
# Oxidative stress and neuroconsequences of the spaceflight environment – A 5-ion GCRsim dose-response study

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*Discipline Session 3: Multistressor Hazard Implementation - GCRsim, Hind Limb Unloading, and Isolation, Abstract No. 1105-002458*

# Artemis and Lunar Gateway

*"the first woman and the first person of color" on the Moon*

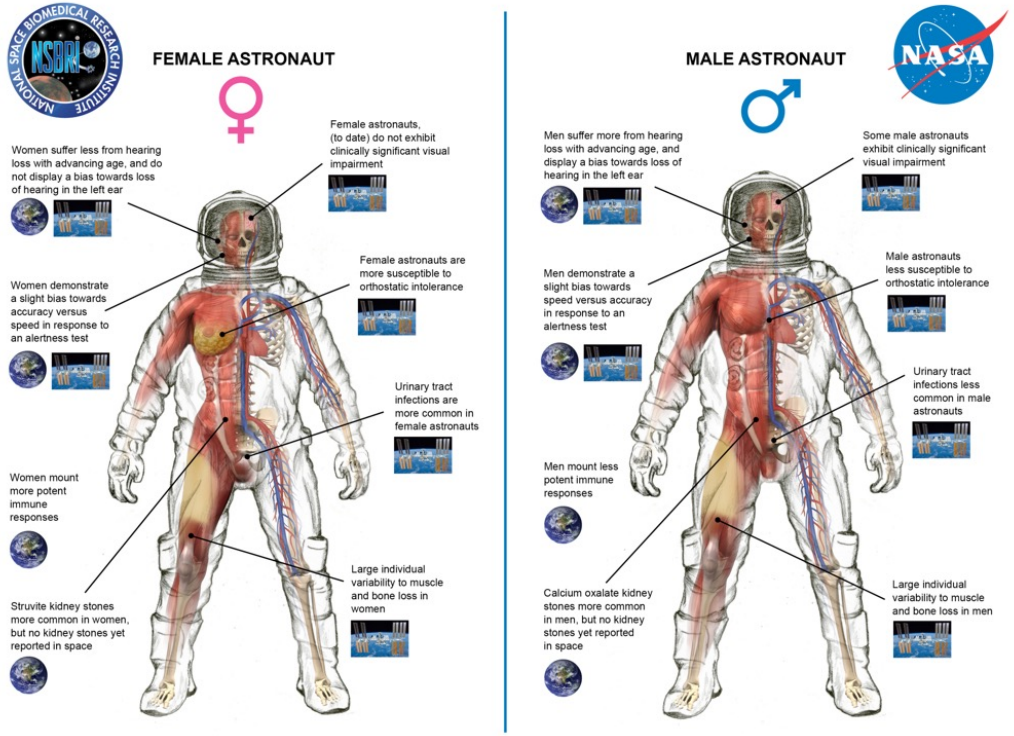


*Figure Credit: Mark et al., JWH 2014*

J Women's Health, 2014

## Effects of Sex and Gender on Adaptation to Space: Neurosensory Systems

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## Effects of Sex and Gender on Adaptation to Space: Behavioral Health

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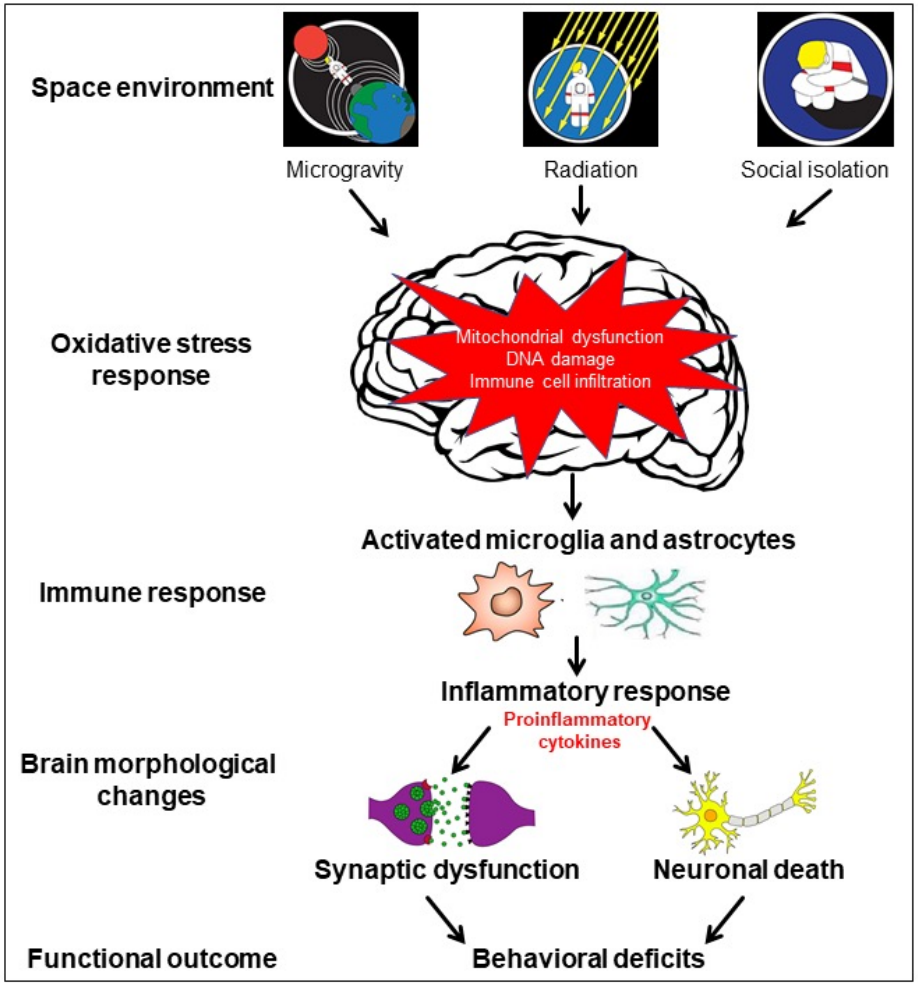
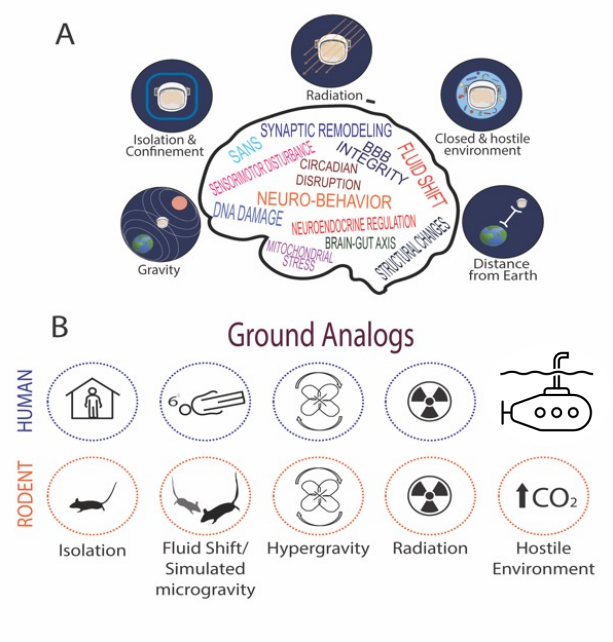
# Central Hypothesis

*Spaceflight hazards combine synergistically to trigger oxidative stress that alters immune homeostasis, brain structure and function, and neurobehavioral and cognitive performance.*




*Mhatre, Iyer et al., Neuroscience and Biobehavioral Reviews Special Issue on Fifty Years of Space Neuroscience as a Prelude to Exploration and Colonization , 2021*

# HU X Low Dose Radiation

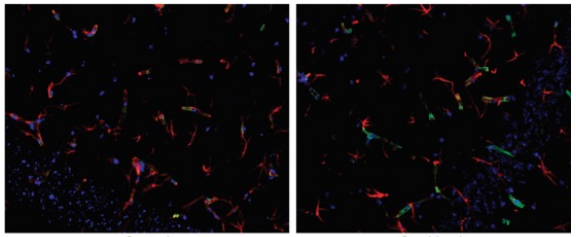



Spaceflight hazards and ground-analog models

*Mhatre, Iyer et al., Neuroscience and Biobehavioral Reviews Special Issue on Fifty Years of Space Neuroscience as a Prelude to Exploration and Colonization , 2021*

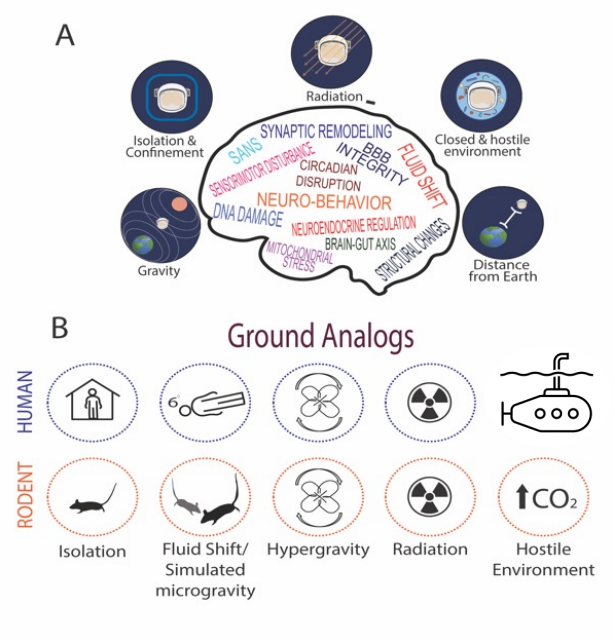
**Brain and Behavior**

- Oxidative damage, increased apoptosis, remodeling of brain microvasculature, increased expression of Nox2 (NADPH oxidase), BBB dysfunction, and increased risk-taking behavior (Bellone et al., 2016; Mao et al., 2016, 2017; Overbey et al., 2019a)
- Increased AQP4 (green) in astrocytes (red) 9mo after return to normal ambulation (Bellone, et al, 2016)
- Space flown mice show similar outcomes in AQP4 and oxidative stress (Mao, et al, 2020)




- Altered pathways in neurogenesis and neuroplasticity, regulation of neuropeptides and cellular signaling revealed by a NASA Transcriptomic analysis (Overbey et al., 2019b)

# HU Alone




Spaceflight hazards and ground-analog models

**Sensorimotor**

- Acute gait impairment (Canu, et al, 1998)
- Inhibition of Righting Reflex (Fox, et al, 1998; Kawano, et al, 2004)
- Somatosensory Cortex adaptation (Dupont, et al, 2010)

**Cognitive**

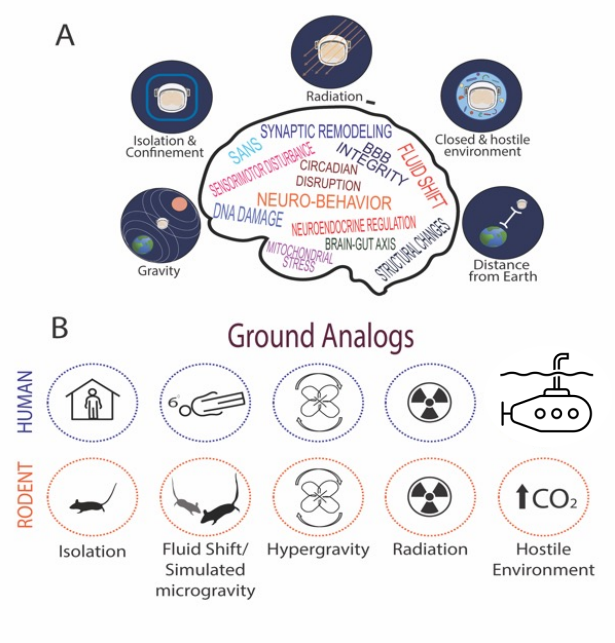
- Learning and memory impairments with MWM & Shuttlebox after 14d HU; Cholinergic and antioxidant dysfunction/oxidative damage (Zhang, et al, 2018)
- Learning and memory impairments with NOR (Zhai, et al, 2020; Xu, et al, 2021); Reduced hippocampal theta oscillation; Glutamatergic / GABAergic imbalance (Zhai, et al, 2020; Xu, et al, 2021)

**Anxiety & Risk Taking**

- Increased anxiety (open field) and risk-taking behavior (Zero Maze); Oxidative damage 9-mo post return to normal ambulation (Bellone, et al, 2016)

*Mhatre, Iyer et al., Neuroscience and Biobehavioral Reviews Special Issue on Fifty Years of Space Neuroscience as a Prelude to Exploration and Colonization , 2021*

# Social Isolation Alone




Spaceflight hazards and ground-analog models

- ROS production and oxidative stress, altered brain morphology (hippocampal neuron replacement; remodeling of dendrites and synapses; prefrontal cortical changes; behavioral alterations consistent with the pathogenesis of neurologic and psychiatric diseases (Sorce and Krause, 2009).
- Decreased NK cell activity, reduced $CD4^+$ T cells (Hawkley and Cacioppo, 2010).
- Weakened BBB → peripheral cytokines cross into CNS (Sylvia and Demas, 2018; Takahashi et al., 2018).
- Chronically elevated sympathetic tone and HPA activation; impaired inflammatory control; immunity; poor sleep quality, and the expression of glucocorticoid-regulating genes → Increased morbidity and mortality rates in older adults (Cacioppo et al., 2011; Holt-Lunstad et. 2015)

*Mhatre, Iyer et al., Neuroscience and Biobehavioral Reviews Special Issue on Fifty Years of Space Neuroscience as a Prelude to Exploration and Colonization , 2021*

# Project Aims

Determine ***sex-specific effects*** of single vs. combined:

- Ionizing Radiation **(IR, 5-ion GCRsim)**
- Simulated microgravity (head-down tilt via hindlimb unloading, **(HU)**
- Social isolation **(SI)**

*Crew age-matched male and female mice*

→ Assess oxidative stress, immune system, central nervous system (CNS) changes, and behavioral health/cognitive performance

***Specific Aim 1 (SA1)***

Determine IR dose dependence

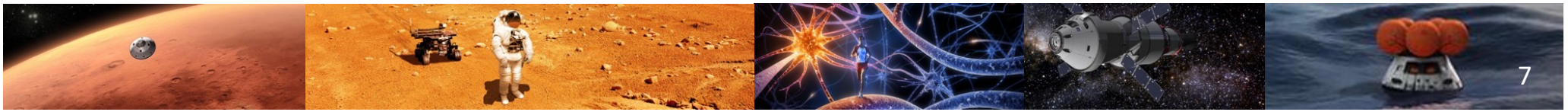
***Specific Aim 2 (SA2)***

Determine combined effects

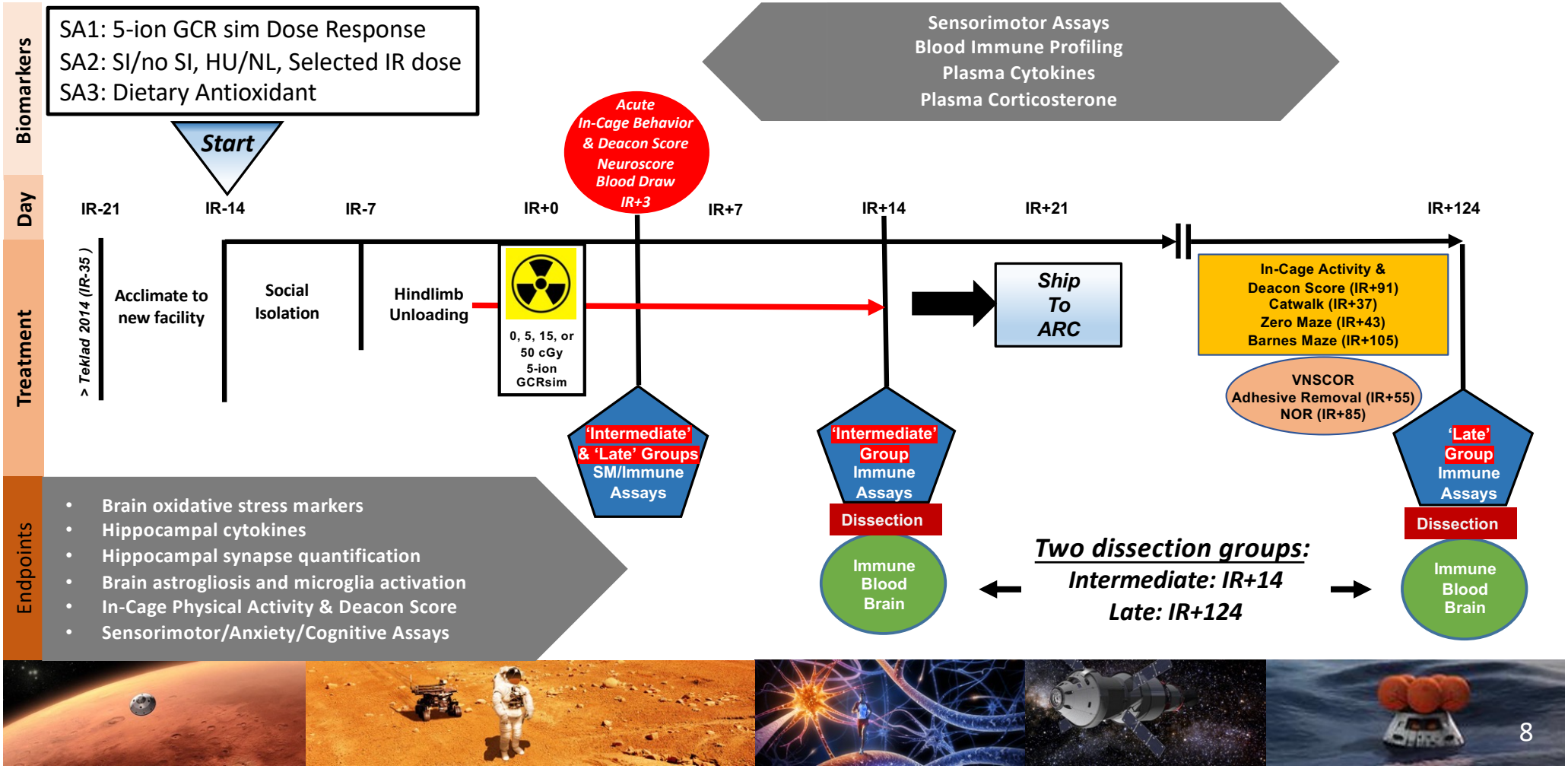
- IR+HU+SI vs single factor

***Specific Aim 3 (SA3)***

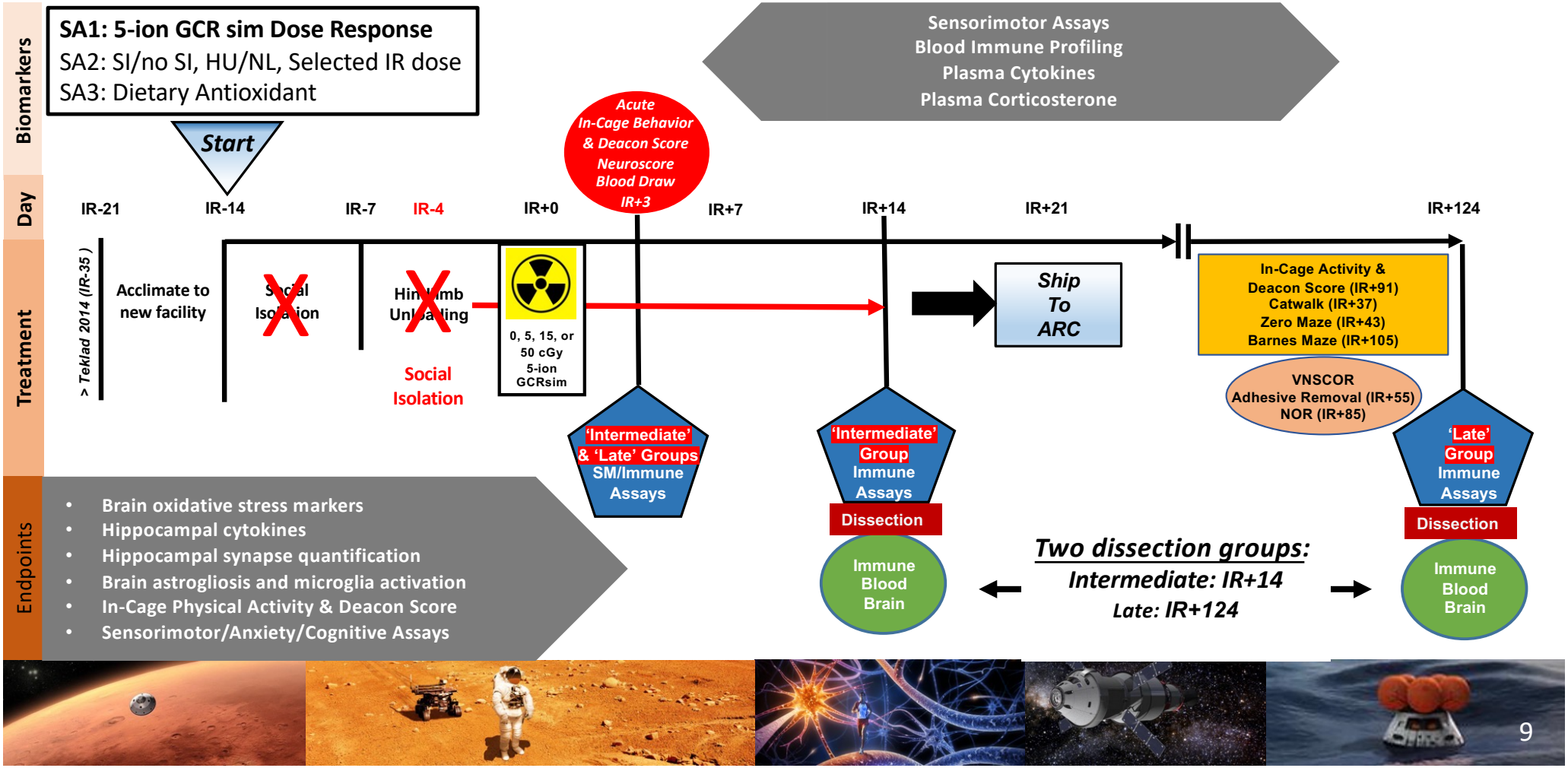
Test dietary antioxidant countermeasure, Nicotinamide Mononucleotide (NMN)



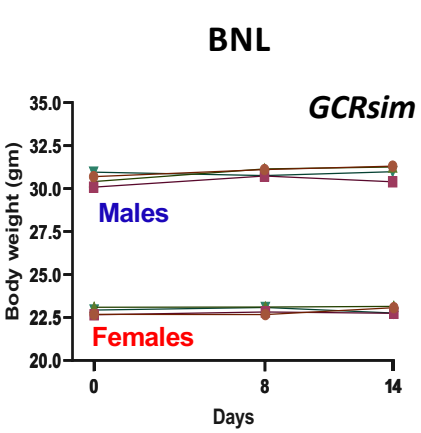
# Overall Study Design

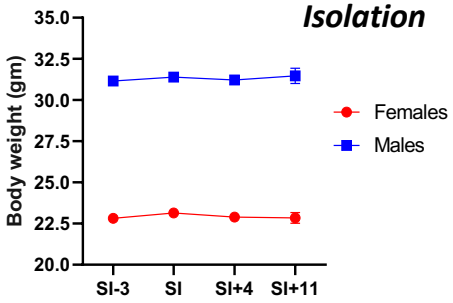


# SA1: 5-Ion GCRsim Dose-Response Study (0, 5, 15,or 50cGy)

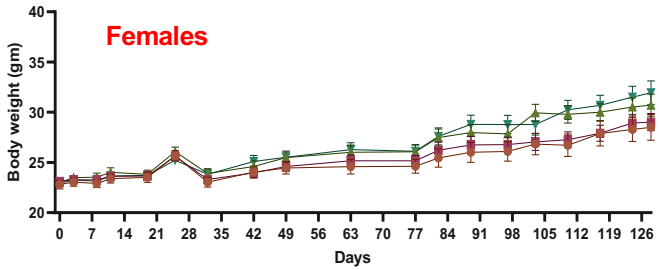


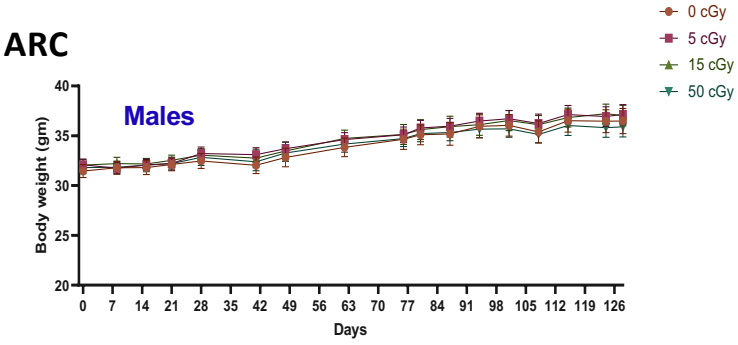
# Mouse Body and Organ Weights

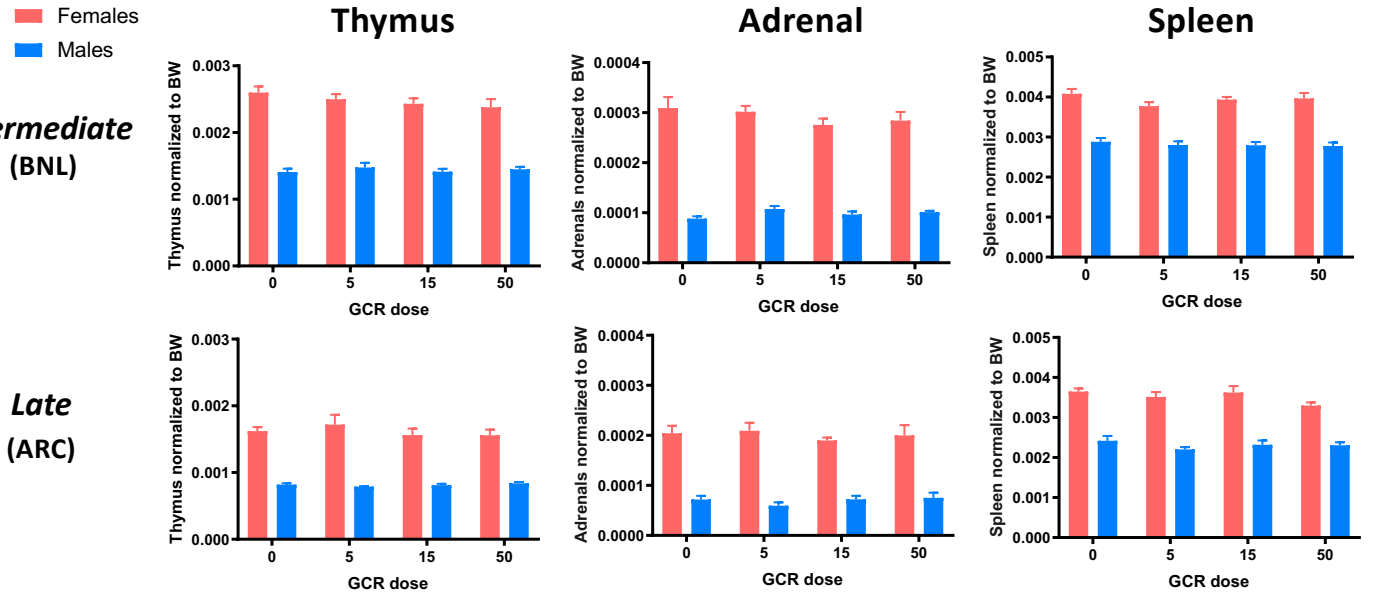


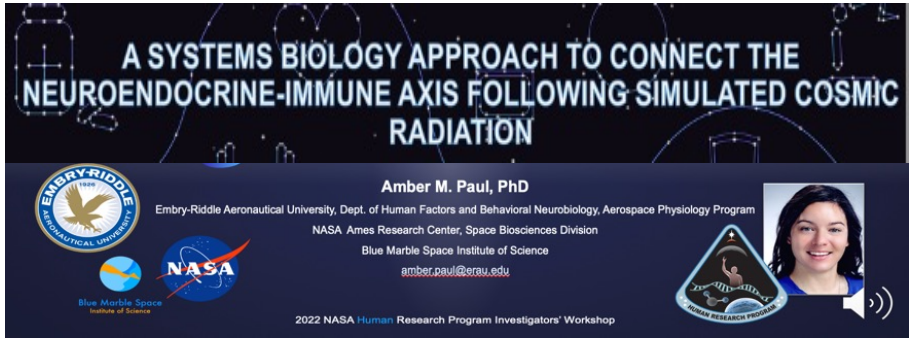

**No 5-ion GCRsim Dose Effects**
**Predictable Sex Differences**

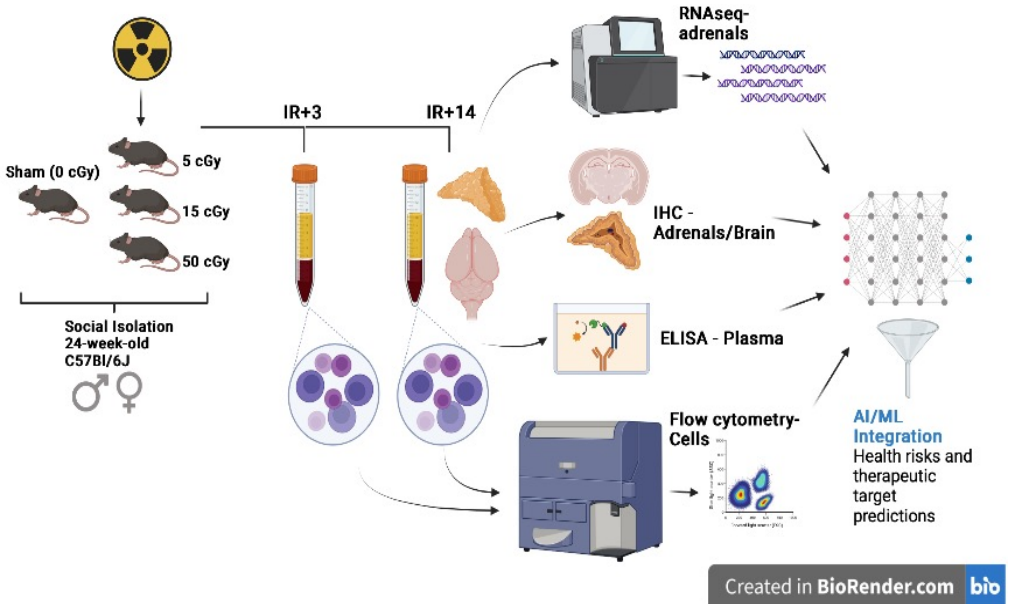


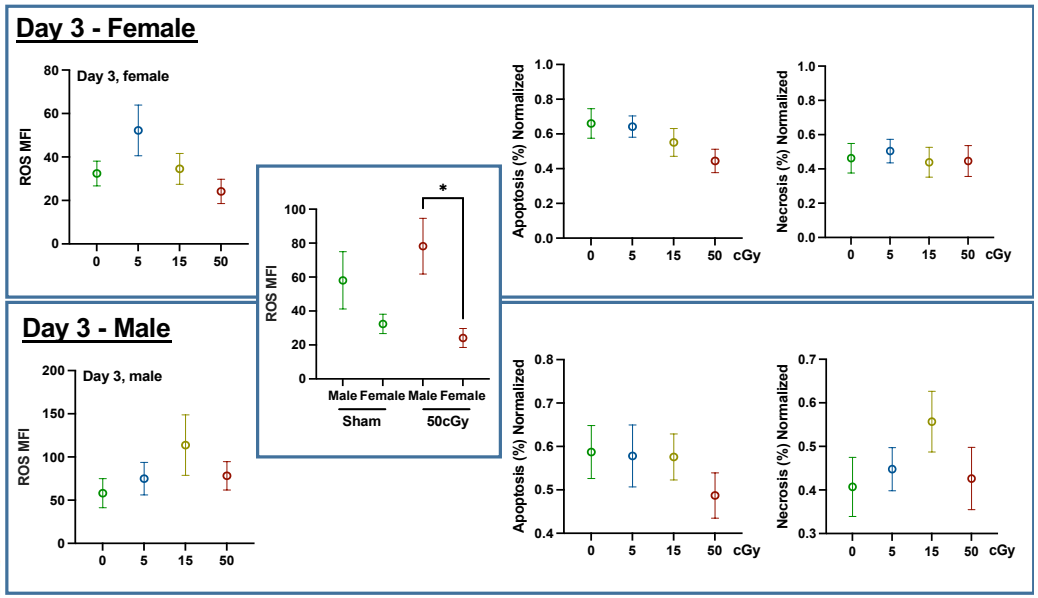



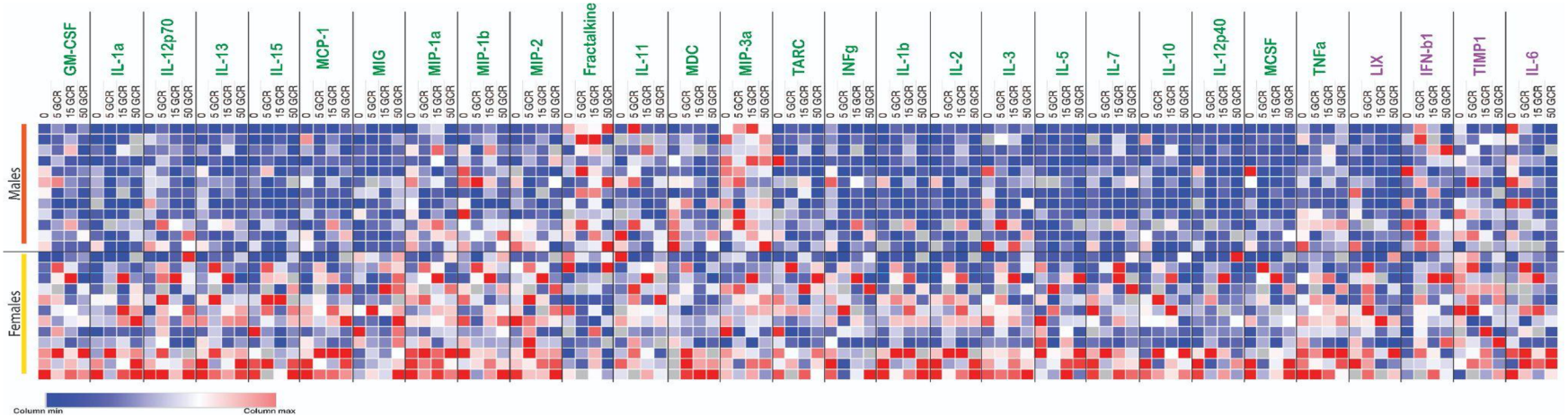
# Immune: Acute (IR+3) and Intermediate (IR+14)

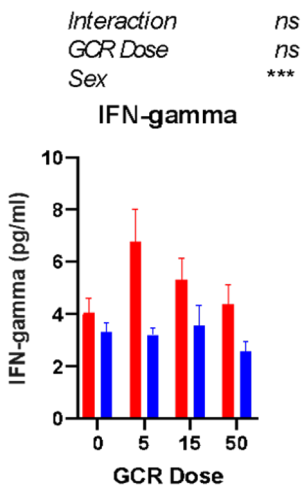


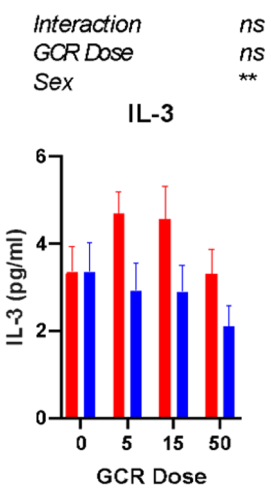



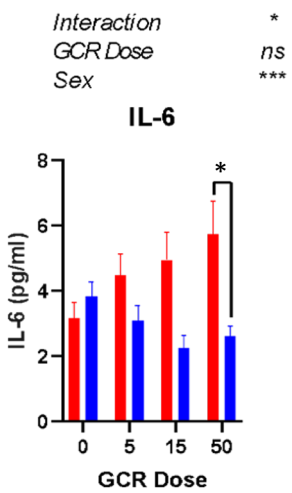



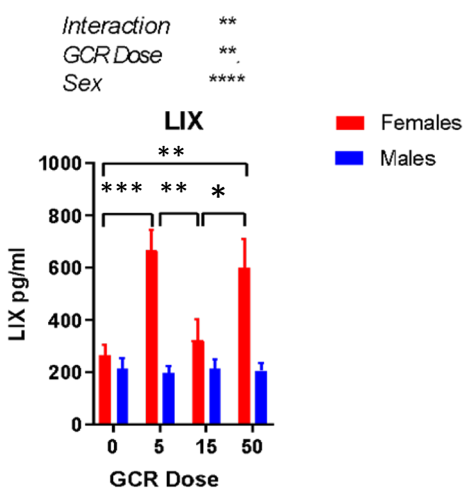
# Cytokine Expression: Intermediate Plasma (IR+14)



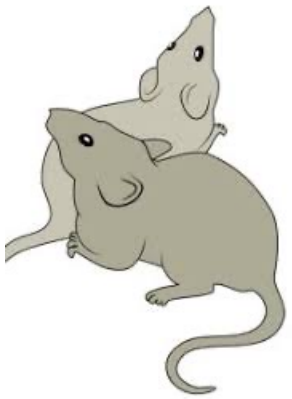



# Neuroscore Test: Sensorimotor Function (IR+3)

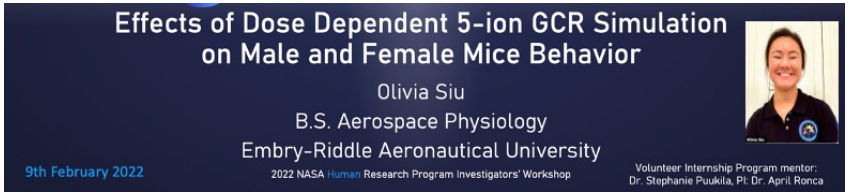


| Subtest | Score | | | |
|---|---|---|---|---|
| | 0 | 1 | 2 | 3 |
| Spontaneous activity (3 min) | No movement | Minimal movement | Touches 1–2 walls | Touches 3–4 walls while standing on hind limbs |
| Side stroking | No response | Unilateral response | Bilateral weak response or strong ipsilateral response | Strong bilateral response |
| Vibrissae touch (stroke whiskers) | No response | Unilateral response | Weak bilateral response | Strong bilateral response |
| Limb extension | Both contralateral; limb completely flexed | One contralateral; limb extended and other flexed | Mid flexion of either contralateral limb | Contralateral forelimbs and hind limbs completely extended |
| Forepaw outstretching | No movement | Moves in circles | Moves to one side | Straight/curved path |
| Climbing (1 min) | Weak grip and fall down | Climbs but does not reach top and weak grip | Climbs to top and weak grip or does not climb to top and good grip | Climbs to top and strong grip |

- Neurological deficits
- Sensorimotor deficits
- Neuromotor skills
- Reflex test
- Limb use

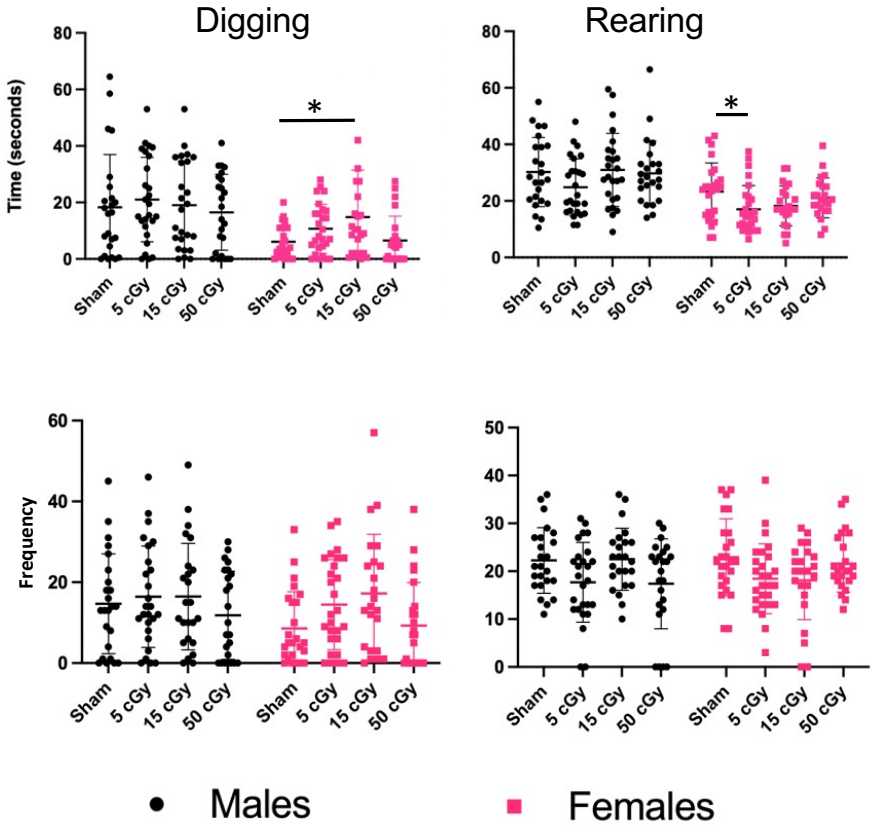
**No 5-ion GCRsim Dose or Sex Differences**

# Neuroscore Spontaneous Activity (IR+3 & IR+91)




Digging and Rearing - 3min samples

Significant duration differences:
- Female sham vs. 15 cGy digging
- Female sham vs. 5 cGy rearing

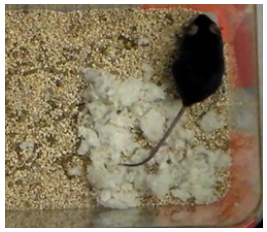


# Nest-Building: Sensorimotor Function (IR+3)

**Deacon Score**

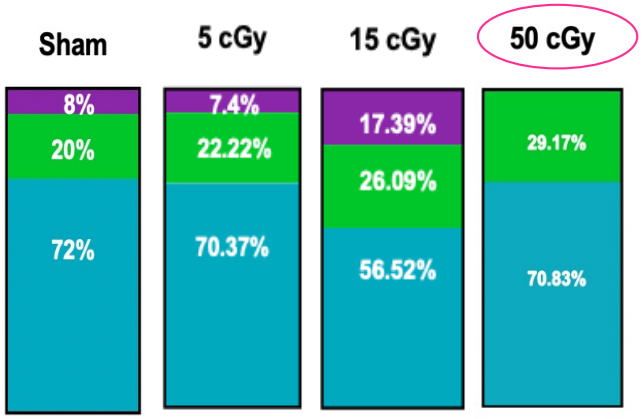
- 3 — Least developed nestlet build
- 4
- 5 — Most developed nestlet build

Deacon Score 3

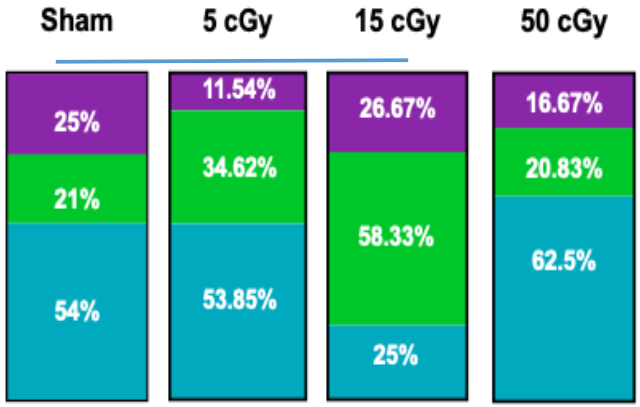


Deacon Score 4

Deacon Score 5




Females

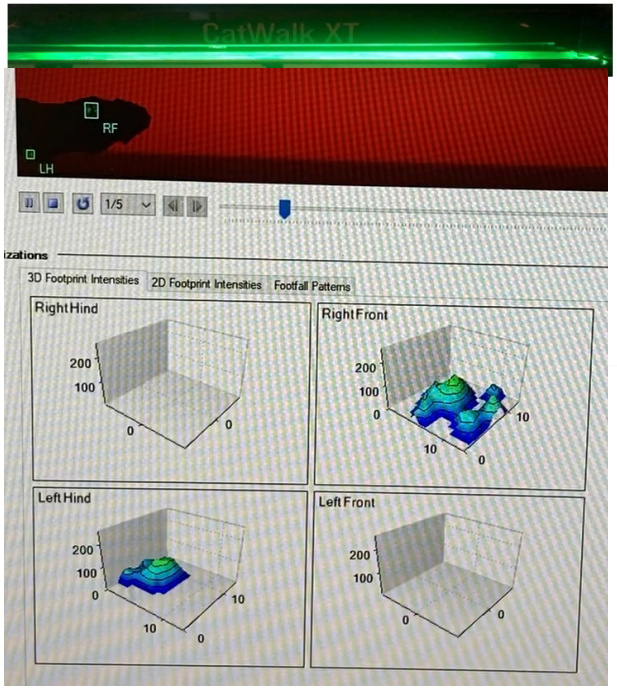



Males

- females > males
- 50cGy females - no '3's
- 15cGy males < sham

Credit: Siu, Rubinstein, Puukila

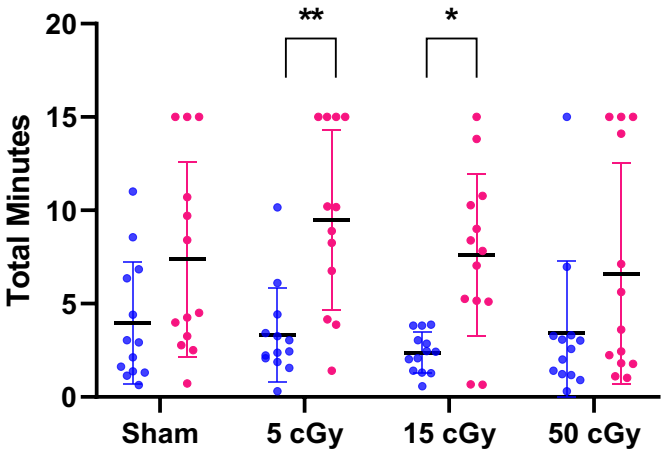
# Catwalk Gait Analysis: Sensorimotor Function (IR+37)

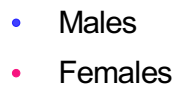



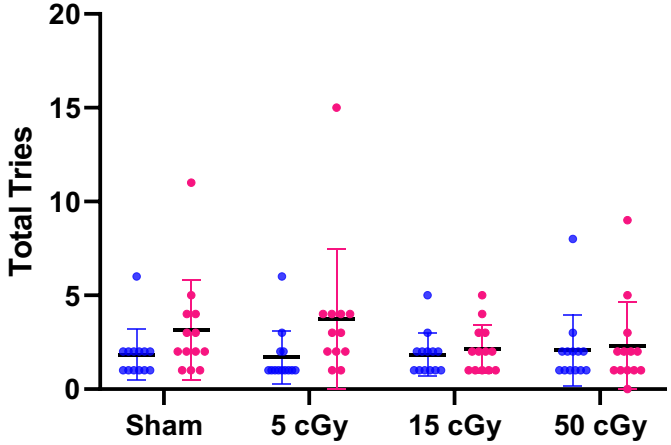

| | | Sham | 5 cGy | 15 cGy | 50 cGy |
|---|---|---|---|---|---|
| Males | Speed (cm/s) | 18.6 ± 2.9 | 15.4 ± 3.7 | 17.1 ± 3.8 | 18.2 ± 2.5 |
| | Regularity Index | 61.7 ± 23.1 | 59.0 ± 21.6 | 69.5 ± 19.1 | 68.8 ± 20.9 |
| | Stance Ratio | 1.2 ± 0.2 | 1.1 ± 0.2 | 1.2 ± 0.3 | 1.3 ± 0.3 |
| | Base of Support | | | | |
| | Front | 1.5 ± 0.2 | 1.5 ± 0.3 | 1.4 ± 0.2 | 1.5 ± 0.2 |
| | Hind | 2.8 ± 0.2 | 2.8 ± 0.3 | 2.8 ± 0.2 | 2.8 ± 0.2 |
| Females | Speed (cm/s) | **31.3 ± 5.2#** | **29.4 ± 4.6#** | **29.3 ± 3.1#** | **28.8 ± 5.6#** |
| | Regularity Index | **94.7 ± 7.1#** | **97.0 ± 3.4#** | **93.6 ± 6.9#** | **91.4 ± 12.8#** |
| | Stance Ratio | 1.1 ± 0.1 | 1.0 ± 0.1 | 1.1 ± 0.1 | **1.1 ± 0.1#** |
| | Base of Support | | | | |
| | Front | 1.6 ± 0.2 | **1.7 ± 0.2#** | **1.7 ± 0.3#** | 1.6 ± 0.2 |
| | Hind | **3.9 ± 0.4#** | **4.0 ± 0.3#** | **3.8 ± 0.4#** | **3.8 ± 0.4#** |

- **Sex differences at all doses**
- **Females > Males:**
  **Speed, Regularity Index & Base of Support**
- **No 5-ion GCRsim effects at any dose**

# Adhesive Removal Test: Sensorimotor Function (IR+63) VNSCOR

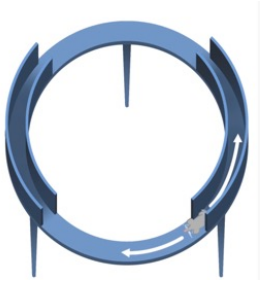


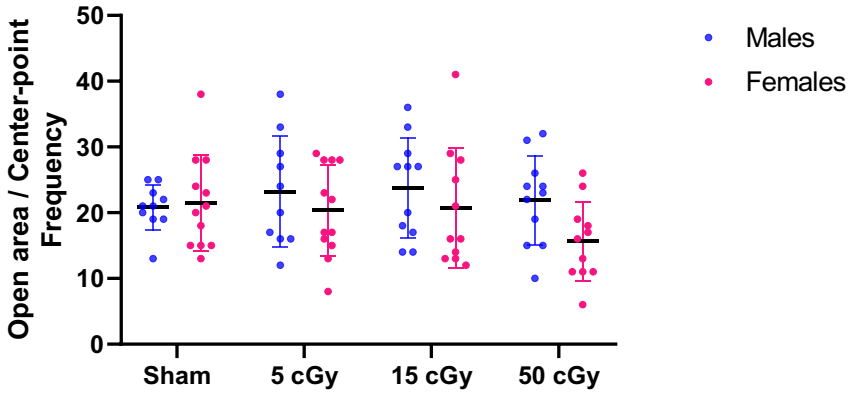



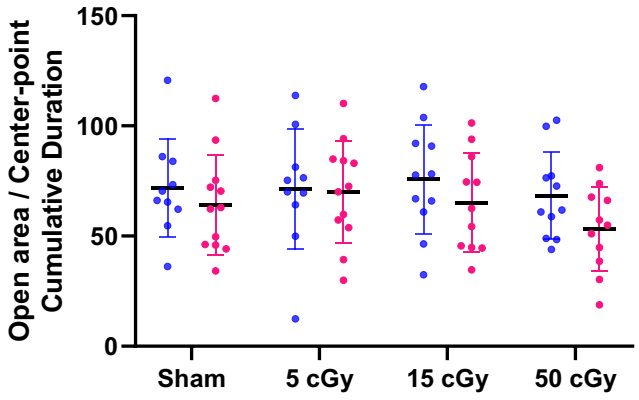

**5-ion GCRsim Dose x Sex Interactions**

# Zero Maze: Anxiety and Risk Taking (IR+42)

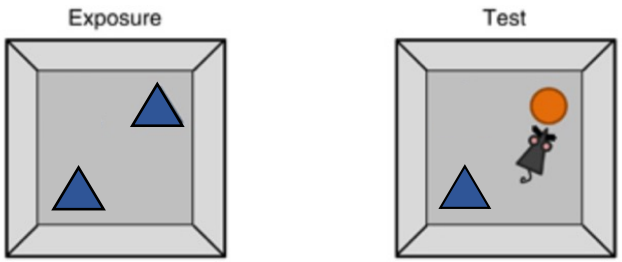


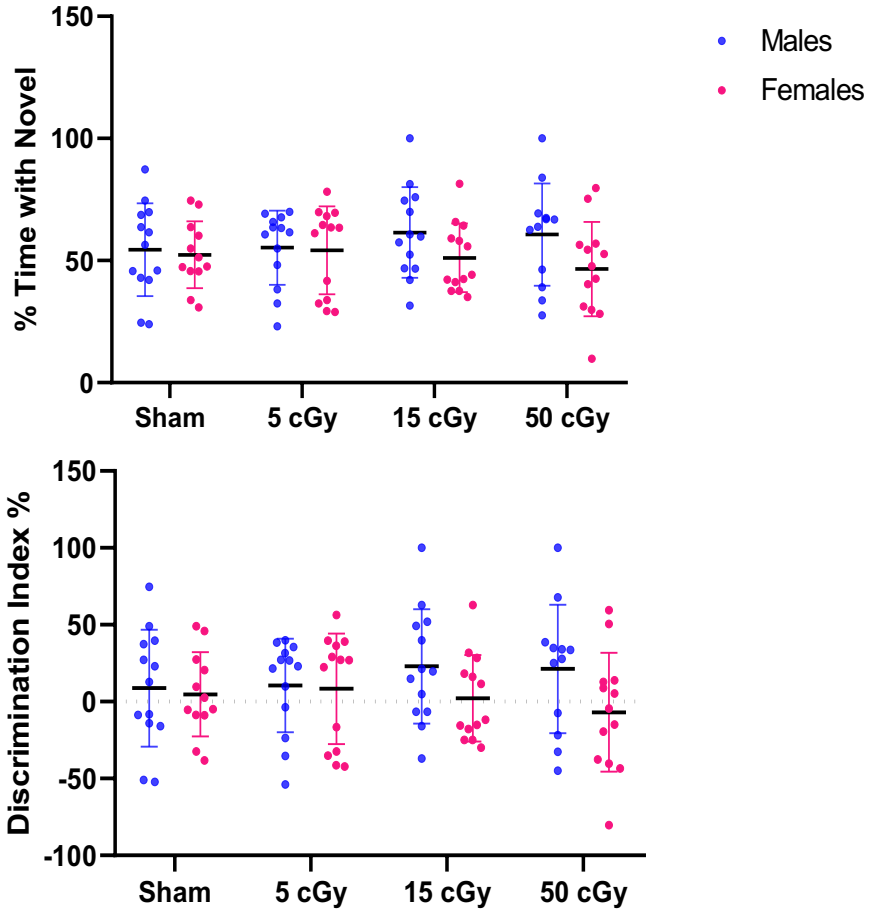
**No 5-ion GCRsim Dose or Sex Differences**



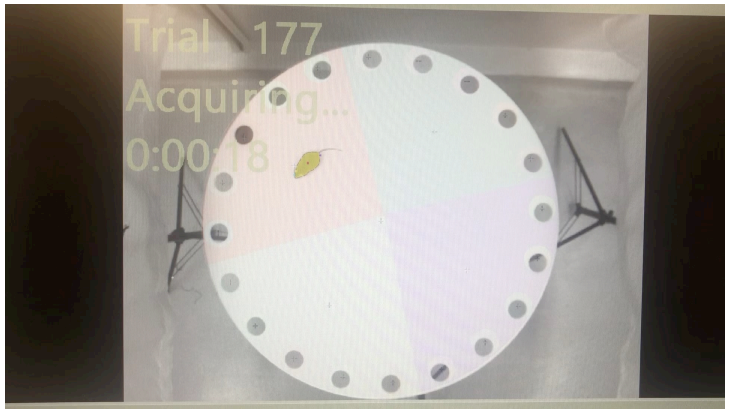
# Open Field + Novel Object: Memory (IR+84) VNSCOR




**No 5-ion GCRsim Dose or Sex Differences**

# Memory: Barnes Maze (IR+115)




| | | Sham | 5cGy | 15cGy | 50cGy |
|---|---|---|---|---|---|
| Males | Total Latency (s) | | | | |
| | Day 1 | 157.8 ± 40.2 | 161.5 ± 36.5 | 157.0 ± 39.8 | 143.2 ± 46.1 |
| | Day 2 | 147.1 ± 48.6 | 143.5 ± 47.0 | 151.3 ± 40.3 | 143.9 ± 48.8 |
| | Day 3 | 153.0 ± 52.1 | 168.0 ± 28.8 | 150.8 ± 44.2 | 164.1 ± 36.1 |
| | Day 4 | **122.3 ± 63.2*** | 140.9 ± 53.6 | 150.1 ± 51.7 | 152.3 ± 43.1 |
| | Probe | 131.0 ± 49.9 | **118.2 ± 60.1*** | **122.6 ± 62.5*** | 142.7 ± 49.1 |
| | Total Errors | | | | |
| | Day 1 | 24.5 ± 14.8 | 26.4 ± 14.9 | 24.1 ± 11.3 | 23.3 ± 11.4 |
| | Day 2 | 25.9 ± 17.1 | 22.3 ± 13.3 | 21.5 ± 9.6 | 23.2 ± 12.5 |
| | Day 3 | 36.9 ± 20.5 | **38.7 ± 22.2*** | 32.8 ± 18.9 | 32.5 ± 12.8 |
| | Day 4 | 24.1 ± 17.0 | 24.8 ± 14.0 | 24.1 ± 14.5 | 27.0 ± 14.1 |
| | Probe | 32.2 ± 20.5 | 27.1 ± 17.8 | 28.2 ± 22.2 | 32.2 ± 18.7 |
| Females | Total Latency (s) | | | | |
| | Day 1 | 161.8 ± 37.4 | 164.5 ± 37.7 | 173.5 ± 30.3 | 157.5 ± 47.3 |
| | Day 2 | 112.0 ± 58.5 | **111.0 ± 62.8*** | **111.9 ± 60.3*#** | 134.7 ± 58.1 |
| | Day 3 | **102.7 ± 61.1*#** | **95.1 ± 62.1*#** | **98.4 ± 57.4*#** | 112.1 ± 65.3# |
| | Day 4 | **77.5 ± 64.0*#** | **86.7 ± 56.7*#** | **116.6 ± 60.4*#** | **112.1 ± 63.0*#** |
| | Probe | **80.0 ± 46.9*#** | **65.2 ± 42.6*#** | **82.7 ± 56.8*#** | **112.1 ± 63.0*#** |
| | Total Errors | | | | |
| | Day 1 | 30.7 ± 14.8 | 28.7 ± 14.6 | 26.6 ± 14.2 | 27.1 ± 14.6 |
| | Day 2 | 20.4 ± 13.3 | 15.5 ± 13.2 | **14.6 ± 9.2*** | 20.0 ± 12.7 |
| | Day 3 | 21.9 ± 14.9# | 20.3 ± 17.1# | 22.9 ± 15.3 | 22.3 ± 17.1 |
| | Day 4 | **10.8 ± 10.5*#** | **14.0 ± 12.7*#** | 19.5 ± 16.4 | 18.1 ± 13.9 |
| | Probe | **15.7 ± 13.2*#** | **13.2 ± 9.6*#** | 17.6 ± 17.1 | 16.5 ± 14.5# |

# = p<.05 vs. males
* = p<.05 vs. Day 1

**No GCRsim Dose but Sex Differences**

- Females faster than males
- Females had fewer errors
- No 5-ion GCRsim dose differed from sham


# Summary

- Early findings from our 5-Ion GCRsim Dose-Response study revealed consistent sex differences in most immune and behavioral analyses, highlighting the importance of male-to-female comparisons.
- 5-ion GCRsim + isolation produced relatively few effects on immune and behavioral endpoints, although some IR dose effects were observed.
- Our next steps include:
  - Full analysis of our data, including correlational comparisons, to facilitate selection of the optimal 5-ion GCRsim dose for our upcoming studies.
  - Collaboration with the VNSCOR Team to apply common predictive modeling approaches.