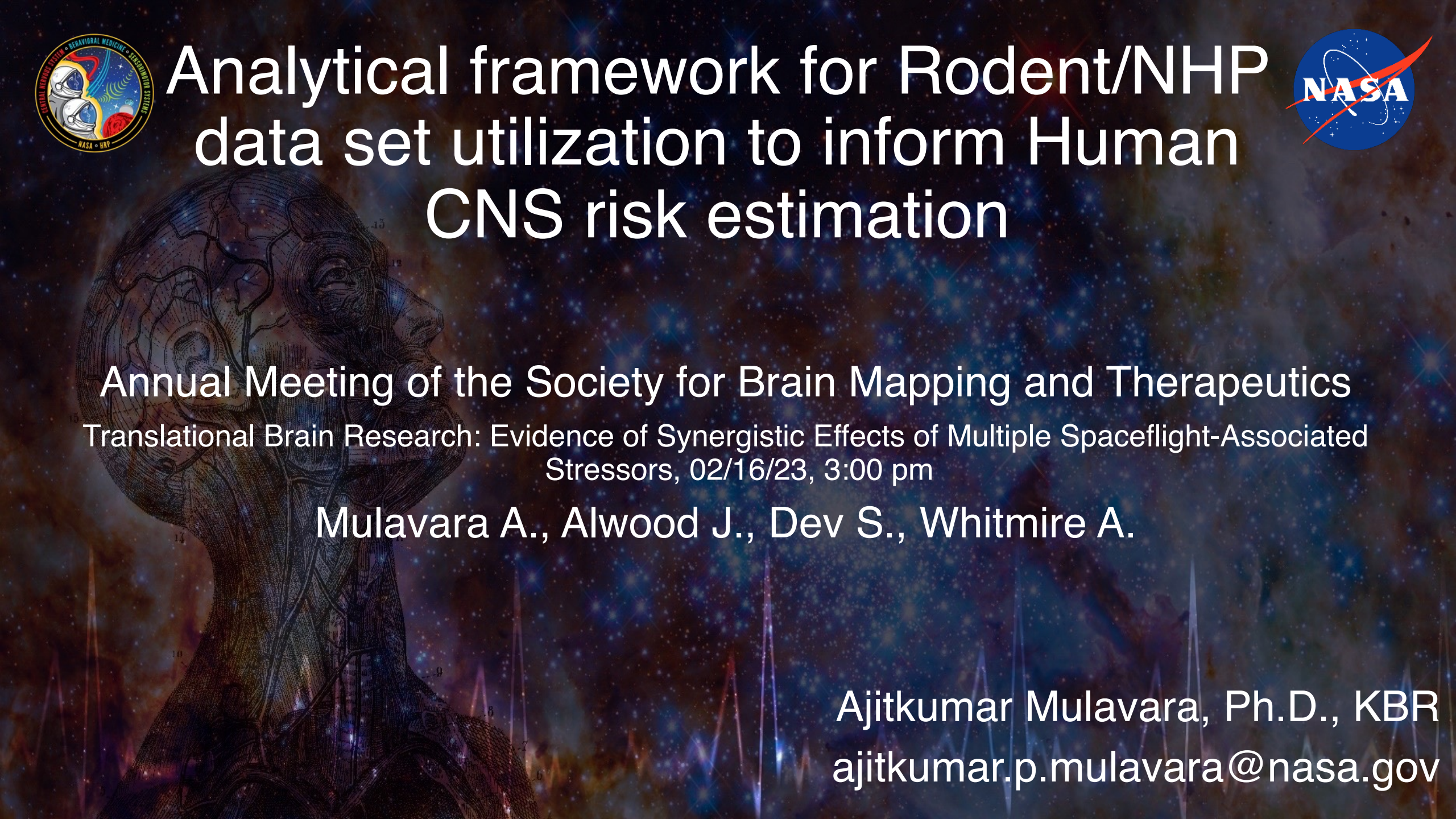
Analytical framework for Rodent/NHP data set utilization to inform Human CNS risk estimation



Annual Meeting of the Society for Brain Mapping and Therapeutics

Translational Brain Research: Evidence of Synergistic Effects of Multiple Spaceflight-Associated Stressors, 02/16/23, 3:00 pm

Mulavara A., Alwood J., Dev S., Whitmire A.

Ajitkumar Mulavara, Ph.D., KBR

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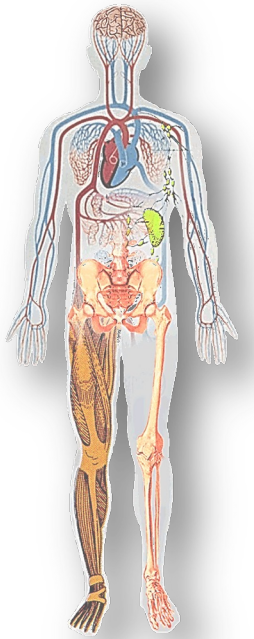
Risks Associated with Spaceflight

HRP seeks to align its research portfolio with high value risk mitigation targets agreed upon by the Human Systems Risk Board

Balance Disorders
Fluid Shifts
Cardiovascular Deconditioning
Muscle Atrophy
Bone Loss

Acute In-flight effects
Long-term cancer risk
CNS-Cognitive



Drives the need for additional "autonomous" medical care capacity – cannot come home for treatment

Vehicle Design
Acceleration/Vibration/ Noise
Environmental – CO_2 Levels, Toxic Exposures, Water, Food
Decreased Immune Function

Behavioral aspect of isolation
Sensory deprivation
Sleep disorders (circadian dysregulation)

https://www.nasa.gov/hrp/5-hazards-of-human-spaceflight

Behavioral Medicine Risk

The Risk of Adverse Cognitive or Behavioral Conditions and Psychiatric Disorders (Behavioral Medicine Risk) states:

"Given that crews of future exploration missions will be exposed to extended duration of isolation and confinement, greater distance from Earth, as well as increased exposures to radiation and altered gravity, there is a possibility that these singular or combined hazards could lead to

(a) adverse cognitive or behavioral conditions affecting crew health and performance during the mission;
(b) development of psychiatric disorders if adverse behavioral health conditions are undetected or inadequately mitigated; and
(c) long term health consequences, including late-emerging cognitive and behavioral changes."

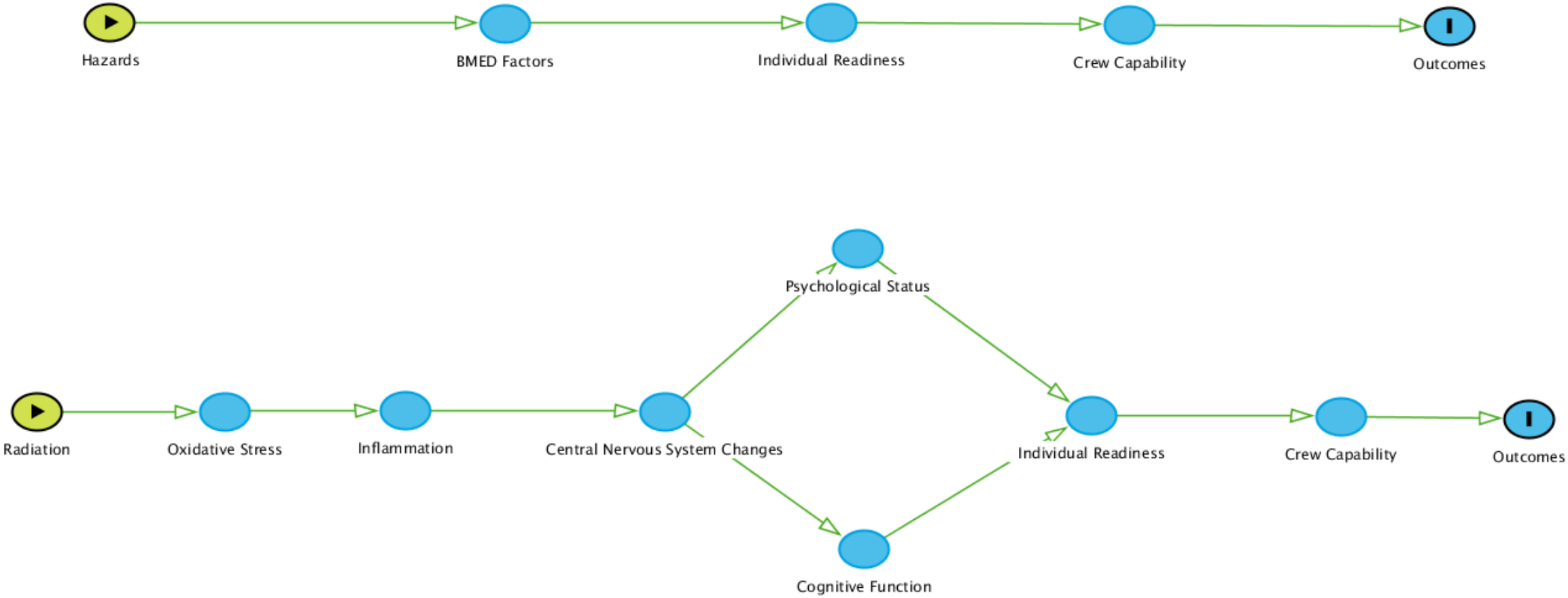
Objectives

Review and inform on tactics to analyze & communicate data from animal research (e.g., rodents, mini-pigs, NHPs, et al) exposed to single and combinations of spaceflight hazards, using NASA's Human System Risk Board (HSRB) process for the Behavioral Medicine Risk

- To quantitatively inform Behavioral Medicine Risk posture - i.e., likelihood (L) and consequence (C)

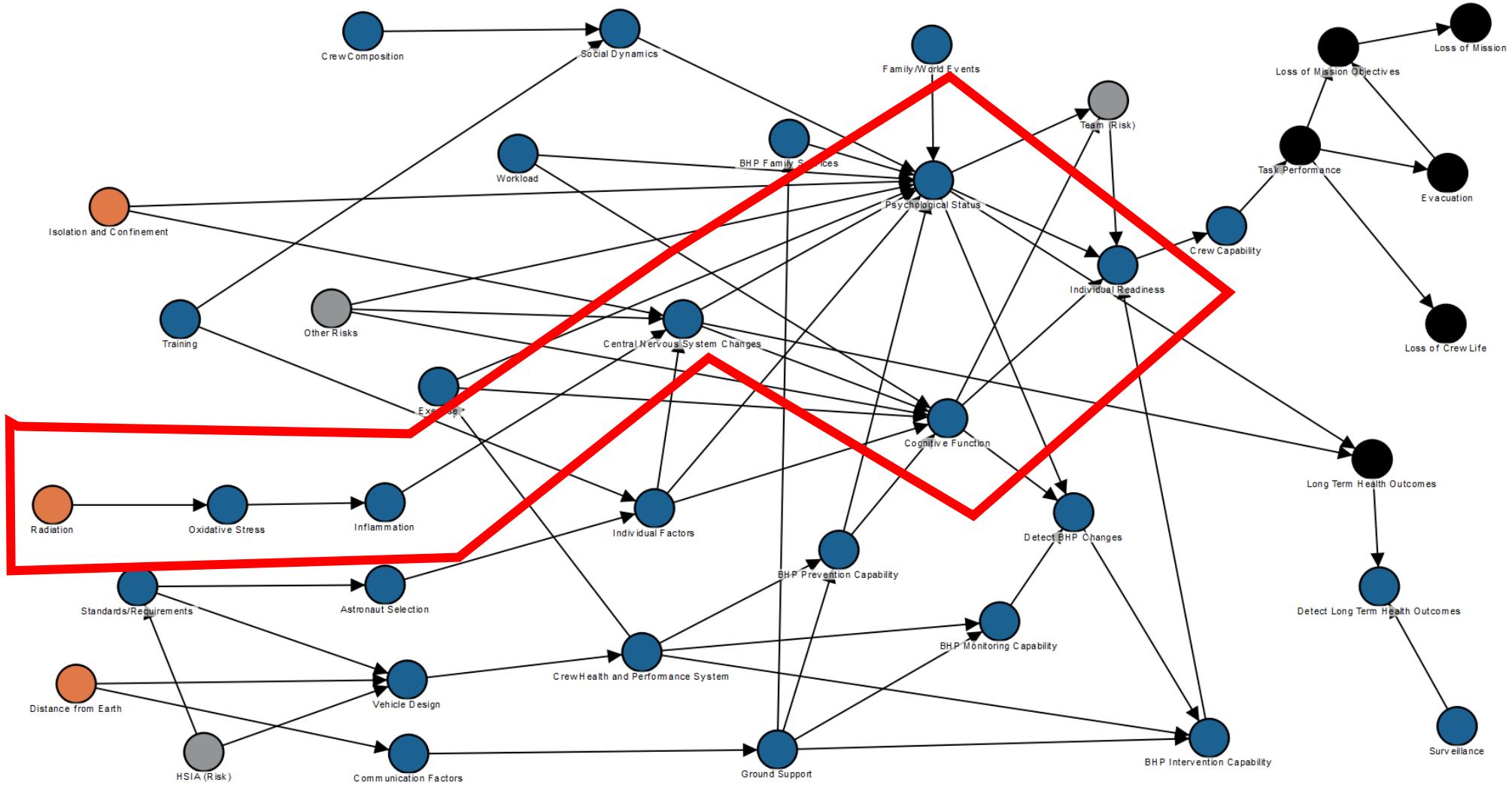
- To help inform estimation of crew health standards for operational task performance (i.e., Fitness For Duty, Space Permissible Exposure Levels, Permissible Operational Limits).

Directed Acyclic Graph (DAG) Behavioral Medicine Risk



- Courtsey of Antonsen E, NASA HSRB
- Antonsen A et al. Directed Acyclic Graph Guidance Documentation Human System Risk Board, NASA/TM– 20220006812, May 2022

Directed Acyclic Graph (DAG) Behavioral Medicine Risk



- Pictures Courtsey of Antonsen E, NASA HSRB
- Antonsen A et al. Directed Acyclic Graph Guidance Documentation Human System Risk Board, NASA/TM– 20220006812, May 2022

Background
HSRB Framework (JSC 66705)

The Level of Evidence (LOE) score to determine LxC score assigned to a Design Reference Mission (DRM) is based on Drivers presented in the risk package for each Impact Category (In-Mission, Return to Flight, Long Term Health)

Bradford-Hill Criteria

LOE	Temporality	Analogy	Mechanism	Reproducibility	Specificity	Coherence
4 - Speculative	✓	✓				
3 - Weak	✓	✓	✓			
2 - Moderate	✓	✓	✓	✓	✓	
1 - Strong	✓	✓	✓	✓	✓	✓

Table F- 1: Sir A. Bradford Hill's causal guidelines employed by the HSRB for level of evidence assessment.

Criterion	Definition	Notes
Temporality	The effect has to occur after the cause (and if there is an expected delay between the cause and expected effect, then the effect must occur after that delay).	This is necessary for all posited causal effects, even speculative ones.
Analogy	The use of analogies or similarities between the observed association and any other associations.	Analogues can be in exposure, population, or both.
Mechanism	If there is a plausible theoretical mechanism that can explain how the causal effect works then the posited causal connection is more likely to be true.	
Reproducibility	Consistent findings observed by different persons in different places with different samples strengthens the likelihood of an observed effect being causal.	
Specificity	Causation is likely if there is a very specific population at a specific site and disease with no other likely explanation. The more specific an association between a factor and an effect is, the bigger the probability of a causal relationship.	This is the classic Person/Place/Time of epidemiology.
Coherence	Coherence between epidemiological and laboratory findings that validate the mechanistic assumptions increases the likelihood of an effect.	This is translational science.

Level of Evidence in ascending order versus Bradford Hill Criteria (from Human Systems Risk Management Plan, JSC 66705, Rev A, 10/1/2020), https://www.nasa.gov/hhp/hsrb

Example of Weak LOE -
Space radiation hazard is considered to be In-Mission LxC Driver for human behavioral health impacts in a Mars DRM

- Animal studies showing the effects of radiation on the brain establish Temporality because the effects are measured after radiation exposure.
- Analogy is established because brain regions are affected that reasonably contribute to behavioral outcomes measured in animals and may extend to humans.
- Studies have identified damage to the hippocampus in mice for example, possibly suggesting Mechanism.
- In some cases they may also approach Reproducibility, though when results are reproduced they are often with radiation types, doses and dose-rates that are not applicable to the exposures astronauts are expected to see in spaceflight and substantial reporting/publication bias of negative results may exist.
- Specificity in these cases is not met because of the lack of generalizability to human astronauts in addition to the lack of specificity with radiation type, doses and dose rates.

Changing from Weak to Moderate LOE – For animal studies to meet criteria for Specificity: demonstrate attention to experimental design and external validation of translational research

- Moderate LOE may trigger a Risk or justify advocating for design impacts or mitigation consideration in vehicle requirements
- Weak LOE has a limited impact on Risk assessment but may indicate the need for additional research to evaluate a concerning contribution to risk

1	Studies demonstrate relevant assessment and selection of animals (e.g., animal age relevant to human age translation; performance screening—higher performing animals selected similar to our astronauts; maintaining regimen of exercise and "fitness" levels).
2.	Studies demonstrate careful matching between experimental and control groups (e.g., sex, age, other characteristics).
3.	Evidence is provided for the appropriateness of animal strains for the question being asked (e.g., for almost all behavioral domains, researchers continue to use inbred isogenic strains such as C57BL/6 mouse strains but these can have both genetic and behavioral differences if coming from different breeders and that should be addressed).
4.	Blind-coding of all analyses (e.g., evidence coding of data by someone other than the researchers so that analysis can be performed in an unbiased manner) is performed and described in the methodology sections.
5.	Statistical approaches are rigorously conducted and adequately documented in methodology sections.
6.	Evidence of experimental results in independent cohorts at different times across different labs are present in the literature.
7.	Studies demonstrate use of multiple outcome measures, including measures that are functionally relevant to humans.
8.	Evidence that researchers have regularly tested their animal models for quality control (e.g., genetic drift, loss of phenotype) is shown and adequately documented in methodology sections.
9.	Evidence of validation across models and in the human condition are available in the supporting literature.
10.	Consideration and addressing of any negative data (e.g., false negatives/false positives) and study limitations are documented.
11.	Obligation to address any evidence or data that seems to contradict research being represented is considered in limitations sections.
12.	If a failure in translation has occurred, that should be addressed within the context of the following: Was it the animal model itself, the analysis, the clinical trial, or another factor?

Recommendations for Animal/Cellular studies (Appendix F, Table-3, Antonsen, Human Systems Risk Management Plan, JSC 66705)

HSRB-Scoring Example # 1

Spaceflight and ground-based combinatorial stressor projects for Blood Brain Barrier (BBB): satisfy at least 5 of 12 animal studies specificity criteria (#2, 4, 5, 7, and 9). These findings improve *mechanistic* understanding of spaceflight effects (*specificity* of combined stressor environment), with *coherence*, and potentially increase the level of evidence from **Low → Moderate**:

- Elevated blood brain barrier (BBB) indices in blood of cosmonauts (n=5) after spaceflight
  - (neurofilament light chain (NfL), glial fibrillary acidic protein (GFAP), total tau, zu Eulenburg, et al, 2021)
- BBB integrity biomarkers changes in the hippocampus
  - (AQP4, GFAP, and PECAM-1, Mao, 2021)
- Changes in expression of 26 proteins in mice after 13 days in space
  - (related to synaptic vesicular activity, protein/organelle transport, oxidative stress, tissue damage responses, activation of catecholamines, and metabolic function, Mao et al., 2018)
- Effects in mice after prolonged combined exposure to simulated weightlessness (hindlimb unloading) and low-dose radiation (gamma) for 21 days when compared to their individual effects
  - (increased oxidative damage, reduced antioxidant defense, increased apoptosis, remodeling of the brain microvasculature , BBB dysfunction, increased expression of Nox2 (NADPH oxidase), increased risk-taking behavior, altered pathways in neurogenesis and neuroplasticity, regulation of neuropeptides, and cellular signaling, when compared to individual effects of stressorswhen compared to their individual effects, Bellone et al., 2016; Mao et al., 2016, 2017; Overbey et al., 2019)

HSRB-Scoring Example # 2

Radiation & Sleep findings meet 9 of 12 animal studies specificity criteria (# 1-5, 7, 9, 10, and 11). Hence, this body of work may meet **Low to Moderate** level of evidence:

- Outbred rat model and cognitive tests of executive function
- Rats selected based on assessment criteria before treatment, with maintained exercise before and after treatment
- Fragmented sleep negatively impacts cognitive ability (McCoy et al., 2007)
- Exacerbation of radiation-induced cognitive impairment in rats that were also subjected to sleep fragmentation, using the attentional set shifting (ATSET) assay of executive function (Britten et al., 2019)
- The ATSET assay is comparable/equivalent to human assay Wechsler Adult Intelligence Scale - WAIS, which has revealed cognitive effects of radiotherapy in humans (Sharma, et al, 2020), thus building some level of *coherence* (though lacking *specificity*).
- Dynamic ensemble prediction of cognitive performance in spaceflight (Tu, Basner et al. 2022), building on *coherence* with *specificity*.

Objectives

Review and inform on tactics to analyze & communicate data from animal research (e.g., rodents, mini-pigs, non-human primates, et al) exposed to single and combinations of spaceflight hazards, using NASA's Human System Risk Board process for the Behavioral Medicine Risk

✓ To quantitatively inform Behavioral Medicine Risk posture - i.e., likelihood (L) and consequence (C)

- To help inform estimation of crew health standards for operational task performance (i.e., Fitness For Duty, Space Permissible Exposure Levels, Permissible Operational Limits).

Survey of Analytical Methods

- National Committees (National Council for Radiation Protection - NCRP, National Academy of Sciences - NAS)
- NASA Space Cancer Risk (NSCR) - 2020 for Cancer Risk
- **Russia's Total Radiation Risk**
- **Meta-analyses using effects sizes**
- Reference and Benchmark Doses from Toxicology
- Graph Theory
- Principal component analyses (PCA) + Topological Analyses
- **AI/ML: Transfer learning**

Potential Human Cohorts for use as benchmarks
(E.g., Epidemiological / Personalized analyses: Mental Health status, Performance and Dosimetry)

Identification of relevant human cohorts would be valuable to assess with cognitive/behavioral testing

- Within-astronaut baseline
  - FFD 4.3.1 Mission Cognitive State (NASA-STD-3001)
- LEO crew members with ISS environment data:
  - Dynamic ensemble prediction of cognitive performance in spaceflight (Tu, Basner et al. Scientific Reports, 2022)
  - Chromosome aberrations in astronauts, peripheral lymphocytes (George, et al, Adv Space Res, 2007)
- Bedrest subjects (Basner et al. 2021)
- Workers in Antarctica
  - Individuals exposed to ICE with primary cosmic rays and secondary neutrons (Kato, et al, J Space Weather and Space Clim, 2021).
- Submariners on nuclear-powered crafts.
  - Isolated confined environments, low-dose radiation, operational (Department-of-the-Navy 2019).
- Airline pilots and crew flown over the Arctic
  - Exposure to primary cosmic rays (NCRP Commentary #12, 1995, https://www.faa.gov/data_research/research/med_humanfacs/aeromedical/radiobiology/cari7/
- Airline Pilots,, Air traffic controllers
  - fatigue/sleep quality indices (Basner M, Rubinstein J, 2011; Hilditch CJ, Flynn-Evans EE. 2022).
- The Million Person Study of radiation workers (Boice. The Million Person Study relevance to space exploration and Mars. Int J Radiat Biol. 2022).
  - Whole brain radiotherapy patients
    - the low linear energy transfer radiation controlled for co-morbidities involved as these may present many potential confounds (NCRP report 183, NCRP 2019) .

Russian Space Program's Total Radiation Risk

- Shafirkin and Petrov 2002*: non-radiation spaceflight factors intensify radiation effects up to 1.3-fold.
  - "… In the case of a prior impact of hyperthermia, a physical overload, strong hypokinesia (restriction of animal movements caused by the small volume of the cage), non-ionizing radiation with power flux of high density and a great heat effect, the subsequent irradiation results in a synergistic effect, and the extent of radiation damage is increased."

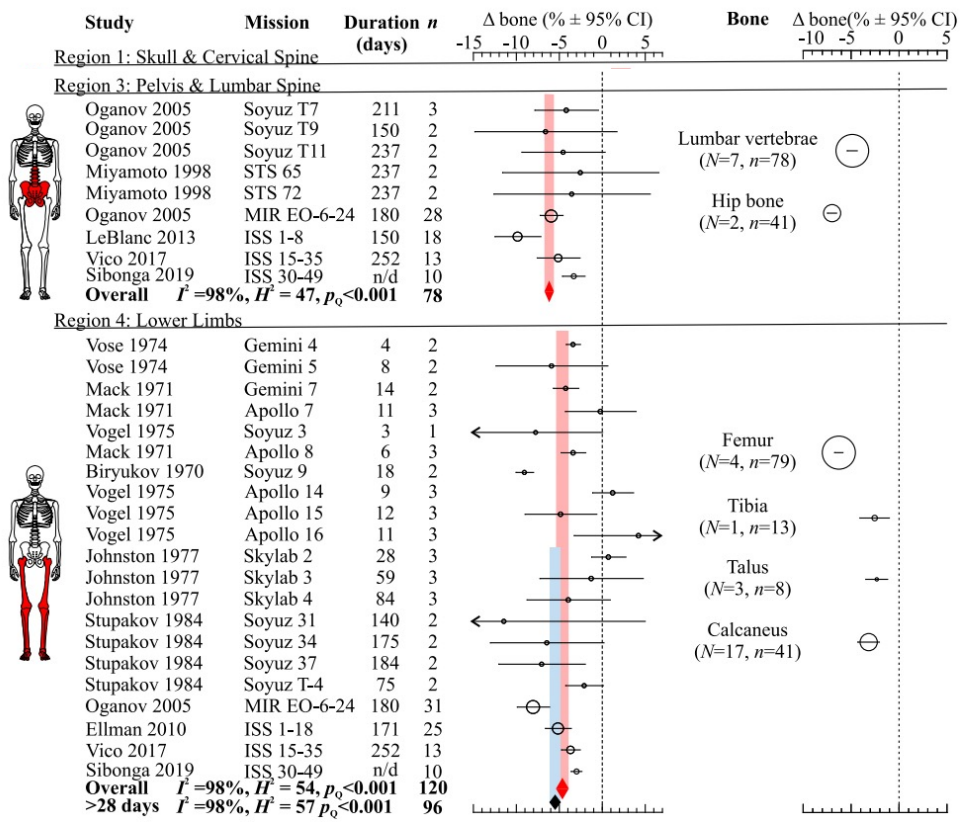
** Review Article: Adv Space Res, 2002*
Cites primary articles using cell and animal data: Saksonov et al., 1968; Antipov et al., 1975; Shafirkin and Farber, 1994

Meta-analyses rely on *effects sizes* to compare across studies and species; susceptible to publication bias

- As an example, skeletal response is quantitatively compared across spaceflight studies and qualitatively compared against astronaut data.

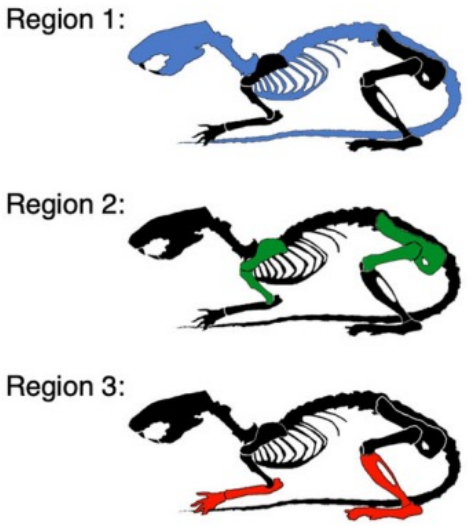
Astronaut bone changes across missions

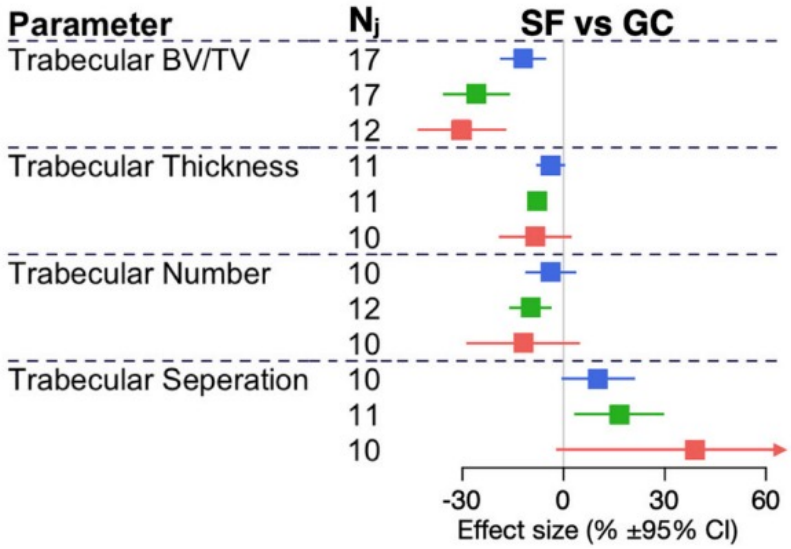
Stavnichuk, Komarova, et al, 2020



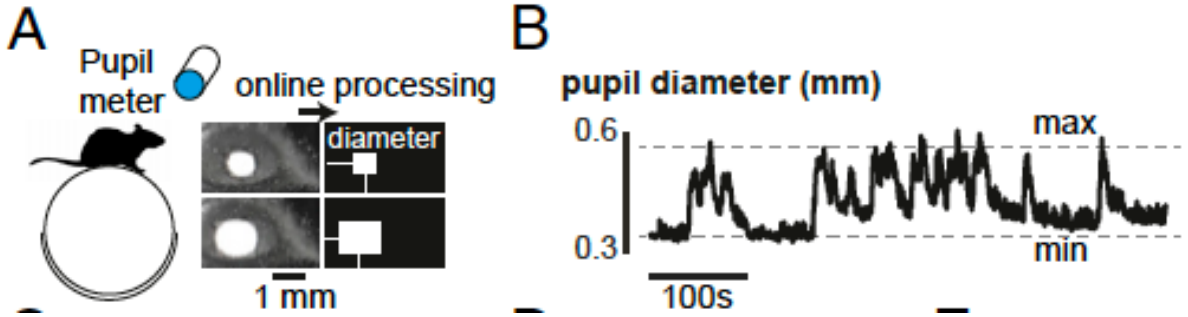
Bone changes in space-flown animals across experiments, i.e., rats, mice, primates (not shown)

Fu, Komarova, et al, 2021
Goldsmith, Komarova, et al, 2022

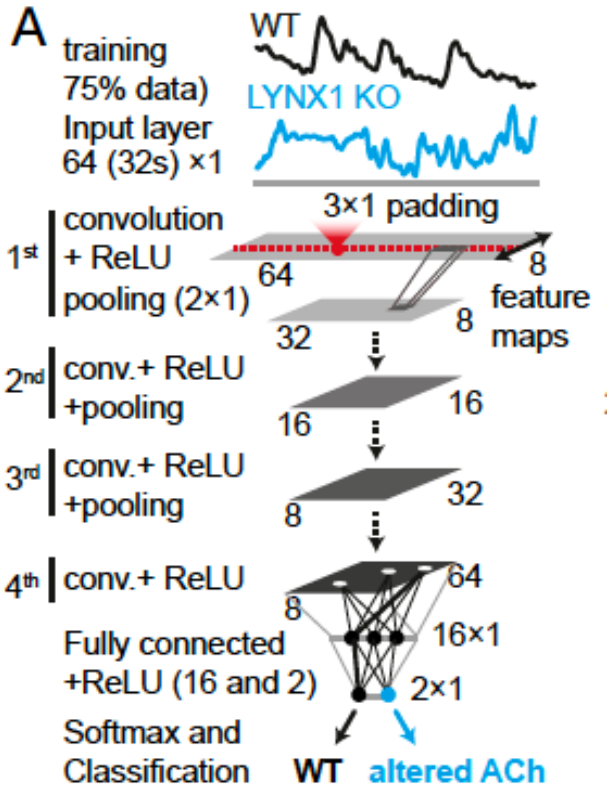


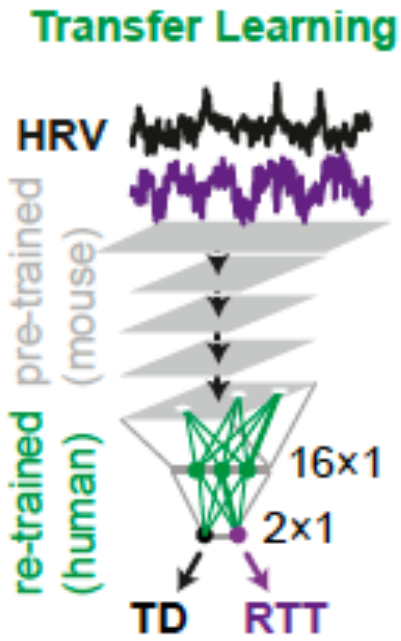


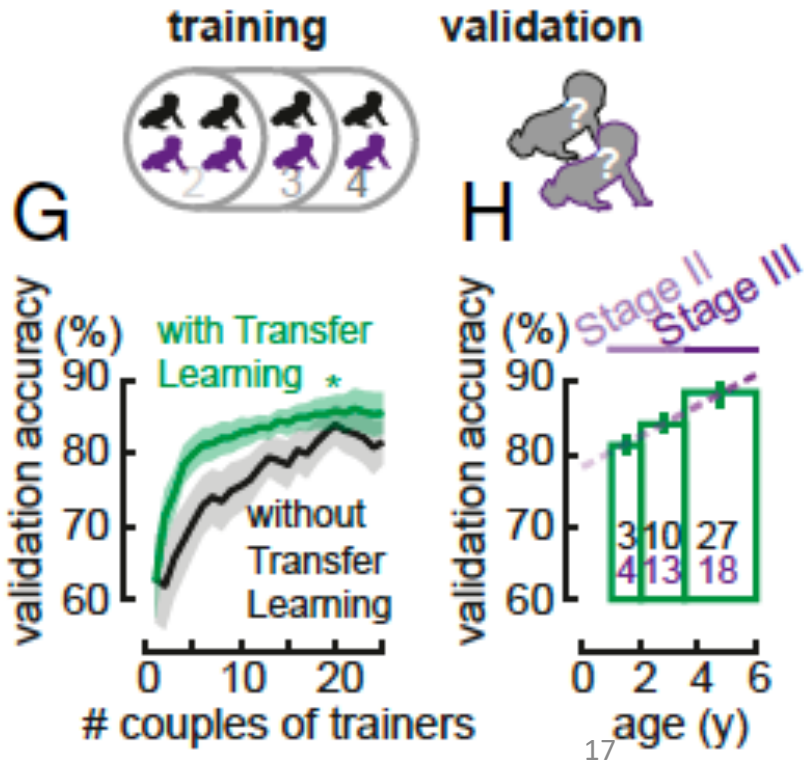
AI/ML Transfer Learning Case:
Arousal monitoring for early Rett Syndrome identification



Artoni, … Hensch, Fagiolini. Deep learning of spontaneous arousal fluctuations detects early cholinergic defects across neurodevelopmental mouse models and patients. PNAS, 2020







Many AI/ML Algorithms

- Ensemble Models
  - SPOKE
  - CRISP
- Review by Sanders and Qutub

Summary

Convergent data from multiple animal species can be leveraged to better inform and manage the spaceflight CNS health risks.

For translating the effects of spaceflight hazards from animal studies to humans:

- Standardized methods to assess behavior and cognition would aid in better translational research
- Utilize enhanced Bradford Hill level of evidence criteria and the considerations for increasing level of evidence recommendation for Animal/Cellular Studies based on Table 3, Appendix F, Human Systems Risk Management Plan, Antonsen 2020) to increase specificity (Weak to Moderate LOE).
- Scaling factors for animal outcomes/metrics equivalent to human response - reverse translation techniques could be used to establish scaling factors at operationally-relevant dose thresholds in animals (Nelson et al. 2021b).
- Test the predictive value for human translation across the different ground-based analogs, spaceflight environment, and in more than one animal species to bolster the quality of evidence in terms of *reproducibility* and *mechanistic*.

Background and Extra Slides

Using data from rodent to NHP cohorts: Considerations for Assessing Levels of Evidence (LOE) within HSRB Framework

Level of Evidence (LoE) in ascending order versus Bradford Hill Criteria (from Human Systems Risk Management Plan, JSC 66705, Rev A, 10/1/2020).

Level 4: Speculative - causal effects that have little to no evidence to support them, but that may make theoretical sense given the current, limited sum of knowledge on a topic.

It can identify potential areas for future research or occupational surveillance.

Level 3: Weak. causal effects that are not well understood either epidemiologically or mechanistically. addition of a theoretical explanation of Mechanism to Speculative.

limited impact on Risk assessment but may indicate the need for additional research to evaluate a concerning contribution to risk.

Level 2: Moderate. Causal effects in this category will have well-characterized epidemiological evidence to support them, though their biological mechanisms may not yet be fully validated. In addition to Temporality, Analogy, and Mechanism, Moderate evidence adds Reproducibility (reproduction of results by others) and Specificity in the evidence, i.e., the effect has been narrowed to a particular person/place/time that is generalizable to the astronaut cohort.

Moderate evidence may trigger a Risk or justify advocating for design impacts or mitigation consideration in vehicle requirements.

Level 1: Strong. causal effects that have attained broad consensus among subject matter experts. Connections that fit in this level will have high-quality epidemiological evidence in humans as well as laboratory studies describing mechanisms. In addition to having all the elements of the lower levels, Strong evidence will also have Coherence, which describes a correspondence between laboratory and human-subject results.

Blood Brain Barrier (BBB) Integrity after Spaceflight

Letters

RESEARCH LETTER

Changes in Blood Biomarkers of Brain Injury and Degeneration Following Long-Duration Spaceflight

Peter zu Eulenburg, MD, PhD
Judith-Irina Buchheim, MD
Nicholas J. Ashton, PhD, MD
Galina Vassilieva, MD
Kaj Blennow, MD, PhD
Henrik Zetterberg, MD, PhD
Alexander Choukér, MD

JAMA Neurology Published online October 11, 2021

jamaneurology.com

- neurofilament light chain protein was significantly elevated compared with preflight levels directly postflight, 1 week, and 3 weeks after return to Earth
- coherent reparatory processes, from cephalad fluid shift, in the brain with subsequent restoration of the blood-brain barrier integrity

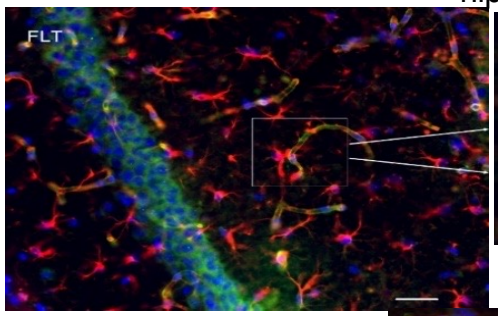
- N = 5 cosmonauts, 20 days preflight and (1 day, 1week, and 21 to 25 days after landing)
- Single molecule array (Simoa) immunoassay quantification of neurofilament light chain (NfL), glial fibrillary acidic protein (GFAP), total tau, and 2 amyloid-β (Aβ) proteins (Aβ40 and Aβ42), hemoglobin as a control protein

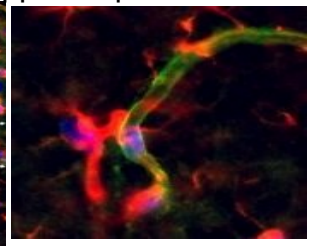
Mao et al., Spaceflight induces oxidative damage to blood-brain barrier integrity in a mouse model. Faseb J, (2021)

Changes in BBB integrity biomarkers including AQP4, GFAP, and PECAM-1 in the hippocampus, impact was less substantial in the cortex

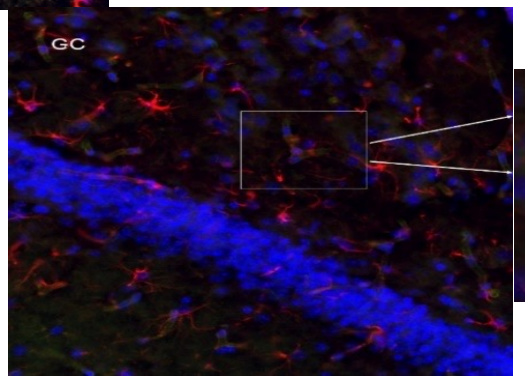
Spaceflight impacts on BBB integrity -AQP4 dysregulation in the hippocampus of mouse brain

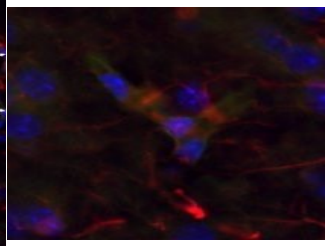
Glial fibrillary acidic protein (GFAP, red) and aquaporin4 (AQP4, green) staining in the hippocampus





Flight



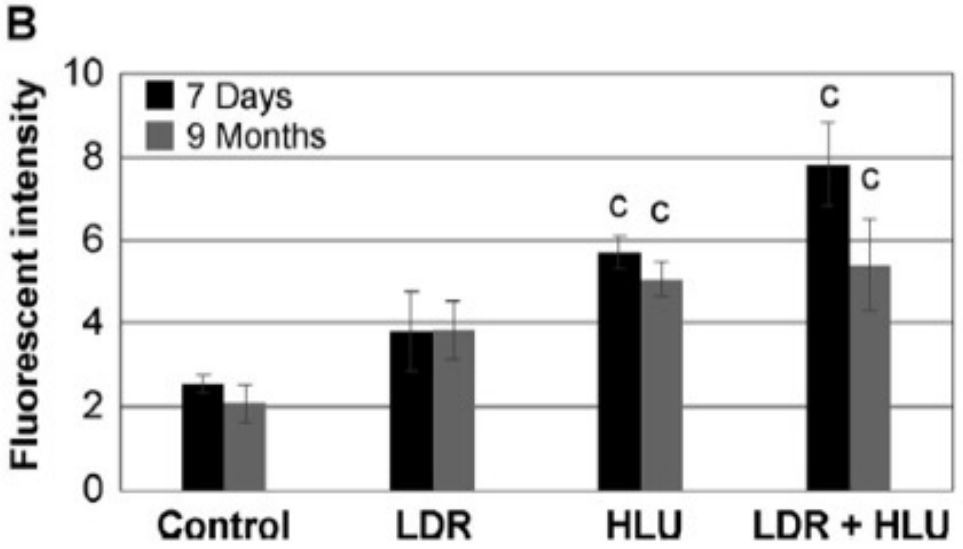


Ground Control

Combined Space Radiation and Altered Gravity

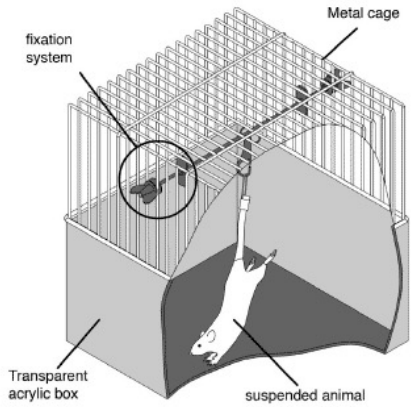
Chronic Gamma Irradiation ± Hindlimb Unloading

Oxidative Stress biomarker

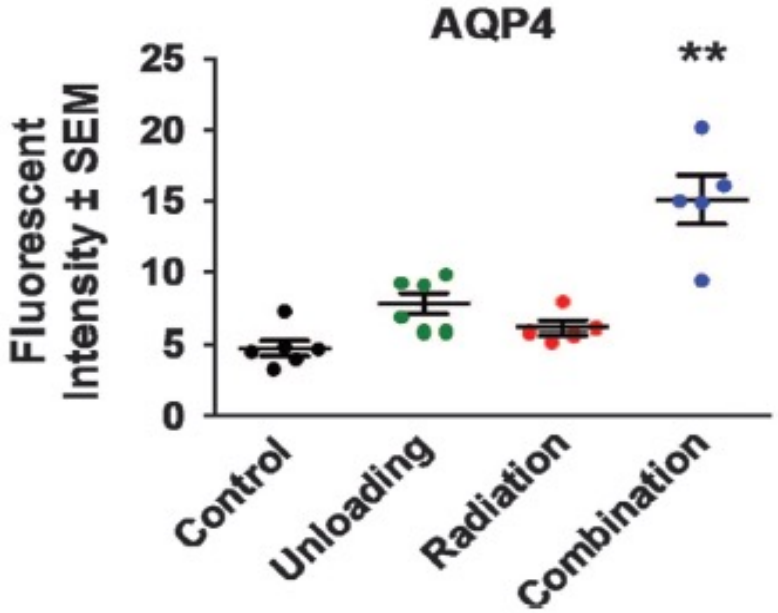


4-hydroxynonenal (4-HNE) staining in the hippocampus (panel B) at 7 days or 9 months.

Hind Limb Unloading



BBB biomarker



Water transporter aquaporin 4, astrocyte foot / endothelium interface marker at 9 months.

Stressors:

- Low dose gamma radiation (LDR) using a ^{57}Co source (0.01 cGy/h for a total dose of 0.04 Gy)
- Hindlimb unloading (HLU)
- Combination of both for 3 weeks

Measurements of LDR+ HLU Effects on:

- Mouse Brain Oxidative Stress increases (4-HNE)
- Blood Brain Barrier modified (AQP4)
- Microvessel changes

Mao et al. (2016), Bellone et al. (2016)

22

Sleep fragmentation unmasks latent ATSET deficits following neutron and Si radiation exposures

Sleep Fragmentation Exacerbates Executive Function Impairments Induced by Low Doses of Si Ions

Richard A. Britten,[a,b,c,d,1] Arriyam S. Fesshaye,[a] Vania D. Duncan,[a] Laurie L. Wellman[c,e] and Larry D. Sanford[c,e]

RADIATION RESEARCH 194, 116–123 (2020)
0033-7587/20 $15.00

DOI: 10.1667/RADE-20-00080.1

Si Radiation
Whole-body irradiation with 600 MeV/n Si ions 5 cGy (n = 11)

Attention Shift assay modeled after the intra-dimensional /extra-dimensional component of the Cambridge Neuropsychological Test Automated Battery (CANTAB) – testing complex decision making cognitive function (Humans & Primates)

EVMS
Pre-screen for ATSET proficiency
↓
Ship to BNL
↓
SR exposure
↓
EVMS
ATSET reassessment 90 days post exposure.
→ "Good Rats" exposed to sleep fragmentation
↓
ATSET reassessment

ATSET – attentional set-shifting
ATRC – attempts to reach criteria
SR – space radiation
EVMS – Eastern Virginia Medical school
BNL – Brookhaven National Laboratory

Original Articles

Sleep fragmentation exacerbates executive function impairments induced by protracted low dose rate neutron exposure

Richard A. Britten, Vania D. Duncan, Arriyam S. Fesshaye, Laurie L. Wellman, Christina M. Fallgren & Larry D. Sanford
Received 25 Jul 2019, Accepted 29 Oct 2019, Accepted author version posted online: 14 Nov 2019, Published online: 06 Dec 2019
Download citation https://doi.org/10.1080/09553002.2019.1694190

INTERNATIONAL JOURNAL OF RADIATION BIOLOGY
https://doi.org/10.1080/09553002.2019.1694190

Neutron Radiation
The rats were exposed to neutrons for 15.9–18.3 h/day, N.30), total neutron dose: 18 cGy

Sleep Fragmentation Chamber (Lafayette Instrument Co., Lafayette, IN)

SD – Simple Discrimination
CD – Compound Discrimination
CDR – Compound Discrimination Reversal
IDS – Intra Dimensional Shift
IDR – Intra Dimension Reversal
EDS – Extra Dimensional Shift
EDR – Extra Dimensional Reversal

Sleep Fragmentation
Rats placed in Sleep Fragmentation chamber, Active/Sleep (Dark/Light), Sweep time 2 mins, Allows dozing but not deep (REM) sleep.

1. Under rested wakefulness conditions, no significant effect of Si or low dose neutron radiation.
2. After sleep fragmentation rats showed a significant increase in the ATRC :
   - for the IDR, and EDS stages of the ATSET test after Si radiation
   - for the IDR stage of the ATSET test after low dose neutron radiation
   - IDR deficits are not typically induced after SR exposure