight risks helped set the stage for the targeted line of research detailed in this review.

3. Strengths, limitations, and translational value of NHPs for spaceflight research

Overall, the prevalence of NHPs used in animal research is less than 1%. Rodents account for approximately 95%. Despite limited use, their study has been linked to major scientific discoveries in medical research and has had an enormous impact on human health. Along these lines, the U.S. Air Force and NASA conducted studies from the mid-1960s to the 1990s in rhesus monkeys to determine the acute and long-term risk of cancer and cataract following exposure to X-rays and protons (see Cucinotta et al., 2014; Dalrymple et al., 1991; Lett et al., 1991; Wood, 1991). As noted by Cucinotta et al. (2014) and the NCRP (1989), observations from these NHP studies provided key information on the acute and long-term risks of cataract and cancer formation after radiation exposure (e.g., protons), information that was critical at the time in preparing for the Apollo missions. With the exception of an early study in rhesus monkeys recording CNS lesions after short-duration flights (Haymaker et al., 1970), to the best of our knowledge, the effects of spaceflight stressors, including exposure to space-relevant radiation on NHP CNS have not been systematically examined.

Table 1
Strengths and limitations of the use of NHP versus rodents for assessing risk of combined spaceflight stressors for humans.

General Strengths:

- NHPs and humans have considerable CNS genetic overlap [>70% for marmosets (*Callithrix jacchus*) (~47 million years removed), >80% (~35 million years removed) for squirrel monkeys (*Saimiri saimiri*), >90% (~25 million years removed) for rhesus monkeys (*Macaca mulatta*)] compared to rodents (20%)
- NHPs and humans have considerable similarity in physiological, pharmacokinetic, neurochemical, neurobiological, behavioral, and cognitive features compared to rodents
- NHPs and humans share some basic biological processes, e.g., emesis not present in rodents; complex visual system and behavior not found in rodents
- NHPs have developed brain structures that are not present or underdeveloped in rodents. For example:
  - Rodents have small brain size, reduced pre-frontal cortex size, reduced development of frontal lobe, and lesser neocortex (e.g., 27% rodents vs 72% NHPs vs 80% humans) compared to NHPs and humans
  - Rodents have reduced brain cells, number of neurons, synaptic connections, axon size, and number of synapses compared to NHPs and humans
  - Rodents have higher levels of neurogenesis compared to NHPs and humans
- Lifespan conducive to needed longitudinal study and post-stressor observation compared to rodents (mouse ~2 years and rats ~2–3 years), marmosets ~10–15 years, squirrel monkeys ~15–20 years, rhesus monkeys ~25–30 years
- Translational value of NHP in CNS-related neurobiological, behavioral, and cognitive function has been validated and established over many decades
- Superior cognitive abilities, identical or nearly identical tasks as those used with humans as clinical diagnostic tools for neuropsychiatric conditions
- Rodents have limited pathological manifestations of neurodegenerative diseases compared to NHPs and humans, i.e., Alzheimer's and Parkinson's diseases
- Within-subject designs similar to those used in human laboratory studies permit meaningful conclusions and inferences based on the evaluation of treatment effects
- Proven reliability as subjects in long-term CNS-related neurobehavioral and cognitive studies
- Recent advances in touchscreen technology for studies of complex cognitive performance in NHP permit the use of tasks that are well-established in human subjects

General Limitations:

- Lower number of subjects per experimental group compared to rodents, which may lead to inferior statistical power for scientific outcomes
- Valuable/limited resource
- Ethical considerations
- Husbandry and maintenance costs substantially higher than rodents
- Greater handling and husbandry training requirements compared to rodents
- Unlike rodents, transgenic and knockout NHP models are being developed, but currently not as readily available

Although studies in other species (e.g., rats, mice) have provided important information on how spaceflight stressors may impact CNS-related neurobiological and behavioral/cognitive function, the translational value of NHP studies in various research domains, including neurobiological, neurobehavioral, and complex cognitive processes has been validated and established over many decades (e.g., Banks et al., 2017; Goldman-Rakic et al., 1992; Harding, 2017; McClure 1984; Weed et al., 1999; Weerts et al., 2007). These successes are based on numerous factors including: 1) the considerable overlap in the genetic, physiological, pharmacokinetic, neurobiological, and neurobehavioral effects in NHPs and humans; 2) the proven reliability of NHPs as subjects in long-term (i.e., longitudinal) neurobehavioral and cognitive studies; and 3) the ability to use powerful within-subject designs that are similar to those used in human laboratory studies, which permit meaningful conclusions or inferences to be based on the evaluation of all treatment effects in individuals as well as in groups. Considerations such as these suggest that NHPs are especially well-suited for ground-based research to study the acute and long-term neurobehavioral effects of spaceflight stressors alone and in combination. Table 1 provides an overview of the general strengths and limitations of NHP research and highlights some important differences between NHPs and rodents that ought to be considered for assessing risk of combined spaceflight stressors for humans.

While the translational value of NHP data is widely-recognized, important caveats involving research in NHPs also deserve consideration (see Table 1). First, most NHP studies have historically employed a relatively small number of subjects per experimental group (typically $n = 4–6$ in neurocognitive studies) compared to rodent studies. This may lead to inferior statistical power for determining translatable scientific outcomes. Second, NHPs are a valuable resource and therefore their use in any research requires considerable ethical and scientific justification with regard to addressing knowledge gaps in a particular research domain (see Section 4 below). Third, the use of NHPs requires significant expenses devoted to ensuring proper handling, training, husbandry, and maintenance compared to rodent research. Finally, characterization of CNS-related disease states through genetic manipulations (transgenic and knockout animal models) are emerging but not yet widely available in NHPs compared to rodents.

4. Ethical considerations of NHPs for spaceflight research

Discussion of future directions in spaceflight stressor research requires careful consideration of ethical issues and constraints. In this regard, it is generally accepted that experiments designed to further our understanding of CNS mechanisms as they translate to humans are essential to NASA's ability to increase the probability of mission success. No matter how pressing the issues may be, however, in order to carry out such experimental work in laboratory animals within an ethical and moral framework, whether rodent or NHP, several important principles must be considered carefully. First, established guidelines related to best practice procedures that emphasize ethical factors in animal research are available and should be consulted (e.g., National Research Council, 2011). Second, ethics committees comprised of scientists, veterinarians, and lay members of the community should be assembled to review experimental protocols and provide recommendations to be adhered to by scientists studying laboratory animals (e.g., Institutional Animal Use and Care Committees). Third, animal research, like all endeavors in biomedical science, requires active participation of society on the local, national, and international level to consider the problem at hand and the role that both human and nonhuman animal subjects can play in finding solutions to unsolved public health concerns thereby improving the quality of human life. Ultimately, critical evaluation of potential benefits in relation to risk should guide the nature of all scientific pursuits.

5. Approach for appraising NASA-relevant NHP studies

5.1. Inclusion criteria

In this review, we were as inclusive as possible, while also striving to ensure that the research reports we reviewed were directly relevant to understanding how the acute and long-term exposure to combined spaceflight stressors including altered gravity, space radiation (low dose/low dose rate), and isolation/confinement, impact NHP CNS function. Below we describe some key issues that were considered.

First, our review is focused on a broad range of endpoints in NHPs that may be impacted by a combination of spaceflight stressors. In particular, we were interested in identifying research that examined whether combined exposure to spaceflight stressors in NHP cause acute and long-term molecular, cellular, physiological, vestibular/sensorimotor, neurochemical and neurobiological abnormalities that lead to CNS-related deterioration in neurobehavioral and complex cognitive processes that may be involved in neurodegenerative/neuropsychiatric conditions. Identifying such data provides key information that will help develop evidence-based countermeasures for spaceflight stressors thereby providing a strong and clear foundation for successful designs of future human deep space missions (e.g., a mission to Mars). Accordingly, we included any references that evaluated multiple endpoints in NHPs. Where possible we have included sources that reported quantitative data as well as sources that provided descriptive case studies and narrative

reviews. Meta-analysis is generally preferred for integrating estimates of the same relationship of interest across studies, i.e., it allows us to generate cumulative knowledge about a set of studies. While the benefits of meta-analysis have been widely appreciated, we recognized early in our review process that there is very limited information on the impact of single or combined spaceflight hazards on CNS function in NHPs. Unfortunately, this prevented us from conducting a traditional meta-analysis.

Second, a major goal of NASA's HRP Integration Research Plan is to identify brain performance pathways that may be synergistically impacted by spaceflight relevant environmental stressors. However, it is also critical that this information be used meaningfully to discover novel strategies (e.g., pharmacological, biologics, nutritional) that can be utilized by astronauts to counter the impact of spaceflight stressors during long-duration deep space exploration. Notwithstanding the view that combined exposure to multiple spaceflight stressors will be problematic for humans during deep space travel, the impact of acute and long-term exposure to space radiation is likely to be the most consequential of any spaceflight stressor, in that the effects of microgravity and isolation/confinement among others may be further exacerbated. Thus, we have included several sources that describe the use of NHPs as animal models for developing countermeasures against radiation, especially acute radiation syndrome (ARS).

Third, although assessment of biomarkers was beyond the scope of this review, we also included any spaceflight stressor sources that involved new areas of scientific research in NHPs. For example, assessment of metabolomic and lipidomic profiling of specific biologic samples provides feasible approaches for identifying potential biomarkers of interest to virtually any physiological outcome. This has successfully been used as a platform for biomarker discovery and biodosimetry following radiation exposure. More recently, several laboratories have assessed the types of metabolomics and lipodomic biomarkers that can be evaluated to assess radiation injury to the brain, and possibly as predictive biomarkers of neurobehavioral outcome in rodents and NHPs. While some examples of sources in NHPs that have used metabolomic and lipodomic approaches to characterize radiation-induced changes, it remains to be determined whether similar biomarkers would be identified following space relevant exposures. Nevertheless, such work does point to the feasibility of this approach, and whether specific biomarker signatures of radiation injury track with adverse behavioral outcomes (either positively or negatively).

Finally, given that limited information is currently available on the impact of spaceflight stressors in NHPs, we have included selected references from other research domains. Although our citations are not exhaustive, we have used certain sources to formulate a list of gaps and research recommendations for NASA to conduct future targeted studies in NHPs.

5.2. Literature search

We used three search strategies to obtain research information on the impact of spaceflight hazards including space radiation, microgravity, and isolation/confinement on CNS function: a) a thorough literature search on NHP using multiple databases; b) a search of the NASA taskbook to identify potential data and technical report leads and researchers to contact; and c) a review of reference lists of key articles and other reviews of similar domains (e.g., Cucinotta et al., 2014; Kokhan et al., 2016; Nelson et al., 2018). The search process for NHP-related research included all dates to the present search date. Overall search strategies are provided in the Appendix.

5.3. Description of variables and accuracy of data sets

In this review, we evaluated a) descriptive, b) correlational, c) quasi-experimental, and d) experimental sources. Given the limited information available on the impact of spaceflight stressors on CNS function, fidelity characteristics for all sources were independently reviewed and discussed by the authors. Data sets were inspected to better understand the nature of the data, to determine the appropriateness of meta-analysis for summarizing the data, and to determine the best way to summarize the available evidence.

All sources (e.g., journal articles, technical reports, books) were used to generate information on how spaceflight hazards alone and in combination during deep space travel impact CNS function in NHP. All studies listed in the report are based on the effects of different types of spaceflight hazards on CNS functioning. Where possible, additional information on the methodology and study characteristics used in each article reviewed is also provided including species, age, radiation dose, length of exposure, and type of CNS function measured. The lack of information in all categories reviewed precluded benchmarking of data sets.

6. CNS effects of spaceflight stressors in NHPs

6.1. Molecular and cellular function

A recurrent theme with the majority of these subsections, is the general absence of data specific to NHP studies in which the effects of spaceflight stressors alone or in combination with charged particle radiation on molecular and cellular processes has been systematically evaluated. With that caveat, investigations of cellular and molecular modifiers of largely acute, whole body photon irradiation sources have been focused primarily on the assessment and/or resolution of the acute hematopoietic syndrome. Single fractions ranging from 2 to 8 Gy have been used to evaluate plasma biomarkers, hematopoietic failure, and the extent that selected countermeasures were successful in ameliorating indications of ARS. For this section, the primary relevance of the majority of the NHP studies selected for the review was that total body exposures were delivered, albeit with doses in excess of LD_{50} and with low LET radiation. In nearly every instance, doses $\geq$5 Gy led to the hematopoietic ARS, with reductions in bone marrow cellularity (Chen et al., 2010; DeBo et al., 2015; Gluzman-Poltorak et al., 2014; MacVittie et al., 2000,) along with systemic indications of inflammation (DeBo et al., 2015; Ha et al., 2014; Rong et al., 2018). These and related studies have analyzed circulating biomarkers and found a number of cytokine/chemokine signaling pathways (Gluzman-Poltorak et al., 2014; Ha et al., 2014) and miRNA signatures in the plasma (Fendler et al., 2017; reviewed in Singh and Pollard, 2017) that exhibited some dose-dependency and were found to be predictive of radiation exposure and/or mortality. Unfortunately, the foregoing studies were not focused on defining CNS specific effects and have limited utility in moving NASA closer to establishing if/how total body irradiation might compromise CNS function during deep space travel at space relevant doses.

Given the high fat content of the brain, studies that analyzed plasma exosomal biomarkers (Cheema et al., 2018) and serum lipids and metabolites (Pannkuk et al., 2016) uncovered widespread perturbations in lipidomic and metabolomics profiles—signatures that may well be informative to NASA if such data can be specifically linked to CNS injury and cognitive decline. Circulating biomarkers, especially plasma-based exosome metabolomics represents a potential beneficial avenue to pursue with NHP research and based on its non-invasive nature and feasibility for longitudinal measurements, may provide significant opportunity for prognostic determinations of spaceflight-induced CNS complications.

A few select studies have implemented fractionated (5 Gy/fraction) whole brain irradiation (Andrews et al., 2017; Hanbury et al., 2015) to total doses of 40 Gy. Despite the fact that this dose is 100 times greater than the total dose expected during an entire mission to Mars, findings were intriguing, and uncovered neurocognitive decline, neuroinflammation, and significant parenchymal and stromal breakdown over protracted post-irradiation times. Of particular note were data finding enhanced cortical sensitivity to whole brain irradiation that tracked

cognitive deficits, effects seen in the relative absence of hippocampal damage (Hanbury et al., 2015). This is in distinct contrast to many data sets obtained in rodents. While this represents a single report, these provocative data suggests potential species-specific differences in radiosensitivity within certain regions of the brain. This may provide one potential starting point for designing future rodent and NHP studies to further validate the significance of these findings and for contemplating how best to exploit such differences in effects among species for minimizing the CNS risks associated with deep space travel.

6.2. Overt behavior, physiological function, and motor performance

After thorough review, it became clear that NHP studies examining the effects of radiation on observable behavior and motor performance in their own right have not been conducted. However, relevant consequences on these important endpoints can be derived to some extent from ancillary analyses presented in several of the papers reviewed in the Neurobehavioral and Cognitive Performance section (see McDowell, 1958). Regarding the effects of spaceflight stressors on physiological function in NHP, few papers were identified. Two papers report important findings on the effects of radiation on cardiac structure and microvascular density in rhesus macaques. DeBo et al. (2016) documented that total body gamma irradiation produced long-lasting myocardial injury, altered heart structure and function, and elevated levels of MCP-1 and LBP consistent with a systemic pro-inflammatory state. Interestingly, no significant differences were observed in standard clinical measures of cardiac health and function, such as systolic or diastolic blood pressure. Fanning et al. (2017) reported that total body gamma irradiation induced changes in skeletal muscle architecture and examines in depth its relationship with metabolic health that can persist many years after radiation exposure. In a recent systematic and quantitative review paper, MacVittie et al. (2015) summarized the literature of hematopoietic syndrome of ARS in rhesus macaques. They concluded that the findings generated by controlled studies in NHP provides support for the targeted use of rhesus macaques, rather than uncontrolled and anecdotal reports from human clinical case reports, for the examination of lethal dose relationships of radiation exposure. This, according to the authors, provides a superior means to develop improved countermeasures and treatments from radiation exposure ranging from medical procedures to nuclear weapons.

6.3. Neurochemical activity

Like other endpoints, we were not able to identify any NHP studies in which the effects of spaceflight stressors alone or in combination on CNS-related neurochemical activity have been systematically evaluated. This is especially surprising, given that for over two decades evidence from rodent studies has strongly suggested that exposure to space radiation can produce profound deleterious neurochemical changes in dopamine (DA) activity (Carey et al., 2007; Carrihill-Knoll et al., 2007; Davis et al., 2014; 2015; Joseph et al., 1992; 1993; 2000; Rabin et al., 2004; Rice et al., 2009). More recently, space radiation-induced alterations in other neurotransmitter systems including glutamate (GLU) (Machida et al., 2010; Marty et al., 2014; Sanchez et al., 2010), γ-aminobutyric acid (GABA) (Lee et al., 2017; Marty et al., 2014), and the cannabinoid system (Lee et al., 2017) has also been identified in rodent studies. Based on this early work, it seems clear that space radiation can have a major impact on neurotransmitter systems within the brain. A large body of evidence from studies in other scientific disciplines has also demonstrated significant alterations in neurochemical activity (e.g., DA, GLU, CRF etc.) following exposure to stressors, such as social stress (e.g., Holly and Miczek, 2016). Taken together, these findings suggest that there is a large unmet need to fully characterize how combined exposure to spaceflight stressors during deep space travel alters in vivo changes in brain neurochemistry in rodents and NHPs in brain regions known to be associated with complex neurobehavioral and cognitive processes.

6.4. Neurobiological structure and function

As with other endpoints, we found little information on how exposure to spaceflight stressors alone or in combination may impact neurobiological structure and function in NHPs. In the only study cited, rhesus monkeys were exposed to X-ray irradiation during early- and mid-gestation and cortical gray and white matter volumes were recorded. Results from this work showed that early-gestation exposure reduced nonfrontal gray matter volume (17%) and white matter volume in frontal (26%) and nonfrontal (36%) lobes, whereas mid-gestation irradiation resulted in smaller gray (frontal: 28%; nonfrontal: 22%) and white matter (frontal: 29%; nonfrontal: 38%) volumes (Selemon et al., 2005). Such observations suggest that changes in NHP brain structure and function may be highly sensitive to radiation exposure. However, it is important to emphasize that radiation exposure in periods of early development (e.g., during cortical proliferation) inhibits brain development. Notwithstanding, early developmental sensitivities of the CNS to radiation exposure have been clearly documented in rodents as well as humans, but such early-gestation responses to radiation exposure (or other spaceflight stressors) on brain structure and function of adult astronauts are not likely to be as consequential.

6.5. Neurobehavioral and cognitive performance

Like the other endpoints surveyed in this report, there is an unfortunate paucity of NHP studies in which the effects of irradiation on cognition-related behavior have been systematically examined. However, notwithstanding diversity in cognitive task and variation of radiation insult, the extant studies in the literature reveal important functional similarities of effects (see Cucinotta et al., 2014; Dalrymple et al., 1991; Kokhan et al., 2016; Nelson et al., 2018 for review). A majority of the studies on neurobehavioral and cognitive performance reviewed can be grouped into those that were conducted approximately 50–60 years ago (~1958–1968) and those conducted within the last 10 years (~2011–2016). A vast majority of these studies were conducted in rhesus macaques using whole body (e.g., x-ray, gamma, neutron) and fractionated whole-brain irradiation. Taken together, the NHP studies surveyed are generally consistent with numerous rodent studies examining cognition-related behavior in that they both demonstrate radiation-induced deficits across several diverse cognitive endpoints (see Nelson et al., 2018 for review). In addition, the consistency of effects observed within a particular NHP cognitive domain across the two time periods in which the studies took place (i.e., 60 years ago vs. last 10 years) is somewhat remarkable. For example, repeated acquisition and discrimination reversal tasks, which are thought to assay, respectively, basic features of learning and cognitive flexibility, show magnitude-related radiation impairment in both eras, despite relatively primitive apparatus and methods available in the 1950s versus the modern technology available today. Nevertheless, functional similarities in impairment are obvious. Importantly, one thread that connects several NHP studies on radiation exposure and cognitive performance across the decades relates to a recurring finding of the impact of *cognitive load* on observed impairments (see Cucinotta et al., 2014; Dalrymple et al., 1991; Kokhan et al., 2016; Nelson et al., 2018 for review). For example, several studies, both old and more recent, varied the task *difficulty* in addition to magnitude of radiation (e.g., Davis, 1961; Hanbury et al., 2016; Robbins et al., 2011). Arranging conditions that varied in cognitive load revealed significant effects of smaller radiation magnitude insults on cognition that might not otherwise have been observed using simple cognitive tasks. Although not appearing in the literature often, this tactic of manipulating cognitive load appears critical for the ability to examine spaceflight stressor effects on neurobehavioral and cognitive performance that, although sometimes subtle, may have long-lasting and significant consequences on astronaut behavior during and/or after the mission. More generally, this highlights another example of the critical need for targeted studies in NHP, as rodents are

not the ideal model systems for within-subject examination of performance implementing tasks that vary in cognitive load. Future targeted studies in NHP designed to examine spaceflight stressor effects on neurobehavioral and cognitive performance should include tasks that examine multiple endpoints (e.g., learning, memory, attention) and with a specific focus on varied cognitive load where the difficulty is parametrically varied in each task.

6.6. Other work

6.6.1. Altered gravity

Extensive evidence suggests that microgravity, a consequence of spaceflight, may induce alterations in cortical structure and function for several reasons, including the otoliths that constitute gravity-dependent acceleration detectors of the vestibular system. Gravity deprived peripheral input could in turn have a profound effect on the vestibular nuclei, as well as on the cortical projections where integration of the different sensory inputs takes place (i.e., parieto-insular region, thalamus, and temporoparietal region) resulting in a negative impact on the cognitive functions supported by these regions (Van Ombergen et al., 2018). Studies in humans have demonstrated that the microgravity phases during parabolic flights causes profound changes in frontal lobe activity; however, this is most likely due to gravity transition, which is probably not a major factor under constant microgravity (Schneider et al., 2008). Importantly, several functions and brain areas have also been associated with symptoms related to weightless/space conditions in humans (De la Torre, 2014). These include: a) a decline in somatosensory and proprioceptive accuracy (primary somatosensory cortex) (Roll et al., 1993; Watt, 1997) as well as mental imagery (Grabherr and Mast, 2010); b) a reduction in executive functioning such as decision-making errors and attention deficits (prefrontal and premotor cortex, frontal eye fields; Lackner, 1985; Pattyn et al., 2005; Roll et al., 1993); c) impaired motor control (motor cortex and cerebellum; Heuer et al., 2003; Pattyn et al., 2005; Semjen et al., 1998); and d) vestibular dysfunction, including motion sickness, malaise, headache, vomiting (Berger et al., 1997; Bock et al., 2001; Heuer et al., 2003). Finally, connectivity across brain regions and gray matter reductions are also negatively affected by microgravity, contributing to impairments in attention, learning, memory, and coordination (Demertzi et al., 2016; Koppelmans et al., 2016; Li et al., 2015). Interestingly, Stahn and colleagues recently reported that a 6° head-down tilt bed rest paradigm was associated with moderate cognitive slowing and changes in emotion recognition performance, but these effects were not mitigated by either continuous or intermittent daily 30 min exposure to altered gravity (Banser et al., 2021). Together these observations in humans are consistent with rodent studies showing that manipulation of vestibular input by microgravity results in hippocampal atrophy and a reduction in hippocampal N-methyl-D-aspartate (NMDA)-receptors, both of which predict a decline in spatial memory performance (Besnard et al., 2012; Brandt et al., 2005; Machado et al., 2014; Strangman et al., 2014). Precisely how radiation or other spaceflight stressor exposure might interface with the foregoing vestibular endpoints is less certain, but certainly represents an area worthy of further consideration if not investigation.

With regard to NHP studies, we found several reports that examined the impact of altered gravity in rhesus monkeys. A majority of these studies involved assessment of various aspects of vestibular/oculomotor function and locomotion before and after spaceflight (14 days) and were compared to changes observed following flight simulation at 1 G (Cohen et al., 1992; 2005; Dai et al., 1996; 1998; Edgerton et al., 2000; Recktenwald et al., 1999; 2000). Although these studies are highly relevant, it is important to emphasize that the sample size in the vast majority of these studies is relatively small (n = 2–4 monkeys), which makes it difficult to interpret outcomes. Nevertheless, results from early studies by Cohen et al. (1992; reviewed in 2005) showed that horizontal and vertical semicircular canal-induced responses of the angular vestibular-ocular-reflex to rotation were largely unaffected. Another study reported changes in vergence in NHPs, which appear to be induced by the sinusoidal component of gravity acting along the naso-occipital axis during off-vertical yaw axis rotation, and that the modulation of the vergence reflex seems to be significantly less sensitive to linear acceleration after spaceflight (Dai et al., 1996). Moreover, Dai et al. (1998) noted both short- and long-term changes in otolith-induced eye movements after adaptation to microgravity. Based on such data Dai and colleagues noted the unique value of the monkey as a model for studying effects of vestibular adaptation in space given that eye movements can be measured in three dimensions in response to controlled vestibular and visual stimulation, and the results can be directly applicable to humans. The authors concluded that studies in monkeys to determine how otolith afferent input and central processing is altered by adaptation to microgravity should be an essential component of future space-related research (Dai et al., 1996; 1998).

In other work, a few studies assessed aspects of locomotor function before and after spaceflight (e.g., Edgerton et al., 2000; Recktenwald et al., 1999; 2000). Results from this work showed that postflight, but not postsimulation, electromyographic (EMG) recordings revealed clonus-like activity in all muscles, i.e., the primary extensors [soleus (Sol), medial gastrocnemius (MG), and vastus lateralis (VL)] and the flexor tibialis anterior (TA). In addition, alterations in mean EMG amplitudes reflected differential adaptations in motor-unit recruitment patterns of flexors and extensors as well as fast and slow motor pools. Compared with pre-simulation, the simulation group showed slight increases in the cycle period and burst durations of all muscles with EMG amplitude decreasing in the Sol, increasing in the MG and VL, and remaining unchanged in the TA. Adaptations observed post-simulation were different from those observed postflight, indicating a response unique to the microgravity environment, in that modulation of the

Table 2
Research recommendations and priorities in NHPs for assessing risk of combined spaceflight stressors for humans.

Priority #1: Assess impact of combined spaceflight stressors on neurocognitive performance in NHPs
Task A: Use touchscreen technology to systematically characterize alterations in neurocognitive performance
Priority #2: Assess impact of combined spaceflight stressors on neurobiological changes in brain structure/function in NHPs
Task B: Use cutting-edge neuroimaging tools to determine a link between abnormalities in brain structure/function and observed deficits in neurocognitive performance identified in Task A
Task C: Use EEG mapping of evoked potentials to study whether deficits in neurocognitive performance identified in Task A are associated with decrements in whole-brain neuronal networks in NHPs
Note: Task A and B or task A and C can be conducted separately in the same subjects or while NHPs are engaged in neurocognitive tasks
Priority #3: Assess impact of combined spaceflight stressors on neurochemical signatures in brain regions that are involved in neurocognitive performance in NHPs
Task D: Use in vivo microdialysis techniques in NHPs to determine whether deficits in neurocognitive performance identified in Task A in specific brain regions identified in Task B correspond to neurochemical abnormalities
Note: Task A and D can be conducted separately or while NHPs are engaged in touchscreen neurocognitive tasks
Priority #4: Assess impact of combined spaceflight stressors on structural plasticity, biochemical, and molecular activity in brain regions that are involved in neurocognitive performance in NHPs
Task E: Using a range of biochemical/molecular tools, brain regions involved in neurocognitive performance found to be impacted by spaceflight stressors in Task B and C can be further interrogated to determine the underlying causes of resultant neurological abnormalities
Note: This work can be conducted at the end of the other experiments in NHPs used in any of the above listed Task A-D
Priority #5: Identify biomarkers in NHPs that may be related to combined spaceflight stressor induced deficits in neurocognitive performance in NHPs
Task F: Biomarkers (e.g., plasma, blood-borne exosomes, metabolomics, lipidomics, proteomics, RNA-seq) associated with neurocognitive performance (Task A) can be further researched
Note: This work can be conducted throughout using NHPs in Task A and C

nervous system controlling locomotion cannot merely be attributed to restriction of movement but maybe due to changes in the interpretation of load-related proprioceptive feedback to the nervous system. Overall, while the above highlighted endpoints are important in understanding the impact of altered gravity on sensory motor function, it is somewhat surprising that assessment of other measures (e.g., complex behavior/cognition, biomarkers, etc.) were not established in monkeys that experienced 14 days of spaceflight. Such information would be critical to developing future human deep space missions.

6.6.2. *Biomarkers*

Although biomarkers were not a major focus of this report, we found a number of studies in NHPs on radiation-induced biomarkers (metabolomics/lipidomics/proteomics/RNA-seq) that could potentially be utilized in future NHP research (e.g., Altadill et al., 2017; Hinzman et al., 2018; 2019) and are discussed in greater detail in Section 7.5.

6.6.3. *Environmental stress*

Social aspects also play a key role in space missions. Impaired physical and social interactions may result in problems for the crew, especially in long-duration missions. These issues may result in several potentially hazardous conditions, such as diminished social interaction, lack of motivation, depression, anxiety, and mood problems (Lupien et al., 2009; Manzey et al., 1998). Social isolation can impact the hippocampus through mechanisms similar to stress, where reduced glucocorticoid receptors (Kamal et al., 2014), disrupted neurogenesis (Cinini et al., 2014), and hippocampal volume shrinkage (Pereda-Perez et al., 2013) can result in learning and memory deficits. In addition to hippocampal impairments, social isolation can impact executive function, with poorer attentional regulation and hypersensitivity to negative social information (Cacioppo and Hawkley, 2009) and altered functional connectivity to frontal brain regions (Layden et al., 2017). Interestingly, there is also some evidence to support the exogenous administration of allopregnanolone (ALLO), a neurosteroid, in alleviating the symptoms of social isolation, with growing use of ALLO in treating post-traumatic stress disorder and traumatic brain injuries (Cacioppo and Cacioppo, 2015). Thus, social isolation from extended spaceflight may impact cognitive and social functioning, both during the mission and for an extended period of time afterward (Palinkas, 2001).

Table 3
Endpoints in NHPs for assessing risk of combined spaceflight stressors for humans.

Key Indicators	Methodology	Endpoints
	Neurobehavioral and Cognitive Performance	
Touchscreen-based tasks	Stimulus-Discrimination	Discrimination learning
	Stimulus-Reversal	Cognitive flexibility
	Delayed matching-to-sample or -position	Working memory
	Psychomotor vigilance task	Attention/motor function
	Probabilistic reward task	Anhedonia/depression
	Behavioral economic demand analysis	Reward responsiveness: consumption changes as a function of changes in cost
	Progressive-ratio performance	Motivation
	Flanker task	Cognitive control
	Neurobiological Structure and Function	
Neuroanatomy and brain functional activity/connectivity	Neuroimaging	Non-invasively characterize changes in: a) white matter microstructure/region-specific gray matter volume (diffusion weighted imaging/ morphometry); b) brain region-specific neurochemistry and metabolite levels (proton [1H] and phosphorus [31P] magnetic resonance spectroscopy); and c) functional brain connectivity/activation patterns (functional magnetic resonance imaging; task-based and resting state)
	EEG recordings	Use high-density scalp EEG recording to non-invasively map somatosensory evoked potentials to conduct long-term investigation of large-scale neuronal networks and their reorganization before and after exposure to spaceflight stressors
	Neurochemical Activity	
In vivo preparations	Microdialysis	Measurement of real-time changes in key neurotransmitter, and other neurochemical in the extracellular fluid using LC-MS in different brain regions involved in complex neurobehavioral/ cognitive processes. Assessment over 5- to 20-min intervals, i.e., long-lasting changes in content of extracellular fluid
	Molecular and Cellular Function	
Neuroinflammation	Immunohistochemical Western blot	Brain microglia, astrocytes, and key receptors (e.g. puringergic receptors)
	ELISA	Blood and brain levels of pro- and anti-inflammatory cytokines (e.g. IL-1beta, IL-6, IL-10, TNFalpha) and chemokines
Gene Expression	DNA microarray RT-PCR	Key brain areas (e.g. prefrontal cortex, nucleus accumbens, amygdala, hippocampus) to align with analyses of other neurobiological, behavioral, and cognitive endpoints
	Biomarkers	
Biomarkers	Metabolomics/lipidomics/ proteomics/RNA-seq	CNS derived fatty acid biomarkers, metabolic/homeostatic stress-induced signatures, blood-borne exosomes and bioactive cargo, (e.g. miRNA)
	Overt Behavior, Physiological Function, and Motor Performance	
Overt behavior	Behavioral observations	Quantal or continuous recording of changes in species directed stereotypic behavior., e.g., grooming, rearing, scratching, eye blinking, etc.
Temperature	Telemetry	Assess changes in core body temperature
Cardiovascular function	Telemetry	Heart rate and blood pressure
Circadian activity	Telemetry	Changes in circadian patterns of activity
Motor activity	Open field chambers	Changes in locomotor activity
	Actigraphy	Assess gross motor activity and rest patterns
	Operant behavior	Assess changes in learned motor performance

7. Critical gaps, research recommendations, and considerations

Each of the sections below provides a brief rationale for our view of the key gaps, recommendations, and considerations for NASA. Note that the organization of this section is based on the overall research gap and the order in which our recommendations/priorities are listed in Table 2. With this in mind, we have grouped our recommendations in NHPs into several orderly categories based on the following overall research question (see Table 2): *Will combined exposure to space radiation, environmental stress, and microgravity cause acute and long-term CNS-related deterioration in behavioral performance and complex cognitive processes that is associated with brain structure/function, neurochemical, and biochemical/molecular abnormalities?* In addition to these recommendations and priorities, in Table 3, we provide a concise list of specific assays in NHPs that could otherwise be utilized to achieve the goals outlined in NASA's HRP Integration Research Plan. This list of endpoints is based on using a systems-biology approach to identify CNS circuitry related to operational performance that may be critical to monitor in astronauts in-flight and upon return to Earth. Finally, the selection of the most appropriate NHP species to study the acute and long-term CNS effects of spaceflight stressors alone and in combination and how they translate to astronauts ought to be carefully considered by NASA in future work. To address this issue, Table 4 highlights some of the strengths and limitations that are associated with using marmosets, squirrel monkeys, and rhesus monkeys for scientific research. Although any of these NHP species can be used to conduct the recommended research described in this report, we appreciate that NASA is likely to conduct targeted studies in NHPs to confirm or advance observations in rodents, which makes selection of the most appropriate NHP-based animal models critical.

7.1. Impact of spaceflight stressors on complex behavior and cognition

Recent advances in touchscreen technology have dramatically enhanced the ability to systematically examine complex behavior and cognition in laboratory animals, including both rodents and NHP (reviewed in Kangas and Bergman, 2017). Regarding NASA's aims, modern touch-sensitive technology would provide an extremely flexible means to expose laboratory animals to a variety of complex behavioral tasks designed to assay dimensions of cognitive function before, during, and after single or combined spaceflight stressors. Future studies in rodents could be designed to examine the effects of irradiation or other spaceflight stressors in custom-designed (Kangas and Bergman, 2017) or commercially available (Bussey-Saksida Rat Touchscreen Chamber, Lafayette Instrument Company, Lafayette, IN) touchscreen chambers. Importantly, over the last decade, there have been several studies focusing on methodological and pharmacological efforts in optimization and standardization and have verified the ability to assess analog task performance in rodents (e.g., Bussey et al., 2008; Cook et al., 2004; Kangas and Bergman, 2017; Mohler et al., 2015; Talpos et al., 2012). Many of these advanced cognitive tasks adapted for rodents are becoming increasingly prevalent in translational neuropsychiatric research (reviewed in Bussey et al., 2012; Dumont et al., 2021; Hvoslef-Eide et al., 2016). However, ultimately what appears needed, are studies in which radiation and other spaceflight stressors are examined individually and in combination under task performance that specifically varies *cognitive load*. It is certainly plausible to implement this approach in rodent subjects; however, there are very limited examples of this in the extant literature (e.g., Parihar et al., 2016; Acharya et al., 2019). Studies in NHP that arrange multiple cognitive tasks that both assay distinct dimensions of cognitive function and difficulty across endpoints have already proven successful repeatedly in the characterization of psychoactive drug effects. In an early example, Weed et al. (1999) examined in rhesus macaques the acquisition and long-term performance of a variety of touchscreen-based models of memory (self-ordered spatial search and delayed non-matching to sample), attention and learning (intra-dimensional/extra-dimensional shift), fine motor performance (bimanual motor task and reaction time), and motivation (progressive ratio). Notwithstanding some individual differences in task acquisition, monkeys were readily able to perform multiple tasks with high levels of accuracy across extended periods of time. Importantly, the use of touchscreen technology allowed the authors to convincingly identify a strong correlation between task difficulty and performance sensitivity to parametric manipulations. In a follow up study, Weed et al. (2004) examined rhesus macaques infected

Table 4
NHP species for assessing risk of combined spaceflight stressors for humans.

Common marmosets *(Callithrix jacchus)*

General Information
- New World Monkey
- Life span: 12–15 years (wild)
- Average height: males ~188 mm (7.40 in); females ~185 mm (7.28 in)
- Average weight: males ~256 g (9.03 oz); females ~236 g (8.32 oz)
- Tendency to give birth to non-identical twins

Strengths
- Small size: limited housing space required; easy handling; less test material required; can be socially housed
- Reproduces well in captivity with the shortest gestation period of any primate
- Inexpensive to maintain
- Wild populations are not endangered so population numbers are high

Limitations
- Small size prevents sampling of large quantities of blood or tissue making it difficult to quantify results
- Small brain reduces cognitive capacity limiting the type of neurobehavioral cognitive research that can be performed, i.e., they cannot complete certain puzzle task compared to other NHPs (e.g., rhesus monkeys)
- Greater evolutionary distance to humans relative to old world monkeys

Squirrel Monkeys *(Saimiri saimiri)*

General Information
- New World Monkey
- Life span: 20 years (wild)
- Average height: males ~318 mm (12.5 in); females ~316 mm (12.4 in)
- Average weight: males 554 and 1150 g (1.22 to 2.53 lb); females 651 to 1250 g (1.43 to 2.76 lb)

Strengths
- Medium size: reasonable housing space; easy handling; less test material required; can be socially housed
- Easily adapt to laboratory housing and handling which reduces stress from manipulation
- Widely available
- Maintained in smaller spaces, less expensive housing than larger NHPs (e.g., rhesus monkeys)
- Minimum risk of zoonotic disease transmission than with macaques and other old world primates (e.g., rhesus monkeys)
- Less expensive personal protective equipment required for handling
- Ability to tolerate high gravitational forces – ideal subject for physiological studies of the effects of spaceflight
- Used extensively by NASA in the 1950s-60 s

Limitations
- Small size prevents sampling of large quantities of blood or tissue making it difficult to quantify results
- Greater evolutionary distance to humans relative to old world monkeys

Rhesus Monkeys (*Macaca mulatta*)

General Information
- Old World Monkey
- Life span: 25–30 years
- Average height: males ~531.8 mm (1.74 ft); females ~468.8 mm (1.54 ft)
- Average weight: males ~7.70 kg (17.0 lb); females ~5.34 kg (11.8 lb)

Strengths
- Easy to keep in captivity and can be socially housed in stable and compatible groups
- Widely available
- Considerably closer to humans anatomically and physiologically
- Use has led to many medical discoveries and breakthroughs (e.g., HIV/AIDS, Parkinson's disease)
- Used by U.S. Army, U.S. Air Force, and NASA in 1950s-60s and Soviet/Russian space program in 1997

Limitations
- Large size: requires large housing space; can be difficult to handle; large test materials required
- Expensive to purchase and maintain
- Aggressive nature
- Risk of zoonotic disease transmission, e.g., Herpes B virus

with simian immunodeficiency virus (SIV) in an animal model of AIDS, and showed that, as with HIV-infected AIDS patients, tasks thought to involve frontostriatal dopaminergic functioning (i.e., self-ordered spatial search, bimanual motor task, and reaction time) were particularly vulnerable. These studies formed an elegant illustration of the great value of touchscreen technology, providing supportive evidence of homology between human and NHP behavioral test batteries (see also Nagahara et al., 2010), and the importance of examining varying levels of cognitive load in SIV and HIV infection models. Other researchers have effectively used touchscreen apparatus in squirrel monkeys (e.g., Kangas and Bergman, 2012; 2014; Kangas et al., 2016a) and marmosets (e.g., Kangas et al., 2016b, Spinelli et al., 2004; Wooldridge et al., 2021). Results show that squirrel monkeys and marmosets are able to master the tasks presented and that performance was comparable to that observed with rhesus macaques in some tasks (e.g., discrimination reversal, 5-CSRT), but not others (e.g., some variants of the delayed match-to-sample task). Taken together, exploratory studies can and should be conducted in rodent subjects using touchscreen apparatus to examine complex behavioral phenotypes relevant to complex behavior and cognitive function. However, targeted studies in NHP that utilizes their ability to engage in multiple tasks, with varied levels of cognitive load and close functional similarities to human tasks, are highly feasible and could be implemented based on NASA priorities.

In consideration of prioritizing targeted cognition studies in NHP, an example of arranging a variety of cognitive tasks designed to assay distinct dimensions of cognitive function and, as well, examine varying levels of cognitive load, has recently been promulgated by Kangas et al. (2016a). In studies using squirrel monkeys to study the psychoactive effect of a variety of cannabinoids, subjects were trained in a touchscreen-based visual discrimination task (learning one simple problem), repeated acquisition task (learning multiple problems daily), discrimination reversal task (response inhibition/cognitive flexibility), and delayed matching-to-sample task (short-term recognition memory). The cognitive functions, noted parenthetically and in Table 3, are diverse and thought to be mediated largely via distinct brain structures. However, although the tasks are diverse, they are also all predicated on arrangements of visual discriminations that vary in their complexity and, as such, can be categorized by their baseline difficulties, i.e., visual discrimination < repeated acquisition < discrimination reversal < delayed matching-to-sample. Viewing baseline task difficulty in this manner provides a parsimonious interpretation of the positive correlation of cannabinoid-related task impairment and cognitive load observed in Kangas et al. (2016a) and subsequently replicated with methamphetamine (Kangas and Bergman, 2016) and cocaine (Kangas et al., 2019). Taken together, as shown in the NHP irradiation studies reviewed and, as well, across several psychoactive drugs that vary in mechanism summarized above, prioritizing diverse touchscreen-based cognitive tasks that can be rank ordered in difficulty (i.e., visual discrimination task, repeated acquisition task, discrimination reversal, and delayed matching-to-sample) would be highly valuable to NASA's mission to predict surprise events using innovative animal models.

7.2. Impact of spaceflight stressors on brain structure and function

Magnetic resonance imaging (MRI) at ultra-high field strength (i.e., 9.4 Tesla) can be utilized to determine the relationship between stressor-induced alterations in brain structure/function and cognitive function (see Table 3). Using a powerful within-subject design, it will be highly beneficial to employ established MRI techniques to measure gray matter volume, white matter integrity, and functional connectivity in fully conscious NHPs before and at several time points after combined exposure to radiation/gravity/stress in the same subjects that participate in the behavioral/cognitive studies. This, in combination with newly developed functional MRI techniques, provides a multimodal neuroimaging approach that can be used to evaluate how combined exposure to spaceflight stressors alters neural activity over time in regions known to play a role in behavioral/cognitive processes (e.g., prefrontal cortex, hippocampus). Such studies will allow for the structural and molecular determination of whether cognitive load deficits are accompanied by abnormalities in brain neural circuits. Additionally, electroencephalogram mapping of evoked potentials can be used to study whether changes in behavioral performance and complex cognitive processes identified in imaging studies are associated with decrements in whole-brain neuronal networks in NHPs.

7.3. Impact of spaceflight stressors on neurochemical function

Extensive evidence from studies in rodents suggests that exposure to space radiation can produce deleterious neurochemical changes in dopamine, glutamate, GABA, and cannabinoid activity (Carey et al., 2007; Carrihill-Knoll et al., 2007; Davis et al., 2014; 2015; Joseph et al., 1992; 1993; 2000; Lee et al., 2017; Machida et al., 2010; Marty et al., 2014; Rabin et al., 2004; Rice et al., 2009; Sanchez et al., 2010). Clearly, space radiation and other spaceflight stressors can have a profound impact on multiple neurotransmitter systems within the brain. It is noteworthy that neurochemical systems (e.g., DA, 5-HT, NE, GLU, GABA, ACh, etc.) within the brain are highly complex and interconnected with multiple neurotransmitters playing a key role by modulating each other's actions to maintain normal CNS function and control, including aspects related to complex neurobehavioral and cognitive processes. Importantly, it is well-established that aberrant DA, GLU, GABA, 5-HT, ACh, and NE neurotransmission are closely linked to neurological diseases (e.g., Parkinson's disease, anxiety, schizophrenia, depression, attention deficit hyperactive disorder) that may adversely impact mission success during deep space travel and astronaut health/well-being thereafter. As stated above, modulation of CNS neuronal activity by DA, GLU, GABA, 5-HT and NE, e.g., in the frontostriatal region, is critical for cognitive function (Altman et al., 1996; Kehagia et al., 2010). Although studies already suggest that space radiation has deleterious effects on DA, GLU, and GABA function (see above), the effects of combined spaceflight stressors on brain neurochemical activity and associated behavioral and cognitive function, over time have not been systematically assessed in rodents or NHPs. Thus, while it will be difficult to directly address neurodegenerative diseases that may develop as a consequence of exposure to spaceflight stressors, alterations in neurochemical systems can provide valuable information that may allow us to predict the likelihood of developing neurodegenerative diseases associated with long-duration deep space missions. Consequently, assessment of how spaceflight stressors alone or in combination may affect key neurotransmitters in brain regions that are associated with varying cognitive load and how they might mediate neurobehavioral/cognitive processes over time will provide critical information that will help predict acute/long-term risk of neurological disorders.

7.4. Impact of spaceflight stressors on biochemical, structural, and molecular activity

A series of biochemical, structural and molecular studies targeted to elucidate how radiation and spaceflight stressors (e.g., microgravity, isolation) disrupt oxidative and inflammatory pathways that impinge of the structural integrity of neurons and supporting stroma have been conducted. Significant rodent data exist showing that space relevant radiation exposure elicits chronic increases in oxidative stress and injury (Giedzinski et al., 2005; Tseng et al., 2014), neuroinflammation (Parihar et al., 2015; Rola et al., 2005; Rosi, 2018), neurotransmitter availability (Lonart et al., 2012), epigenetic reprograming (Baulch et al., 2014; Impey et al., 2017), and importantly, reductions in dendritic complexity, spine density, synapse density, and axonal myelination (Allen et al., 2015; Dickstein et al., 2018; Parihar et al., 2018). Related data has also demonstrated regional-specific differences in the radiosensitivity of specific neuronal subsets. While such data points to a

multifaceted series of diverse yet overlapping mechanisms that define the complexity of the CNS radiation response, these rodent findings point to specific studies that can be conducted in NHPs following the cessation of in vivo measurements.

7.5. Impact of spaceflight stressors on biomarkers

Assessment of metabolomic and lipidomic profiling of specific biologic samples provides feasible approaches for identifying potential biomarkers of interest to virtually any physiological outcome. This has successfully been used as a platform for biomarker discovery and biodosimetry following radiation exposure (Altadill et al., 2017). More recently, work has begun to assess the types of metabolomic/lipodomic biomarkers that can be evaluated to assess radiation injury to the brain, and possibly as predictive biomarkers of neurobehavioral outcome. In an initial study, tissue specific metabolomics were used to identify endoplasmic reticulum stress in the irradiated hippocampus (Hinzman et al., 2018). More relevant to current NASA interests is recent work analyzing plasma-derived extracellular vesicles that was able to yield predictive markers of cranial irradiation exposure in mice (Hinzman et al., 2019). Two days and two weeks post-exposure (9 Gy head only), plasma and plasma-derived extracellular vesicles (EVs) from these mice were collected. Using metabolomic and lipidomic profiling, several markers associated with inflammation were up-regulated in EVs including triglycerides, platelet activating factor, carnitine, and C-16 sphinganine were detected. Additionally, whole-plasma profiling provided further evidence of systemic injury, including moderate dyslipidemia and a significant decrease in systemic β-hydroxybutyric acid, which serves as a neuroprotectant. These studies are the first to demonstrate that metabolomic and lipidomic profiling of plasma-derived EVs may be used to study clinically relevant markers of ionizing radiation toxicities to the brain that typically manifest as late effects. It remains to be determined whether similar biomarkers would be identified following space relevant exposures, but such work does point to the feasibility of this approach. Identifying which potential EV cargo can be cross referenced between samples derived from different species (including humans), and whether specific biomarker signatures of radiation injury track with adverse behavioral outcomes (either positively or negatively) will be critical.

8. Summary and conclusions

NASA's HRP has identified studies of the effects of combined spaceflight stressors on acute and chronic CNS, BMed, and sensorimotor function during and after deep space travel as a major research topic. In particular, understanding the combined impact of space radiation, microgravity, and isolation/confinement during deep space travel on an astronaut's ability to perform both simple and complex CNS-, Bmed- and sensorimotor-related tasks in which they have previously received extensive training is a specified need.

A large body of evidence from studies in rodents suggests that exposure to distinct spaceflight stressors (e.g., low doses of space radiation) can have a profound impact on CNS-, BMed- and/or sensorimotor-related neurobehavioral and cognitive performance. Studies conducted in mice and rats evaluating the impact of space radiation on CNS function have conclusively demonstrated a variety of learning and memory impairments along with the emergence of stress-related behaviors. Based on this research, we highlight below a number of critical issues related to space radiation for NASA to consider in advancing research in laboratory animals:

1) Mice and rats have shown significant impairments in nearly every behavioral task administered after exposure to space radiation, suggesting that exposure to space relevant radiation disrupts neurotransmission in key brain regions involved in behavioral and neurocognitive function.
2) Multiple brain regions are affected including hippocampal, basal forebrain, medial prefrontal, perirhinal, and entorhinal cortices. Thus, it seems likely that no region of the brain is spared after exposure to space radiation, but understanding region-specific susceptibilities and their relation to behavioral performance and complex cognitive processes remains a major challenge.
3) Neurobehavioral and cognitive deficits are persistent and long-lasting following exposure to space relevant-radiation, and left on their own, may never completely resolve.
4) In addition to radiation-induced deficits in adaptive learning and problem solving, radiation-induced psychological disorders (e.g., depression or bipolar disorder) remains relatively unexplored area of research and may portend potential problems in social interaction and decision making under autonomy and stress during long-duration space exploration.
5) Sex-specific differences in behavior and cognition after exposure to space radiation exist but the precise reasons for this remain uncertain at present.
6) Radiation promotes the emergence of significant inter-individual variability. This argues that sample sizes ought to be carefully considered to delineate the cause of such variation – an issue that could also be addressed during judicious pre-selection/pre-screening criteria of the astronaut pool.

Clearly, significant research in rodents has identified that space relevant radiation exposures elicit long lasting behavioral and cognitive changes that suggest potential risks for astronauts manifesting performance decrements during deep space missions and neurodegeneration upon return to the terrestrial environment. However, extrapolating current radiation data sets in rodents to humans is an imperfect science. More importantly, while the above highlighted issues are directly relevant to the impact of space radiation on CNS function, at present, it is unclear how concurrent exposure to other spaceflight stressors (e.g., microgravity, isolation/confinement) and space relevant radiation exposure will impact all of the above issues.

NASA is deeply concerned about how the combinations of these three major spaceflight stressors impact overall CNS-related neurobiological, neurochemical, neurobehavioral and cognitive function in-flight, as well as more chronic neurodegenerative conditions when astronauts return to earth. These knowledge gaps have significantly hindered NASA's ability to realistically estimate spaceflight hazard risk to the CNS associated with human space exploration, an issue that requires considerable refinement before embarking on future deep space missions. Remarkably, notwithstanding the progress to date in rodent studies of space radiobiology and other spaceflight hazards, there have been very few comparable investigations of the impact of these spaceflight stressors alone and, to our knowledge, none in combination using NHP animal models. In view of the accepted translational value of NHP data in advancing medical discoveries, this research deficiency is an unfortunate gap in our efforts toward understanding the impact of individual and/or combined spaceflight stressors on CNS-related neurobiological, neurochemical, neurobehavioral and cognitive function. In this report, we sought to address this knowledge gap by conducting a comprehensive and systematic evaluation of existing research on the acute and long-term impact of exposure to spaceflight stressors on NHP CNS function. In this report, we a) provide a brief background for the review and its objectives; b) offer an overarching view of the strengths, limitations, and translational value of NHP research in its own right and within the existing context of NASA-relevant rodent research (Table 1); c) describe the methodology used to achieve our objectives; d) highlight specific results and conclusions from our efforts; and e) provide a set of targeted areas of NHP research needed for future endeavors (see Table 2).

It is important to set expectations regarding the underlying science that is specific and/or general to mammalian species subjected to ionizing radiation. This is particularly germane to the current focus on

CNS function in NHPs, given the paucity of data sets derived from experiments originally designed to analyze functional changes in the CNS following radiation exposure in combination with other spaceflight stressors. While the connectivity and architecture of mammalian brains differ considerably, at the cellular level, and especially in regard to their response to radiation sensitivity, there is nothing in particular that distinguishes the radiation response of a rodent, NHP, or human neuron (or other CNS cell types). Radiation chemistry is linear with dose, and while biological responses will certainly depend on intracellular differences in chemical constituents, macromolecular structure and gene expression, covalent changes caused by radiation exposure will be fundamentally similar. However, at present, it is unclear whether space radiation-induced changes in CNS activity of a rodent, NHP or human will differ (or not) upon simultaneous exposure to other spaceflight stressors (e.g., isolation/confinement and microgravity) during deep space exploration. Moreover, what is not expected to be similar are the functional outcomes (cognition, neural activity/connectivity, neurotransmission, electrophysiology, perpetuation of oxidative and inflammatory cascades, and metabolomics/lipidomics/proteomics/RNA-seq biomarkers) that can be interrogated by modern day scientific techniques in behavioral testing, in dwelling neurochemical probes and electrodes, structural and functional imaging, and a range of omics-based biomarker discovery platforms (see Table 3). Each of these readouts will then depend greatly on a concerted series of complex processes interacting in cell, tissue, and species-specific ways. That said, we have reviewed a number of existing studies in this report that point to a critical need for research in NHPs, with a series of suggestions pointing toward the best avenues to pursue (see Table 2).

It is important to recognize that studies in NHPs can be designed to explore many of NASA's needs that are outlined in the Integration Research Plan (see Table 2 for research recommendations and priorities and Table 3 for alternative endpoints). However, given that NHPs are a valuable resource, their use in NASA-relevant scientific research *must* be carefully considered and scientifically justified. Accordingly, we have judiciously identified research gaps and provide a set of recommendations in Table 2 for future targeted studies in NHPs that will efficiently and directly provide quantitative, qualitative, and correlative information on the acute and long-term impact of spaceflight environment on a) behavioral performance, b) neuropsychological domains, c) brain performance pathways and mechanisms, d) CNS-related diseases, and e) biomarkers associated with behavioral performance and complex cognitive processes. These gaps and recommendations are guided by several *key* considerations. First, our aim was to establish a solid framework (e.g., key indicators, methodology, and endpoints) of targeted translational studies that can be conducted in both rodents and NHPs. This will allow NASA to realistically relate outcomes across species, i.e., rodents ↔ NHPs ↔ humans. Second, the proven reliability of NHPs as subjects in long-term (i.e., longitudinal) neurobehavioral/cognitive studies together with the ability to use powerful within-subject designs that are similar to those used in human laboratory studies permit meaningful conclusions and inferences based on the evaluation of treatment effects (see Tables 1 and 4). Consequently, we recommend that NASA's research program in NHPs *must* encompass within-subject investigations that will efficiently deliver data sets on multiple endpoints assessing the acute and long-term risk of combined spaceflight stressors for humans (see Table 2). With regard to spaceflight stressors, radiation exposure is another important issue to consider when designing experiments in NHPs. Based on current data sets in rodents, we recommend chronic GCR (1 month, 4 weeks, 6 days/week; 40 cGy total dose. Irradiation implements the "33 beam" GCR simulation conducted at the NASA Space Radiation Laboratory at Brookhaven National Laboratory). This corresponds to the most accurate terrestrial simulation of the space radiation fields; and chronic low dose rate (1 mGy/day) neutron exposures. This is the best simulation of space relevant dose rates on Earth. With regard to other stressors, while isolation/confinement can be easily incorporated into NHP study designs, the ability to simulate microgravity conditions may be more challenging.

Based on the copious available information from rodent studies and the paucity of functionally similar studies in NHPs reviewed, we describe critical research gaps and priorities in NHPs related to the Integration Research Plan that can be considered by NASA moving forward to bridge the key knowledge gaps that currently exist in deciphering how best to interpret rodent data to humans (see Table 2). In Tables 2, 3 and 4, we provide a roadmap of scientific gaps and research recommendations for NASA regarding the availability, validity, strengths, and limitations of NHP models in future targeted research, including points of contact between existing NASA-relevant rodent assays and functional NHP analogs. The main goals of this roadmap are to efficiently and directly address the CNS-related risks due to space radiation, stress, and altered gravity on brain performance pathways that may play a key role in crew behavioral health and performance during long-duration space exploration, i.e., a Mars mission. These risks currently have the potential to catastrophically "surprise" NASA by an unanticipated event or suboptimal response to sudden circumstances (spaceflight stressors) encountered during deep space missions.

Declaration of Competing Interest

None.

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APPENDIX

Literature Search

We conducted a targeted search of specific databases including PubMed and Web of Science as well as space agency databases such as the NASA technical report repositories, Military and Government Collection, and more general databases such as Google Scholar, EBSCOhost and other research websites (e.g., ResearchGate). We included a broad range of search terms and keywords related to the influence of spaceflight hazards on CNS function in NHPs to identify articles: molecular/cellular function; observable behavior, physiological and motor function; neurochemical activity; neurobiological structure and function; and neurobehavioral and cognitive performance. A detailed list of our search terms and keywords is provided in Table 5. We also searched specific spaceflight-relevant journals. In addition, we reviewed previous evidence reports produced by the HRP, i.e., Risk of Acute and Late Central Nervous System Effects from Radiation Exposure, HRP Space Radiation Program Element, Gregory A. Nelson, Lisa Simonsen, and Janice L. Huff; https://ntrs.nasa.gov/search.jsp?R=20160004368 2018–07–25T04:10:47+00:00Z) as well as several recently completed reviews of similar domain areas (e.g., Akoev et al., 1981; Cucinotta et al., 2014; Dalrymple et al., 1991; Kokhan et al., 2016; Singh and Olabisi, 2017; Vazquez, 1998).

7In addition to the approach described above, we sought to obtain unpublished or in-press research on the impact of any of the spaceflight hazards alone or in combination on all of the above described aspects of CNS functioning that is being conducted within the US, i.e., spaceflight relevant research that may be generated by the 7 US National Primate Research Centers (https://nprcresearch.org/primate/research.php). Although these NHP institutions focus on several key areas of research

such as aging, behavior/psychology, neuroscience, that are somewhat relevant to issues that may be associated to NASA's Integration Research Plan, no research domains appear to address specific issues involving the combined impact of three spaceflight environment stressors, e.g., space radiation, microgravity, and isolation/confinement. Consequently, we were unable to identify additional articles or datasets to generate a total number of sources. Yet, since our approach in this report is to provide a wide-ranging view of NASA-relevant research that can be conducted in NHPs, we have identified NHP-based research articles for inclusion in our review.

Table 5
List of search terms.

Search Term Category	"Space Radiation" AND/OR "Microgravity" AND/OR "Stress/Isolation/Confinement" AND "Monkeys" AND:
Overall Search Terms	**1)** "Molecular"; **2)** "Cellular"; **3)** "Observable OR Overt Behavior"; **4)** "Physiological"; **5)** "Motor"; **6)** "Neurochemical"; **7)** "Neurobiological"; **8)** "Brain Structure"; **9)** "Brain Function"; **10)** "Neuroanatomy"; **11)** "Neurobehavioral"; and **12)** "Cognition" OR "Cognitive"
Molecular and Cellular Function	**1)** "Neuroinflammation"; **2)** "Hematopoietic Function"; **3)** "Inflammatory Response"; **4)** "Brain Injury"; **5)** "Immunohistochemical"; **6)** "Western blot"; **7)** "Microglia"; **8)** "Astrocytes"; **9)** "Receptors", e.g., purinergic"; **10)** "Pro-inflammatory cytokines (e.g., IL-1beta, IL-6, IL-10, TNFalpha)"; **11)** "Cemokines"; **12)** "DNA microarray"; **13)** "Gene Expression"; **14)** "Prefrontal Cortex"; **15)** "Striatum"; **16)** "Amygdala"; and **17)** "Hippocampus"
Physiological and Motor Function	**1)** "Adverse Reactions"; **2)** "Emesis"; **3)** "Telemetry"; **4)** "Temperature"; **5)** "Cardiovascular Function"; **6)** "Heart Rate"; **7)** "Blood Pressure"; **8)** "Circadian"; **9)** "Motor Activity or function"; **10)** "Actigraphy"; and **11)** "Operant"
Neurochemical Activity	**1)** "Neurotransmitters"; **2)** "Binding"; **3)** "Microdialysis"; **4)** "Dopamine"; **5)** "Serotonin"; **6)** Norepinephrine"; **7)** "Glutamate"; **8)** "γ-aminobutyric acid or (GABA)"; **9)** "Acetylcholine"; **10)** "Cannabinoid"; **11)** "Monoaminergic"; **12)** "Cholinergic"; **13)** "Opioid"; **14)** "Trace Amines"; **15)** "Purines"; **16)** "Gasotransmitters"; **17)** "Neuropeptides"; **18)** "Corticotrophin Releasing Factor"; and **19)** "Metabolites"
Neurobiological Structure and Function	**1)** "Brain Connectivity"; **2)** "Myelination"; **3)** "Synaptic Density"; **4)** "Diffusion Tensor Imaging (DTI)"; **5)** "Neuroimaging"; **6)** "White Matter Microstructure"; **7)** "Region-specific gray Matter Volume"; **8)** "Diffusion Weighted Imaging/ Morphometry"; **9)** "Brain Region-Specific Neurochemistry and Metabolite Levels"; **10)** "Proton and Phoshorous Magnetic Resonance Spectroscopy"; **11)** "Functional Magnetic Resonance Imaging" and **12)** Task-based and Resting State Imaging"
Neurobehavioral and Cognitive Performance	**1)** "Complex Behavior"; **2)** "Cognitive Performance"; "Cognitive Function"; **3)** "Behavioral Tasks"; **4)** "Spontaneous Activity"; **5)** "Fear Conditioning"; **6)** "Extinction"; **7)** "Attention/Vigilance e.g., Psychomotor Vigilance Test"; **8)** "Attentional Set-Shifting"; **9)** "Working Memory" e.g., Delayed-Matching-To-Sample – or –Position Task"; **10)** "Touchscreen-Based Task"; **11)** "Motivation e.g., Progressive-Ratio Task OR Behavioral Economic Demand Analysis"; **12)** "Learning e.g., Stimulus Discrimination"; **13)** "Cognitive Flexibility, e.g., Stimulus Reversal"; **14)** "Cognitive Control, e.g., Flanker Task"; **15)** "Depression OR Anhedonia, e.g., Probabilistic Reward Task"; **16)** "Social Stress"; "Response Inhibition"; **17)** "Metabolic Stress"; and **18)** "Nutritional Stress"

Next, we conducted a more detailed review of all the identified sources and applied the inclusion criteria described above. To better appreciate the nature of the available data, sources were first sorted into nine categories: 1) space radiation review articles/reports; 2) molecular and cellular function NHP research articles; 3) physiological and motor function NHP research articles; 4) neurochemical activity NHP research articles; 5) neurobiological structure and function NHP research articles; 6) neurobehavioral and cognitive performance NHP research articles; 7) altered gravity NHP research articles; 8) other spaceflight NHP research articles; and 9) general NHP research articles. Subsequently, these reports were further categorized into the type of spaceflight hazard, amount of exposure, inclusion of quantitative and/or qualitative data on radiation dose, and sample size. Given that, to date, only limited information exists on the impact of spaceflight hazards on CNS function in NHP, sources that did not included full quantitative information were also reviewed further. Where possible quantitative data was further assessed as needed. All sources were reviewed for accuracy characteristics and other moderators along with the quantitative data.

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