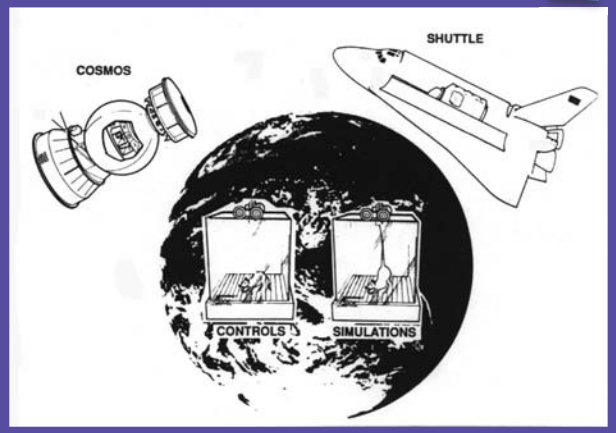
Rodent Research-1
Validation of Rodent Hardware

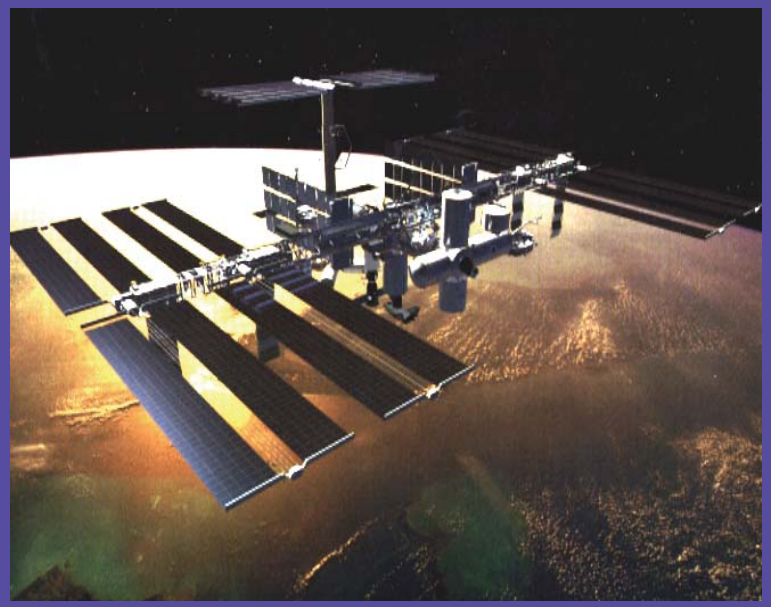
Ruth Globus, Ph.D.
Project Scientist

Janet Beegle
Project Manager

Direction for Rodent Research on ISS

NRC Decadal Survey for NASA (2011) : panel members unanimous in recommendation that ‘an animal habitat should be incorporated as soon as possible into the ISS’





Rodent research focus areas needed per NRC:

Bone, Muscle, Cardiovascular, Immune, Endocrine

Interacting systems and factors (including Radiation)

Importance and Reason for ISS

To achieve novel science objectives, validation of a rodent habitat on ISS will enable:

- In-flight analyses during long duration spaceflight
- Use of genetically altered animals
- Application of modern analytical techniques
  (e.g. genomics, proteomics, and metabolomics)

Scientific knowledge to be gained from Rodent Habitat capabilities on ISS:

- Better understanding of how long term habitation in space affects **mammalian physiology throughout lifespan:** 180 days is ~ 1/3 adult rodent lifespan
- Determine basic cellular and molecular **mechanisms** of gravity responses.
- Better define factors that impact crew health **risk and** develop effective **countermeasures**

Overarching Hypothesis & Goals for Rodent Research on ISS

Research conducted using rodents is foundational for advancing basic biological and biomedical scientific progress in space as on Earth.

Research conducted using the Rodent Habitat on ISS provides the capability to:

- Determine biological and health effects of long duration spaceflight.
- Develop mitigation and countermeasure strategies for adverse effects

Background and New Capabilities

- Heritage Animal Enclosure Modules (AEM) flew rats or mice successfully 27 times on the U.S. Space Shuttle.
  Longest flight: 19 days
- Extend for ISS capability: up to 180 days
- New hardware and operations requirements to conduct research on ISS:
  - transit on unmanned vehicle (SpX Dragon)
  - life support
  - telemetry for matching Ground Control group
  - long duration (30 days minimum per unit)
  - video observation to minimize crew time
  - on-orbit sample collection, preservation and return to Earth for analysis

RR-1: Validation Mission Objectives

Validate that:

#1 the Rodent Habitat can deliver and maintain healthy animals deemed suitable for scientific research

#2 on-orbit activities to support hardware operations can be performed successfully

#3 a set of generic on-orbit operations can be performed which are needed to support science objectives, including euthanasia, dissection and sample preservation

- demonstrate capability of obtaining high quality science return for application of modern analytic methods

Hardware Overview

TRANSPORTER

- Short duration housing during ascent/descent) on Dragon
- Accommodates 20 mice, (up to 12 rats)

HABITAT

- Long-duration housing for rodents on the ISS
- Accommodates 10 mice (up to 6 rats)

ACCESS UNIT

- On-orbit animal access and transfer

Additional kits and support equipment

RR-1 Validation Methods: Animals

- Mice: females, adult (4-mo old), C57Bl/6J strain
  - common background strain for transgenic/knockout animals
- Flight mice loaded into a Transporter (total 20; 10 for Validation, 10 for CASIS)
- Control mice (cohorts of flight mice)
  - Ground Controls-housed in Transporter on time delay and in environmental simulator to matched Flight conditions (Temperature, Carbon Dioxide, Humidity)
  - Vivarium controls (standard housing)

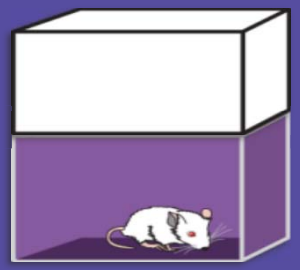
Methods: Basic Concept of Operations

Access Unit

4. ISS Dock/Animal transfer (1 day)

5. ISS Habitat: up to 1 month

Habitat



7. Sample Cold Stowage

3. Ascent (~ 3 days)

2. Launch

1. Late Load on Dragon

Transporter

6. Euthanasia and tissue retrieval

Glovebox

8. Descent

Measurement Approach

- Animal health observations:
  - Direct: crew visual observations made during animal transfer and dissection
  - Indirect: video record reviewed by experts on ground shortly after collection
- Body weights:
  - Advantage: quantitative indicator of general health
  - Achieved by measuring body weights post-flight (euthanized animals).

Measurement Approach
Sample Recovery

- Rationale for tissues selected:
  - tissues affected by spaceflight
  - simple to dissect intact

- Liver and Spleen: quantitative assays for RNA quality (spleen) and enzyme content and activities (liver) to:
  - validate crew & handling operations on ISS:
    - preservation
    - past freezing
  - provide samples for future in-depth genomic and proteomic analyses

- Ground controls (delayed): tissues harvested with similar timing as flight mice

Validate Tissue Preservation Capability

- Spleen: preserved on-orbit in RNA*later* then frozen (≤80 ˚C) for ~6 months
  - -total RNA purified from the entire spleen after return
  - -quality of tissue preservation evaluated by:
    - -measuring the amount of total RNA recovered per organ (variance/mean compared to ground controls)
    - -analyze the quality of RNA (degradation)

- If adequate sample preservation, conduct post-flight global gene expression analysis (Genelab) and other tissue sharing capabilities
  - -science-enabling and informative about animal health/ spaceflight responses

Validate Tissue Freezing Capability

- Liver: quick frozen on-orbit and stored for up to 6 months
  - -Activity levels of enzymes are highly sensitive to temperature/storage conditions.
- Candidate enzymes: selected as either regulated by spaceflight or constitutively expressed.
- Assays performed on tissue after return of all samples:
  - -total protein content of the liver
  - -enzyme amounts and activities measured after return

Expected Outcome

- The Transporter and Habitat are expected to support the health & welfare of adult mice through all phases of the mission (ground-based verification tests)
  - -Launch simulation (vibration, hypergravity) in Transporter
  - - Biocompatibility in Habitat (32 days)

- Results from tissue samples analyzed for RNA and protein (enzyme activities and content) will establish science-enabling capability of all on-orbit mission operations related to sample fixation and freezing

Earth Benefits/Spin-off Applications

- New insight and possible treatments for major human diseases, e.g.
  - Osteoporosis
  - Muscle wasting
  - Cardiovascular disease
  - Immune dysfunction

- Customer base includes
  - NASA funded, peer reviewed research
  - Commercial: Pharmaceutical and biotech industries
  - Other government agencies: DoD, NIH, USDA, etc.
  - International Partners