ADVANCES IN RODENT RESEARCH MISSIONS ON THE INTERNATIONAL SPACE STATION

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BACKGROUND

• Rodent Research is important for biomedical discovery on Earth and in space.
• National Research Council’s Decadal Survey emphasized the importance of expanding NASA’s life sciences research for long-duration rodent experiments on ISS
• New hardware, operations, and science capabilities developed at NASA ARC to support rodent missions in space
Three Rodent missions flown to date

1. **Rodent Research-1**: launched on 9/21/14
   - 20 female adult mice (10 for NASA’s validation mission and 10 for the National Lab’s science experiment)
   - Mission duration: 33 days (NASA’s Validation) and 16-17 days (Novartis) on the ISS

2. **Rodent Research-2**: launched on 4/14/15
   - 20 female adult mice for Novartis/US National Laboratory
   - Mission duration: various time points – up to 8 weeks on the ISS

3. **Rodent Research-3**: launched on 4/8/16
   - 20 female adult mice for Eli Lilly/US National Laboratory
   - Mission duration: 6 weeks on the ISS
Rodent Research-1
# RR-1 Objectives

## NASA Validation flight
1. Validate hardware and on-orbit operations to provide reliable housing for the mice for long duration missions (> 1 month)

1. ISS’s capability to perform and support scientific activity
   - Animal health and behavior
   - Sample retrieval including dissection and tissue preservation

## Novartis experiment
1. Validate ability to conduct commercial rodent research on the ISS

1. Evaluate a specific transgenic strain to prove the mechanisms of spaceflight-induced muscle wasting
**NASA VALIDATION MICE**
10 C57BL/6J mice (female, 16wk old)

**5 CASIS Control (WT) strain**
5 CASIS Genetically modified strain (MuRF1 KO) (female, 32 wk old)

**RR1 Experimental Design**

- 2 mice → Dissect for Spleen + Liver
- 8 mice → Freeze intact for **Body weight** & sample retrieval post-flight

- 10 Hind limbs → Formalin-fixed
- 5 WT mice → Liver and Spleen for Validation

Liver: fast frozen: RNA analysis and enzyme activity measurement
Spleen: preserved in RNAlater: RNA analysis

Daily video monitoring and post-flight behavior analysis
Measurements of body weights and organ weights to assess stress responses
RR1: Concept of Operations

1. Late Load on Dragon

2. Launch

3. Ascent (4 days Launch-Transfer)

4. ISS Dock/Animal transfer via Mouse Transfer Box

5. ISS Habitat: (10 mice: 17/18d 10 mice: 33d)

6. Euthanasia and tissue retrieval

7. Sample Cold Stowage

8. Descent

Transporter

Animal Access Unit

Microgravity Science Glovebox

Habitat

Access Unit Transporter
Rodent Hardware

- Transporter
- Animal Access Unit
- Mouse Transfer Box
- Kits (many)

Habitat
Mice behavior on ISS: Validation mice

Qualitative and Quantitative analyses made using daily health check videos

- Upon initial introduction into the Habitat, mice actively explored the compartments
- Mice were observed eating, drinking, grooming and socially interacting while in the Habitats
  - No major differences in numbers of mice observed feeding across spaceflight conditions
  - FLT mice initially spent more time self-grooming relative to GCs, but the occurrence of grooming was similar in FLT and GC mice throughout the remainder of the flight
- Mice propelled themselves freely and actively throughout the Habitat using their forelimbs
  - Mostly by ‘pulling’ along cage grate with their forelimbs or by ‘floating’ from one location to another; later on orbit, began to use hindlimbs also to move along cage walls.
- As time went on, the mice moved more quickly around the compartment, moving with ease through open spaces and anchoring themselves using tails and/or paws
- “Race-tracking” behavior observed exclusively in FLT mice during the dark cycle
Video clip
Summary of RR1 findings

Body Weights: no difference between Flight and Ground Controls

No signs of chronic stress based on the tissue weights

Body Weight (g)

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal</th>
<th>Viv</th>
<th>GC</th>
<th>FLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (g)</td>
<td>20.0 ± 2.0</td>
<td>21.5 ± 1.5</td>
<td>20.5 ± 1.0</td>
<td>21.0 ± 1.5</td>
</tr>
</tbody>
</table>

Adrenal Glands (mg/g BW)

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal</th>
<th>Viv</th>
<th>GC</th>
<th>FLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal Glands (mg/g BW)</td>
<td>0.30 ± 0.05</td>
<td>0.30 ± 0.05</td>
<td>0.30 ± 0.05</td>
<td>0.30 ± 0.05</td>
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Thymus (mg/g BW)

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal</th>
<th>Viv</th>
<th>GC</th>
<th>FLT</th>
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<tbody>
<tr>
<td>Thymus (mg/g BW)</td>
<td>2.8 ± 0.2</td>
<td>3.0 ± 0.1</td>
<td>2.9 ± 0.1</td>
<td>3.2 ± 0.3</td>
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Summary of RR1 findings (cont’d)

• High quality samples suitable for applying cutting edge molecular biological methods were recovered
  ➢ Livers and spleens dissected on orbit post-euthanasia (RIN≥8)
  ➢ Livers dissected from frozen carcasses after return to Earth (RIN≥8)
  ➢ Liver samples collected from NASA’s validation mice and Novartis mice were provided to GeneLab and analyzed for transcriptomics, epigenetics and proteomics
    o the data are currently publically available:
      https://genelab-data.ndc.nasa.gov/genelab/search_studies/?q=RR1

• Activity of liver enzymes (catalase, glutathione reductase and glyceraldehyde-3-phosphate dehydrogenase) were preserved in samples collected from frozen carcasses
Expanding science return from RR1 through Biospecimen Sharing Program (BSP)

- RR science team recovered **32 tissues** from 40 RR1 Validation mice including flight, ground controls, baseline and vivarium controls, yielding total of **3280 vials** of tissues
- **Additional 7 tissues** were recovered from second thaw of the carcasses

1) BSP tissues were distributed to the scientific community through the Ames Life Science Data Archive (LSDA)

2) Select samples were provided to Russian research colleagues at the Institute for Biomedical Problems (IBMP)

3) NASA GeneLab project
   - Various tissues were provided for “omics” analyses
RR1 Conclusion

• Hardware performed as expected and operations completed successfully on orbit for animal transfer, euthanasia, dissection and sample preservation

• Established baseline mission systems and biological database to help guide future rodent research on ISS

• Provided tissue samples for the NASA Space Biology-Biospecimen Sharing Program and the GeneLab’s omics (transcriptomic, epigenomic and proteomic) analyses
NASA ARC collaborates with commercial partners and the U.S. National Laboratory through CASIS

Rodent Research-2

Rodent Research-3
## RR-2 and RR-3 Achievements

<table>
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<tr>
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<th>RR-2 (Novartis)</th>
<th>RR-3 (Eli Lilly)</th>
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<tr>
<td><strong>Mice</strong></td>
<td>20 C57BL/6 Female</td>
<td>20 BALB/c Female</td>
</tr>
<tr>
<td><strong>New capabilities</strong></td>
<td>Bone densitometry scan</td>
<td>Bone densitometry scan with Anesthesia Recovery System (ARS) Grip strength assessment</td>
</tr>
<tr>
<td><strong>Hardware</strong></td>
<td>Standard RR Hardware</td>
<td>Standard RR Hardware Bone Densitometer, ARS, Grip Strength Meter</td>
</tr>
<tr>
<td><strong>On-orbit Dissection</strong></td>
<td>Blood draw and separation Hindlimb and eye fixation Hindlimb dissection and freezing Carcass freezing</td>
<td>Blood draw and separation Hindlimb fixation Carcass freezing</td>
</tr>
<tr>
<td><strong>Days on ISS</strong></td>
<td>Various (up to 54 days)</td>
<td>6 weeks</td>
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New capabilities developed for use on subsequent missions since RR-1

• RR-2 and RR-3 missions:
  - Bone densitometry scans
  - Grip strength measurements
  - Recovery from anesthesia

• RR-4:
  - Male mice
  - Increase the number of mice from 20 to 40

• RR-5: Live animal return

• JRR-1 (Joint Rodent Research-1): Joint mission between NASA SLPS and Russian scientists
Rodent Research-4

- Objectives: to study bone healing in microgravity
- First mission using male mice
- 40 C57BL/6J mice will be flown on SpaceX 10
- On-orbit dissections:
  - Blood collection
  - Hindlimb dissection/fixation
  - Carcass freezing
Conclusion

• NASA ARC Rodent Research Project continues to:
  – expand capabilities to perform long-duration missions on the ISS
  – maximize science return through Biospecimen Sharing Program
  – contribute to understanding of the effects of microgravity on human health as strongly emphasized in the NRC’s 2011 Decadal Survey, “Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era”
Acknowledgements

- RR Project Team, ARC
- KSC
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Rodent Habitat: RR Scientific subject matter experts

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Alexander Dunlap, D.V.M., M.D.: NASA HQ
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  - Ruth Globus, Ph.D.: NASA Ames
  - David Tomko, Ph.D.: NASA HQ
EXTRA
Bone Densitometer (RR-2 and RR-3)

- Quantitative measures of bone and muscle loss in mice during orbital space
- Dual Energy X-ray Absorptiometry (DEXA)
  - X-ray System for Measuring Bone Density
  - Measures soft-tissue density, lean/fat ratio and total animal mass (i.e. weighing mice in space)
- Fast imaging in less than 5 minutes, allows faster access to important data and safer on animals
Anesthesia Recovery System

- Mice become rapidly hypothermic due to anesthesia
- Supplemental heating provided to support recovery
- Consists of a heated block holds up to 5 tubes at a time
- Each tube (clear polycarbonate, 6” long x 1.5” in diameter) holds a single mouse and vented on both ends to ensure oxygen and carbon dioxide circulation
- Used for the first time for RR3 mission
Grip Strength Meter (RR-3)

- Records the peak force required to remove the mouse from the grid
- To be repeated up to 4 times for each mouse and averaged of 3 or 4 peak force measurements be used as the grip strength per mouse