NASA’s Rodent Research Project: Validation of Capabilities for Conducting Long Duration Experiments in Space


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Background

• Animal research is an essential tool for understand the impact of space flight on the ISS.

• Importance of performing animal research on the ISS was strongly stated in National Research Council Decadal survey report issued in 2011.

• The Animal Enclosure Modules (AEM) have flown successfully 27 times on the U.S. Space Shuttle.
  – The AEMs were modified to support animals:
    • Transporter (AEM-T): provides housing for rodents during ascent/descent on Dragon
    • Habitat (AEM-X): provides on-orbit housing for rodents in an EXPRESS rack on the International Space Station (ISS)
    • Animal Access Unit (AAU): provides the capability to access the animals on orbit and transfer the animals from one habitat to another or to the glove box

• Provide reliable, long duration habitat for rodents on the ISS
Validation Flight Mission Objectives

1) Validate that the Rodent Habitat Hardware can deliver and maintain healthy animals:
   • Determined on basis of:
     ✓ Daily animal health checks (video, direct)
     ✓ Body weights

2) Validate that on-orbit activities to support hardware operations can be performed:
   • Animals transferred, euthanized, and dissected humanely and safely

3) Validate that a limited set of generic on-orbit operations can be performed to support future science objectives including but not limited to euthanasia, gross dissection and sample preservation
The RR Hardware

Transporter

Animal Access Unit

Mouse Transfer Box

Kits (various)

Habitat
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

Animal Access Unit
Transporter
Habitat
Microgravity Science Glovebox
RR1 Experimental Design for on-orbit sample retrieval for Validation

**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)

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

Hind limb → 10 legs → Frozen
10 legs → Formalin-fixed

Liver and Spleen for Validation

**Liver:** fast frozen: RNA analysis and enzyme activity measurement
**Spleen:** preserved in RNAlater: RNA analysis
Experimental groups of mice

- 4 separate groups to better understand observed responses to this unique habitat and environment.

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Group</th>
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<tbody>
<tr>
<td>Age</td>
<td><strong>Basal</strong> (time of launch= starting, in standard cages)</td>
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<tr>
<td></td>
<td><strong>Vivarium</strong> (in standard cages)</td>
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<tr>
<td>Cage</td>
<td><strong>Ground Controls</strong> (in flight hardware on Earth; matched to ISS)</td>
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<tr>
<td>Gravity</td>
<td><strong>Flight</strong> (flight hardware on ISS)</td>
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Post-Flight Sample Analyses

• Body weights from the 8 intact NASA carcasses were measured to assess animal health

• To confirm on-orbit sample preparation and storage capabilities:
  ➢ Spleens: RNA quality analysis
  ➢ Liver: RNA quality analysis and enzyme activity measurement

• After return, livers and spleen from frozen carcasses of NASA mice were dissected and analyzed in support of the validation objectives.
  ➢ Spleen: RNA analysis
  ➢ Liver: RNA analysis and enzyme activity measurement
  ➢ Remaining tissues: stored in the Ames Life Science Data Archive and made available for biospecimen sharing through the NASA Research Announcement (NRA) process
**NASA animal body weights**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Body Weight (g)</th>
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<tbody>
<tr>
<td>Viv at launch</td>
<td>20</td>
</tr>
<tr>
<td>Viv after 37 days</td>
<td>20</td>
</tr>
<tr>
<td>GC at launch</td>
<td>20</td>
</tr>
<tr>
<td>GC after 37 days</td>
<td>20</td>
</tr>
<tr>
<td>Flight at launch</td>
<td>20</td>
</tr>
<tr>
<td>Flight after 37 days</td>
<td>20</td>
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Data: mean+/−SD, n=8/group

* P.C. group: Age-matched mice from separate cohort, euthanized at time of dissections

** Basal group: Euthanized shortly after launch

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**Note:**
- **Viv** at launch and after 37 days
- **GC** at launch and after 37 days
- **Flight** at launch and after 37 days

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**Legend:**
- **Viv**
- **GC**
- **Flight**
High RNA quality achieved from tissues dissected on-orbit (NASA)

- **qPCR** = Quantitative Polymerase Chain Reaction
- **RNA-Seq** = RNA Sequencing
- **RIN** = RNA Integrity Number, index of quality/degradation of total RNA from value of 1 (lowest) to 10 (highest)
High RNA quality achieved from tissues dissected from frozen carcasses (NASA)

![Graph showing RIN values for different conditions including Positive Control, Basal, Vivarium, Ground Controls, and Flight. The graph includes bars for RNAseq, Gene profiling, and qPCR methods, with mean±SD (n=5/group).]
Validation Mice:
Livers dissected from frozen carcasses after return

Catalase Activity

Statistical analysis was performed without the Positive Control group because the PC mice were not from the same cohort as the Basal, GC, or FLT mice.

Mean+/− SD, n=7/group, One factor ANOVA, Tukey’s post hoc test
Specific Catalase Specific Activity

PC=Positive Control
Mean +/- SD, n=5/group

p>0.05
Wet Tissue Weights from frozen carcasses

Liver (mg/g BW)

Spleen (mg/g BW)

N=6 for Basal, Viv, and FLT; N=7 for GC
Wet Tissue Weights from frozen carcasses

Adrenal glands (mg/g BW)  
Thymus (mg/g BW)

N=8/group, Mean+/−SD  
One factor ANOVA, Tukey’s post hoc test
Wet Tissue Weights from frozen carcasses

N=8/group, mean±SD
One factor ANOVA, Tukey’s post hoc test
No significant changes in masses of other muscles (gastrocnemius, tibialis anterior, quadriceps, EDL-extensor digitorum longus)
Expanding science return from RR1

• RR science team recovered for distribution to scientists: **32 tissues** from 40 RR1 Validation mice, yielding total of **3280 vials** of tissues

1) Biospecimen Sharing Program-Space Biology
   – to provide samples to various scientists, including Russian research colleagues at the Institute for Biomedical Problems (IBMP)

2) NASA Genelab project
   – large scale data set analyses (‘omics) then provide open access

Ames Life Science Data Archive (LSDA)
Summary

• Mice thrived through 37 days in microgravity
  - Some common indices of stress were **not** observed
e.g. body weights and adrenal gland weights did not differ
  - High quality samples recovered: suitable for applying cutting edge molecular biology methods

• Findings on tissues contrasted sharply to those of shorter duration, Shuttle experiments
  - Tissue weights (liver, adrenal gland, thymus, spleen)
  - Interesting up-regulation of catalase, oxidative stress-related liver enzyme activity

• Speculate responses to spaceflight depend on duration; multiphasic?
Conclusion

✔ Validated hardware, ops, and science for acceptable science return

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

✔ Provided samples for Space Biology-Biospecimen Sharing Program and the GeneLab’s omic’s analyses
Validation mice behavior on ISS

Qualitative observations made during daily health checks:

• Upon initial introduction into the Habitat, mice actively explored the compartments
• Mice were observed eating, drinking, grooming and socially interacting while in the Habitats
  All considered normal behaviors of healthy mice
• Mice propelled themselves freely and actively throughout the Habitat using their forelimbs
  o Mostly by ‘pulling’ along cage grate with their forelimbs or by ‘floating’ from one location to another
• 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

Quantitative behavioral analysis in progress: April Ronca, ARC
Video clip
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Rodent Habitat: Science Working Group
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