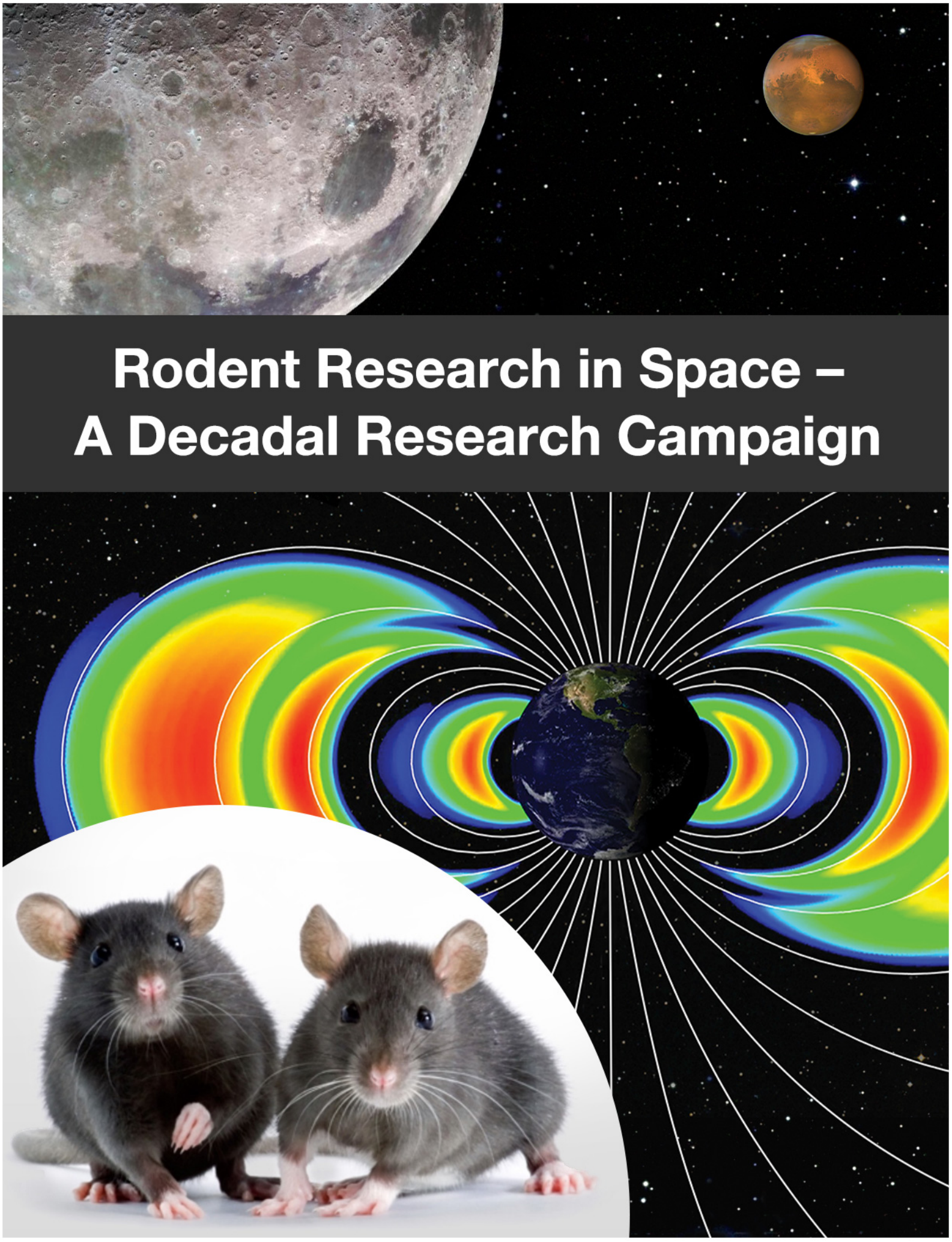
Rodent Research in Space – A Decadal Research Campaign



Rodent Research in Space: A Decadal Research Campaign

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Abstract: Studying the physiological effects of spaceflight in rodents is imperative to our understanding of adaptation and to providing countermeasures. We posit that studies of the reproductive system, development, stem cells, and behavior/cognition are critically important and provide outstanding opportunities as these are sentinel tissues and systems for radiation exposure and overall health. To improve understanding of the environmental stressors (weightlessness, space radiation, isolation/confinement) that impact space travelers, rodent research should be continued within and extended beyond LEO.

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List of Abbreviations
CNS: Central Nervous System
EEG: Electroencephalogram
ISS: International Space Station
GCR: Galactic Cosmic Ray
JAXA: Japanese Aerospace Exploration Agency
LEO: Low Earth Orbit
MRI: Magnetic Resonance Imaging
NASA: National Aeronautics and Space Administration
NSRL: NASA Space Radiation Lab
PVT: Psychomotor Vigilance Test
SLS: Space Launch System
STS: Space Transportation System

Statement on Animal Care and Use: All NASA missions and procedures involving rodents are conducted with cognizance for animal welfare and within the guidelines of the NASA's Institutional Care and Use Committee.

Introduction

For centuries, scientists have used animal models to understand various aspects of human biology. Within the NASA space program, rodent research models have been fundamental and irreplaceable to the continued advancement of basic biology and medicine; as well as for: (1) defining biomedical risks, (2) revealing underlying physiological mechanisms, and (3) developing and testing countermeasures without endangering the health of human subjects. Additionally, scientists can use rodent models in controlled environmental conditions employing elaborate experimental protocols to collect data that are difficult, if not impossible, to achieve in human subjects (Maggiacomo & Stegmaier, 2021). Examples include radiation exposures, immune system dysfunction, microbiome manipulation, cancer formation and development, healing processes after wounds or fractures, hazardous exposures, and use of biotelemetry and detailed tissue analyses post-mortem. In-flight and post-flight rodent biologic and behavioral data have provided critical insights into the fundamental effects of space flight on physiologic and nervous systems that impact human biology and commercial research objectives (*Translational Cell and Animal Research*, 2015; *The Neurolab Spacelab Mission,* 2003; Haymaker et al, 1975). Rodents are excellent surrogates for extrapolating to humans the consequences of long duration spaceflight on growth, development, reproduction, multigenerational reproductive fitness, and aging throughout the lifecycle; since only six months in space for a rat is ~38% of its life expectancy, which approximates 27 years for an adult human. Together, these advantages motivated >60 years of productive rodent research in NASA's Life Sciences programs and by international organizations (*Translational Cell and Animal Research*, 2015; The Biology of Spaceflight, 2020). Science is an iterative process and thus, despite decades of impactful and meaningful studies, critical knowledge gaps remain in our understanding of how animals including humans adapt to life in space. In the following paragraphs, we briefly summarize several of these gaps and our recommendations on how to best address these critically important questions vital for improving the health of future space travelers.

These gaps in our knowledge include: 1) the combined effect of space radiation and microgravity on physiologic systems, 2) limitations in long-term exposures to space, 3) comprehensive in-flight behavioral and neurological evaluations, and 4) limited reproductive/ endocrine, developmental, and stem cell studies, all of which impacts our base of understanding regarding how spaceflight impacts the human body's ability to adapt and thrive in space.

Outside of the Apollo missions of the 1970's, most missions have occurred in LEO within the protective shield of the van Allen belts against space radiation. In LEO, the effects of microgravity are readily studied; however, inferring insults of space radiation, another major environmental impactor to mammalian health, is limited on ISS given the GCR exposure in deep space, on the Moon, or Mars, will be different. Potential ways to enhance our understanding of the potentially synergistic effects of space radiation with microgravity exist including: 1) designing orbits for free-flyer satellites outside the van Allen belts (e.g., polar or other highly-inclined orbits) and 2) implementing pre- and post-flight exposures to high-fidelity galactic cosmic ray (GCR) simulations at NSRL or neutron exposures at the Neutron Radiation Facility at Colorado State University (White Paper by Zawaski et al, 2021; Norbury, et al, 2016). Ultimately, rodent missions beyond LEO would allow combined exposure to true space-irradiation fluences and weightlessness (*Life Beyond Low Earth Orbit*, 2018). Experiments beyond LEO necessitate iterations to the requirements and capabilities of existing rodent habitats (e.g., videography, audio, telemetry, cognitive assays) through the Science Working Group for Rodent Research (Chairs: Y. Shirazi and C. Fuller).

In our view, a robust campaign with rodents over the next decade should be continued and include three elements: 1) augmented experimental designs in LEO to enhance exposure to deep-space radiation; 2) plan, build, and commence missions for extension of key studies beyond LEO; and 3) strong support for ground-based science programs, e.g. gravity as a continuum (White Paper by Alwood et al, 2021), to strengthen basic and translational science at NASA (Alwood et al, 2017; Sides et al, 2021). Our long-term vision is to include a mammalian biological sentinel for the astronauts on exploration class missions to the Moon and eventually to Mars.

Vision for the Coming Decade

During the next ten years, we envision thriving missions continuing within Earth's orbit (e.g., ISS and commercial space stations) and at the Moon with NASA's Artemis Program, which opens new domains for rodent research with the Space Launch System (SLS), Orion, and new commercial vehicles. To effectively leverage these opportunities, NASA must develop Science and Implementation Plans specifically for sending rodents beyond LEO, including designing reference missions, as soon as possible, along the lines of the Science Definition Team report for the Artemis III mission within Planetary Sciences (*Artemis III,* 2020).

Sending rodents beyond LEO provides new opportunities for scientific discoveries and for verification and validation of our understanding of mammalian systems at the organismal level (*Life Beyond Low Earth Orbit*, 2018). Specifically, using rodents as concurrent sentinels for astronauts allows the investigation of true spaceflight effects across species, critically including exposure to space radiation in the weightlessness of microgravity or altered lunar gravity. We highlight reproductive, developmental, stem cell, and CNS studies as these sentinel tissues are critical for the health and function and are those first to be compromised in cases of environmental stress (Rajkovic et al, 2017). We make the following recommendations:

1. Reproductive & Developmental Studies (Summary of White Paper by Ronca et al, 2021)

We recommend that NASA conduct systematic reproductive and development studies within and beyond LEO leading to multi-generational experiments to determine the viability and heritable characteristics of rodents reared in space and to assess epigenomic effects.

Reproductive and developmental biology have been at the forefront of many fundamental biomedical advancements over the last several decades, including stem cell technology, treatments for genetic disorders, influence of epigenetics and genetics, and are likely to play key roles in preservation of species as our world changes. Studying mammalian life cycles in space promises to uncover astounding new insights into how gravity shaped life on Earth and the adaptability of humans and animals to life beyond Earth. The reproductive system (i.e., germline) is the most sensitive tissue to irradiation, hence is a bellwether. This has been recognized by multiple National Research Council (1988, 1991, 1998, and 2011) panels convened to guide NASA Space Biology research. These panels and others have emphasized the fundamental importance of research on development and reproduction of mammals in space, specifying that animals should be studied both within and across generations, completing two full life cycles in space to produce truly space-developed progeny. Some of the most fundamental questions concerning viability and health require these multigeneration studies.

A detailed reproductive-developmental research roadmap and relevant high-priority spaceflight experiments were generated in the NASA Rodent Mark III Habitat Workshop (Ronca et al, 2013 and White Paper by Ronca et al, 2021), including knowledge gaps in fertility, pregnancy and parturition, neonatal development and weaning, development of key sensorimotor systems,

and, importantly, lifespan and multigenerational studies. It was noted for successful multigenerational studies of rodents in space to commence, significant knowledge gaps related to impacts of long-term space flight on reproductive health of males and females must be addressed. A recent analysis of post-mortem vaginal wall tissue from the RR-1 validation mission, indicates that estrous cyclicity of female mice resumes during long-term microgravity exposure (Hong et al, 2021). This work paves the way for the upcoming RR-20 mission (presently slated for 2023) to address whether females in-flight can ovulate, produce normal levels of ovarian steroids, and elicit normal steroidal actions in recipient tissues such as brain, uterus, bone, and muscle. Exposures to both longer duration flights and to elevated radiation levels combined with microgravity are needed in both males and females to characterize and understand germline and supporting somatic cell effects. Further, multiple habitats with differing capabilities will be required to meet requirements for successful breeding, birthing and nursing, maturation, and aging. NASA undertook the design and testing of such hardware but abandoned the program when it deleted the U.S. research module from the ISS. It is time to revive these priorities so they can contribute needed data to contemporary goals.

Two recent innovative reproductive and developmental studies have been conducted by other space agencies (Lei et al, 2020; Matsumura et al, 2019). The recent JAXA study, showed that after 35 days on orbit and subsequent return to Earth, sperm harvested from males exposed to 0g or 1g (via chronic centrifugation) were successful in fertilizing and siring healthy offspring (Matsumura et al., 2019). More recently the Chinese Space Administration (Lei et al, 2020) launched preimplantation mouse embryos aboard a SJ-10 recoverable satellite. The study demonstrated that embryo development continued during short-term spaceflight, but the rate of blastocyst formation and blastocyst quality were compromised. Embryonic cells contained severe DNA damage and the DNA was globally hypomethylated and presented with a unique set of differentially methylated regions. These initial studies emphasize our need to complete better experimentally designed studies of early mammalian development where combined effects of microgravity and radiation exposure can be properly analyzed.

We believe that the study of reproduction and development of mammals in space can serve as a springboard for better understanding how gravitational forces and radiation impacts life on Earth. Studies in these two areas have been instrumental in much of our current understanding of genetics, epigenetics, stem cell, and organismal biology. Moving forward, no agency is better suited than NASA for establishing Earth's impact on biological systems by studying mammalian development in the weightlessness of space. In the White Paper by Ronca et al, 2021, we have defined the key intellectual foundations and believe that the ISS and emerging space platforms beyond LEO can be equipped for these important scientific pursuits.

2. Stem Cell Health and Function (Summary of White Paper by Almeida & Juran, 2021)

We recommend that NASA conduct tissue regenerative health studies within and beyond LEO to understand the responses the various tissue regenerative stem cell niches in humans and relevant model organisms. Special emphases of future work should focus on how regenerative deficits in whole-organism stem cell niches may lead to tissue degeneration and premature aging within the deep-space environment outside of LEO.

The maintenance of healthy adult tissues in mammals requires a complex homeostasis of molecular, cellular, tissue, and metabolic processes which are fundamentally different from the development and aging processes that bookend life. Cellular homeostasis in the adult requires molecular maintenance and repair of non-dividing cells such as cardiomyocytes and neurons, but

also stem cell-based tissue regeneration via direct replacement of cell loss, such as in the blood, immune system, bone, skin, liver, intestine, and other tissues. Because stem cell based tissue regenerative health requires constant proliferation and differentiation of stem cell progenitors in the bone marrow, and other adult stem cell niches, these niches are uniquely sensitive to the stresses of spaceflight including exposure to space radiation and mechanical unloading in microgravity (Blaber et al, 2014). A key central hypothesis in this field is that spaceflight stress factors can have profound negative effects on long-term tissue regenerative health mediated by adult stem cells, and that unmitigated, these lead to premature tissue aging and functional failure. Specifically, it is thought that mechanical unloading due to lack of weight-bearing in space reduces mitogenic mechanotransduction necessary to promote adult stem cell proliferation and differentiation, and that space radiation can also lead to activation of cell cycle arrest mechanisms, further reducing adult stem cell proliferation. These hypotheses have been tested in LEO using a variety of cellular and whole organism tissue model systems, suggesting that spaceflight consistently interferes with stem cell tissue regenerative processes such as in mammalian embryoid bodies (Blaber et al, 2015), regenerating newt tails, and mouse bone marrow hematopoietic and osteoprogenitor cells.

We believe that future stem cell work should probe molecular mechanisms integrating both space radiation and mechanical disuse using leading technology. One example is Almeida et al's investigation of the role of oxidative stress and the cell cycle inhibitor Cdkn1a (Blaber et al, 2013) in the skeletal system using single cell (scRNAseq) expressome analysis of bone marrow osteoprogenitors (Juran et al, 2021) including in various mouse transgenic null backgrounds relevant to these mechanisms. These are data likely to yield new insights into the development of next generation biomarkers, to inform disease diagnostics and to establish effective countermeasures for use on Earth and in Space.

3. Central Nervous System Studies (Stems from White Paper by Zawaski, et al, 2021)

We recommend NASA comprehensively study CNS effects in rodents within LEO and beyond LEO to assess space radiation and microgravity effects on operationally-relevant cognition and behavior.

In the human, newly acquired data reveal that brains of astronauts show concerning ventricular and white-matter changes (Jandial et al, 2018; Stahn & Kühn, 2021; Van Ombergen et al, 2019) and changes in electrical rhythms of the CNS (Petit et al., 2019). We largely do not know if rodent brains show similar changes, though many neurological and biomarker outcomes have been observed (Mhatre et al, 2021; Alwood et al, 2021). In rodents, microgravity causes adaptation in the vestibular nuclei (Pompeiano et al, 2002), adaptation of hippocampal neurons and behavior (Knierim et al, 2000), and evidence of altered permeability of the blood-brain barrier (Mao et al, 2020). From ground-based studies using disuse or simulated space irradiation, rodents show impaired spatial learning and memory consolidation, some which can be rescued by transcranial magnetic stimulation (Shukitt-Hale et al, 2000; Zhai et al, 2020; and Xu et al, 2021). Emphasis should be given to establishing and validating translatability between rodents and humans where feasible (White Paper by Nelson et al, 2021).

Internationally, not one of the Space Life Science Programs has a means to assess cognition and behavior in rodents on orbit using gold-standard targeted assays like the PVT which can be conducted in both rodents and humans (White Paper by Zawaski et al, 2021), despite the value. Learning and plasticity are critical areas to assess in humans and animals during a mission to gauge the general level of cognitive and sensorimotor adaptability. The 30-day Bion-M1 mission

illustrated the value of linking behavioral outcomes with neurotransmitter and molecular analyses (Andreev-Andrievskiy et al, 2014; Andreev-Andrievskiy et al, 2022; Tsybko et al, 2015; Naumenko et al, 2015; Popova et al, 2015). Lastly, daily videography is currently used to assess animal health and welfare during space missions. However, few studies (Ronca et al, 2019) have analyzed these data for behavioral outcomes and audio remains unavailable.

We therefore recommend before and after spaceflight cognitive/behavioral analyses (e.g., PVT used in the COGNITION battery for astronauts Casario et al, 2022) be assessed longitudinally in individual rodents. This would include EEG and/or imaging (e.g., MRI) and be paired with post-mortem intra-regional molecular and neurotransmitter status to further improve our mechanistic understanding of spaceflight adaptation in the mouse model and develop countermeasures against cognitive impairment. We also recommend that cognition/ behavior and/or EEG or other imaging modalities be assessed in individuals during the mission. This will require new hardware and software platforms to enable success. We also recommend observational studies of rodent behavior be standardized and routinely assessed in each mission using modern semi-automated tools (Markowitz et al, 2018) to catalyze this process. Additionally, use of sonic and ultrasonic microphone arrays should be considered for use in the rodent habitat to assess individual and cohort stress levels. Lastly, breakthrough studies on the ground have been conducted with optogenetic experiments to better understand and manipulate CNS outcomes. We recommend NASA consider using optogenetics for modifying cognition and behavior in rodents during spaceflight.

We believe that, by studying the CNS and better understanding how gravitational forces and irradiation impact this critically important system, Earth- and space-based research will benefit from significant translational improvements that will: (1) Enhance the ability of future astronauts and space travelers to cognitively function in the confines of outer space, and (2) manage the biological effects of environmental stressors associated with such travel. Rodent studies play a clear and necessary role in facilitating our knowledge base as well as the development of key countermeasures that can be successfully adapted to humans.

Closing

In closing, as astronaut-led exploration-class missions expand in distance from Earth and duration, there is so much more to learn in biology and physiology. Wherever humans go, rodents are needed on this journey as sentinels to continue our scientific inquiry into the biological and physiological effects of spaceflight.

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