**Topical: Recommendations to Accelerate Translation of Animal Experimental Findings to Humans**

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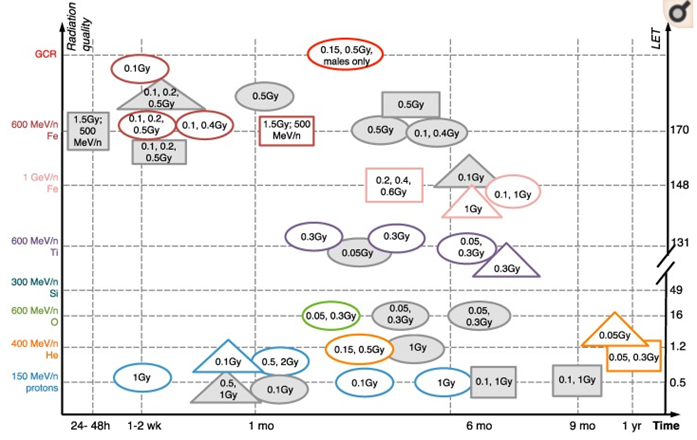
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**Introduction:** Characterization and management of human health outcomes associated with in- mission exposure to space radiation, microgravity, and isolation and confinement, among other stressors, are vital to protect astronaut health and wellbeing and support mission success. Beyond Earth’s magnetic field astronauts will be exposed to the full spectrum of chronic galactic cosmic rays (GCR), acute solar particle events (SPE), albedo neutrons on lunar or planetary surfaces as well as stressors. Due to ethical considerations of conducting radiation research on humans and the availability of a relatively small cohort of astronauts, space radiation research relies heavily on terrestrial radiation epidemiology to form the basis for most risk estimates. Epidemiology evidence is combined with ground-based animal studies to achieve appropriately powered experiments with total doses relevant to exploration missions with the goal of translating findings to humans. For cardiovascular disease a well-established epidemiological association exists at moderate to high doses of radiation1–5, however no such associations exist for impacts to the central nervous system (CNS) and behavioral health (BMed) for mission-relevant radiation doses. Therefore, the National Council on Radiation Protection and Measurements (NCRP) is exploring the Million Person Study that comprises multiple occupationally exposed cohorts for terrestrial epidemiological evidence6; and rodent studies have observed changes in a variety of cognitive and behavioral assays after simulated space radiation exposure7–10. Using these foundational associations, translation of animal experimental findings to humans can be done only if the models utilized are appropriate11 and data obtained comprehensive. To inform our understanding of astronaut physiology both ground- and spaceflight-based experiments have been utilized since the beginning of the space program12,13 but efforts to evolve animal to human translation have been hampered by several issues ranging from experimental design to lack of validation in spaceflight. ***This paper provides recommendations for accelerating translational animal findings to humans with the primary focus on risks to behavioral health (BMed) and the cardiovascular (CV) system.***These risks can impact the long-term health of crewmembers; however, recommendations have implications beyond these specific risks to immune status, bone effects, and carcinogenesis.

**Experimental Design Fundamentals:** Generalizability of past and current BMed and CV research findings to astronauts has been limited by numerous experimental design features. Changes in the delivery systems for the radiation and fiscal restrictions have limited the number of animals, sex, radiation types, radiation doses, dose rates, combined stressors, measurements performed, and time points analyzed. Thus when integrating and assessing the body of knowledge across experiments, data is heterogeneous, and sometimes incongruous which has limited the conclusions and translational value (Fig. 1). Increased experimental standardization and efforts to bolster experimental reproducibility (e.g., repeated experiments across labs) and generalizability (e.g., repeated experiments across species) will improve translatability to astronauts. A comprehensive and coordinated experimental design approach to assess ‘omics to clinically-relevant functional measures will provide the necessary framework to support robust experimental translation. In addition, due to the known interactions between organ systems, investigators should also be encouraged to study multiple organ systems and/or collect tissues from multiple organs for tissue sharing initiative to optimize research funds14.

Fig. 1. Literature review summary of space radiation effects on the central nervous system.Grey shaded shape indicates no impairment and lack of shading a radiation induced impairment. (Ovals=novel object recognition, Squares =spatial memory, Triangles=fear conditioning task. Cekanaviciute, E. et al, IJMS, 2018



A large proportion of past BMed and CV disease (CVD) investigations have utilized *ex-vivo* techniques without corresponding physiological functional endpoints or the inclusion of clinically/operationally relevant techniques. Functional measurements, and preferably clinically relevant, including cognitive and behavioral tests are needed even during strictly mechanistic studies to inform the relevance (construct validity) of the mechanism being studied, increase translatability of findings to humans, and identify practical, relevant biomarkers15. Previous human studies have included clinically-relevant functional imaging (MRI, ultrasound, PET/CT) for outcomes relevant to BMed and CV risks. Specific to BMed are operationally-relevant tasks, including those identified in NIMH’s RDoC criteria11 that have successfully linked neurological disorders to multi-level functional, structural, and cellular assessments which may help facilitate comparison of underlying neural circuitry across species. A recent NASA study16 recommended behavioral measures relevant to 1,125 operational tasks associated with a Mars mission and included in its assessment an animal analog rating. Seven high priority measures were identified: Longitudinal Actigraphy, Psychomotor Vigilance test, Stop-Signal Reaction time, Delayed Non-Match-to-Sample, Delayed Match-to-Sample, Effort Expenditure for Reward Task, and Penn Emotion Recognition. **Recommendation: Develop a comprehensive holistic experimental design approach that includes clinically- and ethologically-relevant functional endpoints.**

**Reverse Translation:** To support the translation and harmonization of animal responses to spaceflight stressors to the astronaut corps, a coordinated suite of measurements, with the accompanying stressor exposure-response curves for humans and animals needs to be established. The identification of permissible outcome levels and permissible exposure limits for spaceflight stressors (i.e., space radiation, altered gravity, isolation and confinement, sleep disruption) individually and combined will depend on defining scaling factors or transfer functions that can be used to relate human and animal response effect sizes. For further information see Nelson G.A. et al.. Topical: Reverse Translation Strategies to Support Cognitive and Behavioral Risk Characterization. **Recommendation: Exploit reverse translation techniques to establish scaling factors at operationally-relevant dose thresholds in animals.**

**Larger Species Animal Models:** Rodent models have many advantages which make them ubiquitous throughout scientific research and have contributed substantially to advances in understanding of the mechanistic underpinnings of risks relevant to spaceflight. However, rodents have significant limitations with respect to translating to humans. Observations in rodent models of CV disease (CVD) at space-relevant doses trend toward subclinical and non-functional outcomes related to endothelial cell biology that are not causally linked to CVD17. The use of a high fat diet to promote cardiovascular pathologies to improve statistical associations is not applicable to the astronaut population. The use of hindlimb unloading (HLU) as a microgravity analog for CV and BMed risks is controversial due to the limited amount of cephalic shift in body fluids compared to humans but does result in muscle atrophy, bone demineralization, changes in blood vessel structure and region-specific changes in blood flow18,19. Quantification of vascular gross anatomy is impeded due to the small size of rodent structures and costs associated with high-resolution imaging required. The small size of rodents limits longitudinal biological sample collection. Mouse models poorly mimic the genetic alterations in human inflammatory disease, which is highly relevant to radiation exposures. Rodent brains differ substantially with respect to structure and function (e.g., brain, pre-frontal cortex, neocortex size; frontal lobe development; brain cell and neuron numbers; synaptic number and connections; axon size; neurogenesis) as highlighted in Table 1 of Desai et al20.

Minipigs are a well-characterized translational animal model used in biomedical research and development to study a number of important aspects of human health including skin, metabolism, major organs, and immune status due to their anatomical, physiological, and biochemical similarities to humans. A well-characterized genotype and gene sequence homology between swine and humans is of added advantage21. Compared to rodents, coronary circulation in minipigs is anatomically similar to young human hearts with no anastomoses between branches of the vasculature, making it a robust model to conduct CV research after exposure to spaceflight stressors22. In addition, anatomical and physiological characteristics tested in different strains of minipigs accurately reflect the response of the human cardiovascular system in clinical and toxicity testing23. Minipigs are used as a model organism for multiple radiation-induced impacts including acute radiation syndrome24,25, cutaneous radiation injury (CRI)26, single-high-radiation-dose-induced lung injury27, and thoracic CT-based measurement of radiation-induced lung damage progression28. Furthermore, similar body thickness to humans allow characterization of radiation depth-dose relationships and volume effects.

Minipigs have also been developed as model systems for neurodegenerative diseases and cognitive and behavioral studies29,30. Brain size and structure are more comparable to primates and standard imaging techniques facilitate comparison to humans. Spatial and recognition memory can be evaluated with mazes analogous to rodent tests and delayed match or non-match to sample tasks have been developed31. Social recognition skills are highly developed and utilize multiple sensory modalities. Effects on pig brains from environmental toxin exposures have not been extensively characterized32, but they have been evaluated for traumatic and radiation brain injury33,34 and radiotherapy35 and found to be more radiosensitive than humans. Targeted behavioral and imaging studies as part of multi-system radiobiology investigations with space-like radiation exposures would provide valuable translational insight.

Furthermore, minipigs provide a robust model for compound-based countermeasure development, testing, and validation given the use of swine models in the pharmaceutical industry as a non-rodent model for toxicity studies and pharmacokinetics due to the human physiology similarities36. If compounds are successfully tested in both rodent and swine models, it may be possible to follow the FDA Animal Rule37 to approve use without conducting an efficacy study in humans. **Recommendation: Implement use of larger animal species, such as minipigs, where the organ(s) of interest is more comparable to humans to improve translation.**

**Innovative In-Silico Techniques:** Due to the abundance of space radiation rodent research but lack of corresponding human datasets, one approach to accelerate animal to human translation would be to create coordinating rodent and human tissues-on-a-chip (TOC) and organoids from different organs (or organ systems) to establish dose response curves and inter-species scaling factors following space hazard exposure and assess current assumptions. TOC/organoid models mimic true multi-cellular tissue structures and are sufficiently high throughput to evaluate physiological, molecular, and cellular hallmarks as well as changes in gene expression. Brain organoid systems express complex functional features, and over the course of many months, some can even exhibit evolving neural network electrical activity quantified by microelectrode arrays38–43. Similarly, bioengineered and highly structured 3D human cardiac organoids44,45 and human pluripotent stem cell-derived heart-forming organoids46 have been used to derive functional contractile tissue that resembles native adult heart tissue. Current techniques used to analyze organoid and TOC responses to stressors include high-resolution (single-cell) transcriptomic analysis47, simultaneous analysis of multiple organ responses to the same stressor48, and live, quantitative, extended-time course cellular network assays49, which yield relevant data for modeling the complex effects of spaceflight on human health. Advances in biomedical engineering have also led to bio-micro-electromechanical systems to enable incorporation of 3D microelectrode arrays and integrated photonic biosensors for detecting chemical/physiological changes or modulate responses by actuating changes. TOC technologies have already been deployed on the International Space Station50 as a model to study different tissue responses in the space environment. Therefore, the opportunity exists to validate ground-based research and assumptions by performing comparative flight and ground experiments.

A major strength of organoids is their longevity51: an organoid system that is stable for over one year would be suitable for payload adaptation for long-duration missions where crew time is limited or for uncrewed platforms. In contrast, TOC models currently have shorter lifespans, but greater potential for high throughput testing using, for example, 96-well plate formats,52 a benefit for countermeasure screening and validation. For more information refer to submitted white paper “Topical: Advancing telemetry-based biology for the Artemis era and beyond.” By Cekanaviciute E. et al..An important strength for both organoids and TOC models is the ability to develop and compare responses across multiple donors, possibly with pre-selected sensitivity levels, for personalized risk assessment and evaluation of individual sensitivity for deeper fundamental understanding of the variability of human responses to space radiation and estimation of risk confidence limits. For additional information on personalized medicine refer to submitted white paper "Topical: Enabling a Precision Health System for Deep Space Exploration" - Theriot, C. A., et al.. **Recommendation: Incorporate and leverage in-silico techniques to establish scaling factors at operationally-relevant dose thresholds in animals and validate ground-based analogs.**

**Inclusion of Spaceflight Experiments:** NASA has previously conducted research in animal models in low Earth orbit (LEO)12,53 and even on a single lunar mission. However, risk estimates of spaceflight-induced health effects depend heavily on ground-based research that cannot fully reproduce the fidelity and multiplicity of spaceflight hazards. The space radiation hazard is possibly the most difficult to accurately reproduce and incorporate into ground-based studies due to the chronic very low-dose rate dosing profile as well as the inability to use human subjects in experimental research. Charged particle radiation doses of between 10-25 cGy are typically sufficient to produce reliable outcomes in appropriately powered animal models of cognition, behavior, DNA damage, inflammation, and oxidative stress. Therefore, targeted spaceflight experiments could be used to verify observations/predictions from ground-based studies and assess risk model uncertainties for both animal and TOC/organoid models, given spaceflight exposures are robust enough to observe relevant outcomes. Repurposing of existing or development of requisite hardware needed for the prolonged habitation would be required to support animal research54. Spaceflight animal research offers the unique opportunity to interrogate cognition and behavior in mission as exposures accumulate – a research gap that has not been assessed by any space agency. Spaceflight could produce sufficient ambient exposures to GCR on 6-12 month Gateway or Lunar missions55 with modulation of radiation exposure/dose controlled by mission duration. To address this limitation, pre- or post-flight exposures to GCR simulations at the NASA Space Radiation Laboratory (NSRL) with transport of animals from launch and recovery sites could be implemented to interrogate interaction of radiation with other spaceflight stressors. Additionally, multiple dose profiles (e.g., multiple doses, fractionated or protracted exposures) could be used with this strategy. Importantly, robust ground controls need to be implemented for these flight studies to ensure results and conclusions are valid56.

**Recommendations: 1) Incorporate spaceflight experiments in LEO and beyond that validate/add to ground-based findings; 2) Leverage NSRL and NASA Rodent Research Program to better characterize the response to combined spaceflight hazards; and   
3) Monitor relevant outcomes in-flight.**

**Incorporation of Computational Methods:** Improvements in computing power, software, and data availability, have brought computational methods ranging from machine learning (ML) to virtual brain models to the forefront of biomedical research. ML is a branch of artificial intelligence (AI) that trains computational or mathematical models to predict aspects of new samples by learning from sample data. These approaches are uniquely suited for situations in which predictions are needed in an area where data is limited (e.g. human space radiation exposure data), but a large amount of relevant data exists in a related area (rodent space-like radiation exposure data). In addition, these could also map markers of disease states from existing clinical datasets to radiation exposure datasets.

Specific to neuroscience research, new methods such as neuromorphic computing (NC) are rapidly enabling iterative, real-time learning and simulation of the brain57. The European Human Brain Project has leveraged neuromorphic computing along with other computer platforms to integrate huge amounts of neuroscience data into models of the human brain58,59. Virtual brain models that reflect network and nodal representations of mass neuronal synaptic responses coupled with connectome information could help predict changes expected from exposures to hazards and facilitate forward and reverse translation based on changes routinely measured in crewmembers after spaceflight60. For additional information please refer to submitted white paper by Sanders L. & Qutub A. Topical: Development of New Algorithms for Space Biology.

Lastly, there are numerous initiatives focused on the assimilation and harmonization of data (Genelab, Life Science Data Archive) which highlight the importance of data management, infrastructure, and processing capabilities to accelerate finding translation especially in the context of deploying computational methods that generate large quantities of data. Computational techniques should be encouraged in future studies but it is important to note that techniques can be applied to already-acquired data61. For example, in animal behavior video analysis can identify micro-movement behaviraol patterns62 not detectable by conventional measurements. Radiomics is always evolving, histological and clinically relevant imaging can be reanalyzed due to advances63–65. Without the infrastructure to house, harmonize, and access datasets, the ability to leverage any computational method on past or future data will be severely limited. **Recommendations: 1) Incorporate computational methods into future studies and apply to previously collected data. 2) Continually update data management processes and enable robust data repositories to assimilate harmonized datasets for current and future use.**

**Conclusion:** In summary, over the last decade substantial radiation research on the CV and BMed have established the foundation to accelerate future findings and conclusions. To build on past studies we recommend **encouraging a comprehensive holistic experimental design approach that pairs human and animal (rodent to mini-pig) ground and spaceflight studies with standardized measures to utilize forward and reverse translation techniques with inclusion of clinically relevant functional endpoints, computational methods, and in-silico techniques when applicable.**

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