NEW DEVELOPMENTS IN NASA'S RODENT RESEARCH HARDWARE FOR CONDUCTING LONG DURATION BIOMEDICAL AND BASIC RESEARCH IN SPACE

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Abstract

Animal models, particularly rodents, are the foundation of pre-clinical research to understand human diseases and evaluate new therapeutics, and play a key role in advancing biomedical discoveries both on Earth and in space. The National Research Council's Decadal survey emphasized the importance of expanding NASA's life sciences research to perform long duration, rodent experiments on the International Space Station (ISS). To accomplish this objective, flight hardware, operations, and science capabilities were developed at NASA Ames Research Center (ARC) to enhance science return for both commercial (CASIS) and government-sponsored rodent research. The Rodent Research program at NASA ARC has pioneered a new research capability on the International Space Station and has progressed toward translating research to the ISS utilizing commercial rockets, collaborating with academia and science industry, while training crewmembers to assist in performing research on orbit.

Starting with a validation mission in 2014, the Rodent Research team has successfully completed four missions to the International Space Station (ISS). In collaboration with commercial, academic, and government entities, the Rodent Research team has enabled researchers to study effects of the space environment on the musculoskeletal and neurological systems of mice as model organisms of human health and disease, particularly in areas of muscle atrophy, bone loss, and fracture healing. Results from these studies contribute to the science community via both the primary investigation and banked samples that are shared in publicly available data repository such as GeneLab. Following each flight, through the Biospecimen Sharing Program (BSP), numerous tissues and thousands of samples will be harvested, and distributed from the Space Life and Physical Sciences (SLPS) to Principal Investigators (PIs) through the Ames Life Science Data Archive (ALSDA). Every completed mission sets a foundation to build and design greater complexity into future research and answer questions about common human diseases.

Throughout phases of these missions, our practices, hardware and operations have evolved from tested to developed standards, and we are able to modify and customize our procedure and operations for mission specific requirements. The Rodent Research Habitat is capable of providing a living environment for animals on ISS according to standard animal welfare requirements. Using the cameras in the Habitat, the Rodent Research team has the ability to perform daily health checks on animals, and further analyze the collected videos for behavioral studies. A recent development of the Rodent Research hardware is inclusion of enrichment, to provide the animals the ability to rest and huddle. The Enrichment Hut is designed carefully for adult mice (up to 35 week old) within animal welfare, engineering, and operations constraints. The Hut is made out of the same stainless steel mesh as the cage interior, it has an ingress and an egress to allow animals move freely, and a hinge door to allow crewmembers remove the animals easily. The Rodent Research team has also developed Live Animal Return (LAR) capability, which will be implemented during Rodent Research-5 mission for the first time. The animals will be transported from the Habitat to a Transporter, which will return on the Dragon capsule and splashes down in the Pacific Ocean. Once SpaceX retrieves the Dragon, all powered payloads will be transferred to a SeaVan and transferred to the Long Beach pier. The NASA team then receives the transporter and delivers to a PI-designated laboratory within 120mile radius of Long Beach. This is a significant improvement allowing researchers to examine animals within 72hrs of re-entry or to conduct recovery experiments.

Together, the hardware improvements and experience that the Rodent Research team has gained working with principal investigators and ISS crew to conduct complex experiments on orbit are expanding capabilities for long duration rodent research on the ISS to achieve both basic science and biomedical objectives.

Objectives

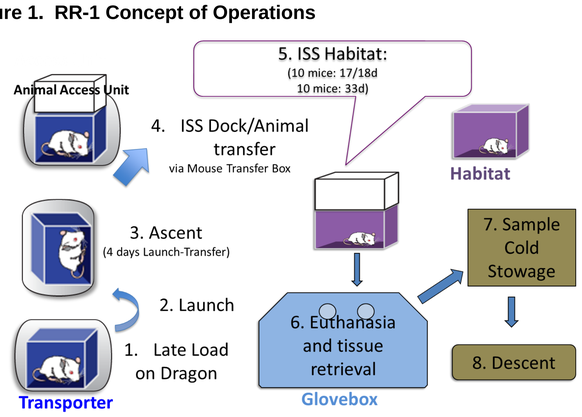
- To support long duration flight experiments with mice as a translational model for human risks in space
- Maximize science return through biospecimen collection and sharing
- Expand science capabilities with each mission, including recent advances to enable live animal return, cage enrichment, male mice as study subjects.

Introduction

- Animal studies are a valuable translational model because they facilitate extensive experimentation and application of techniques that cannot be applied to human subjects (e.g. extensive tissue sampling).
- To better understand how mammals adapt to long duration habitation in space, a system for performing rodent experiments on the ISS has been validated by the RR-1 mission and expanded in 4 subsequent missions that included on-orbit animal support and tissue preservation.
- To maximize science return, we developed methods to recover multiple tissue types from frozen carcasses following prolonged storage of carcasses that are now being applied to multiple ISS missions

Choi S, Ray HE, Lai SH, Alwood JS, Globus RK. Preservation of Multiple Mammalian Tissues to Maximize Science Return from Ground Based and Spaceflight Experiments. PLoS One. 2016 Dec 1;11(12):e0167391

Figure 1. RR-1 Concept of Operations

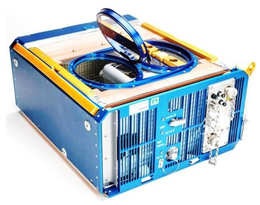


Materials

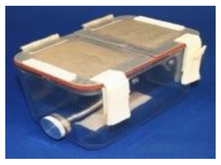
Flight hardware

Rodent Transporter
- transports mice from Earth (KSC) to ISS
- accommodates up to 10 adult mice in each of the 2 compartments

Animal Access Unit
- used by crew to move mice between:
  Transporter and Habitat OR
  Habitat and Microgravity Science Glove Box for on-orbit operations, including euthanasia and dissection



Rodent Habitat
- houses mice long-term on the ISS
- accommodates up to 5 adult mice in each of the 2 compartments
- continuously records humidity, temperature
- video capability to monitor health and behavior

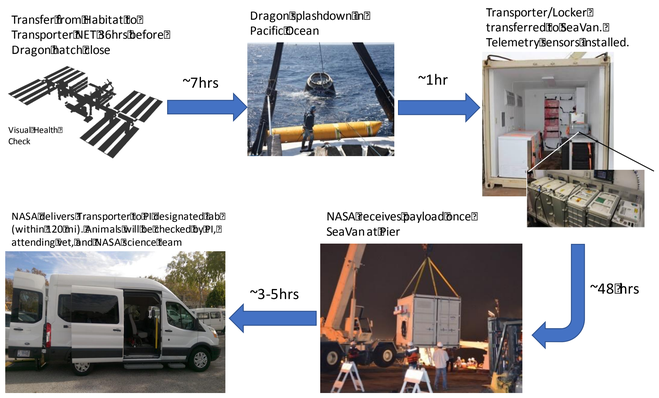


Mouse Transfer Box
- used to transport mice between:
  - Transporter and Habitat OR
  - Habitats and Microgravity Glove Box (MSG)
- also used to hold mice during on-orbit activities including injection, food bar changeouts, dissection, and other activities requiring temporary housing

- Samples recovered by on-orbit dissection or after return of frozen carcasses post-euthanasia, then distributed to PI or retained in via Life Sciences Data Archive for later distribution to and additional investigators (Russian IBMP, academic and government scientists)

Approach

Live Animal Return: Concept of Operations



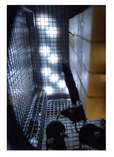
Experimental Groups of Mice

Comparison of results between the experimental group (Flight) and various other possible control groups (basal, vivarium, ground control) facilitates interpretation of which independent variable accounts for differences observed.

Independent Variable	Group
Time	**Basal** (time of launch= starting, in standard cages)
	Vivarium (in standard cages)
Cage	**Ground Controls** (in Flight hardware on Earth; matched to ISS)
Space	**Flight** (Flight hardware on ISS)

Progress

On-orbit video collection
Health checks and behavioral analysis*

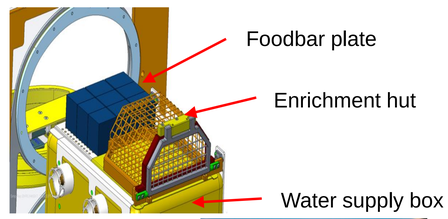


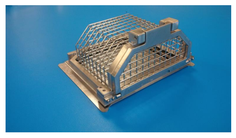
- 5 mice per compartment, 10 per Habitat
- Grating on all sides
- Air flow to entrap waste in filters
- Food supplied in form of bars
- Water supply (not in image)
- Lighting (dark-light cycle)
- Video cameras (infrared)

Rodents inside Habitat on Earth

*results also presented at 2017 ISS R&D annual meeting by Dr. April Ronca (NASA ARC)

Enrichment: hut





Summary of Missions Completed to Date

	RR1: National Lab - Novartis, and Verification of Rodent Research Hardware	RR2: National Lab - Novartis	RR3: National Lab - Eli Lilly	RR4: Department of Defense
Launch Date/rocket	September 21, 2014 (SpX-4)	April 14, 2015 (SpX-6)	April 8, 2016 (SpX-8)	February 19, 2017 (SpX10)
Primary Science Objective	Rodent Research Hardware and Operations Validation	Evaluate muscle atrophy in microgravity and identify molecular pathways and targets that could be used to develop novel therapies for muscle disease.	Evaluate effects of a drug aimed at preventing muscle loss	Characterize events associated with bone healing/tissue regeneration in a microgravity environment
PI	Dr. Samuel Candena (Novartis) and Dr. Ruth Globus (NASA)	Dr. Samuel Cadena	Dr. Rosamund Smith	Dr. Rasha Hammamieh
Mice	20 - C57BL/6 Female: 10 NASA mice 16 weeks old at launch, 10 CASIS mice 32 weeks at launch	20 - C57BL/6 Female, 16 weeks old at launch	20 – BALB/c Female, 12 weeks at launch	40 – C57BL/6 male 10 weeks at launch with pre-flight surgery
Duration	Transporter: 4 days Habitat: 33 days (NASA), 17.5 days (CASIS)	Transporter: 5 days Habitat: 50 days	Habitat: 45 days	20-23 days habitation in µG
On-orbit Measurements	None	Bone densitometry scan	Bone densitometry scan and Grip strength assessment	None
Dissections	Hindlimb dissection & fixation (1 leg/mouse) Spleen dissection and preservation in RNAlater Liver dissection Carcass freezing Froze with Mini Cold Bags, and then inserted into MELFI	Blood draw and separation Hindlimb and eye fixation Hindlimb dissection and freezing Carcass freezing Direct insert to MELFI	Blood draw and separation Hindlimb fixation Carcass freezing Direct insert to MELFI	Blood samples (frozen) Carcasses (Frozen) Hindlimb fixation planned for 20, one leg from each mouse Direct insert into MELFI
Ops Timeline	CASIS: 5 mice/day, 2 days NASA: 10 mice/day (2 dissected and 8 intact carcasses frozen)	5 mice/group processed at each timepoint (week 1, 2, 4 and 8)	Injection at L+2 weeks 2nd Injection, Grip Strength, Bone scan at L+4 weeks Grip Strength, Bone scan, Dissection at L+6 weeks	Bone Defect Surgery & Therapy, tail tattoo, x-ray
Firsts	Rodent Habitats on ISS, Transporters on Dragon, AAU, dissections in MSG, new dissection table, 33 d on orbit,+ many more, tissue fixation kit, carcass freezing kit, daily health check with video downlink	Soft Tissue Fixation Kit and Cardiac Puncture Kit, 60 d on orbit, Bone densitometry with live mice, food bar changeouts, Water refill	Injections every 2 weeks Grip strength measurements Anaesthesia and recovery on mice 5 consecutive days of crew time	Dissections from L+20 to L+23 over 4 consecutive days

RR5 Mission in progress

	RR5: National Lab - UCLA
Launch	6/3/17 (SpX-11) Preposition on OA-7
Primary Science Objective	Osteoporosis drug evaluation
PI	Dr. Lillian Choo, University of California, Los Angeles
Mice	40 - BALB/c Female, 30-40 weeks old at launch (32 week old mice preferred)
Duration	20 mice - ~30 days (Live Animal Return) 20 mice - ~9 weeks (63 days)
On-orbit Measurements	For 20 mice: Bone densitometry scan
Dissections	For 20 mice: Blood draw and separation Hind limb dissection, fixed, fixative swap Carcass frozen Direct insert into MELFI
Pre-Launch	Tail tattoo, microchipping, DXA scan
Ops Timeline	IP Injections (treatment and bone marker) to occur every 2 weeks on-orbit, except no bone marker during first session. LAR for 20 mice at L+30. L+4 to 5 weeks bone densitometry with recovery. Euthanasia at L+56d (min), bone densitometry scans just prior to euthanasia (to occur in minimum possible consecutive days). Samples return on Spx12.
Firsts	Live return Frozen syringes, Light blocking syringe bags 7 day foodbar changeouts Enrichment

Biospecimen Sharing

Biospecimen sharing enhances science outcome.

RR1 BSP (April 2015)
- 32 tissues from 40 RR-1 Validation mice
- 3280 vials of tissues

Available for sharing with Genelab and other investigators via Biospecimen Sharing (LSDA) (http://lsda.jsc.nasa.gov/ https://c3.nasa.gov/genelab/projects)

RR3 BSP (September, 2016)
- 25 tissues from 30 untreated mice (10 mice each from the basal, ground control and flight groups)
- ~1800 vials of tissues

RR4 BSP (April, 2017)
- 18 tissues from 40 sham operated mice (10 mice each from the basal, vivarium control, ground control and flight groups)
- ~1200 vials of tissues

Summary & Conclusions

- Hardware and operations, including on-orbit dissection, was performed successfully through sample return and thus, a new capability for long duration habitation of group-housed rodents on the ISS was validated,
- Experiments performed on five separate spaceflight missions enables achievement of both basic and translational research objectives.
- Recently added on-orbit capabilities include anesthesia recovery, DXA bone scanning, blood collection by cardiac puncture, group-housing for male mice, habitat enrichment, and functional grip strength testing.
- Science return is maximized through the Biospecimen Sharing Program (BSP), which is sponsored by NASA's Space Biology Program
- This work continues to advance translationally-relevant science discoveries on the ISS.

Acknowledgements

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