National Aeronautics and Space Administration

Rodent Research

Implementation of an Immunology Experiment on the ISS

Date: May 11th, 2015

Stanford University

The Immune System in Space – previous studies

- STS-77: FACS after 10-day shuttle flight (rats). Total T-cell populations decreased; fewer Th cells, fewer Macrophages
- STS-108: 12-day mission (mice), lower levels of INF-γ, TNF-α, IL-2, IL4
- STS-118: 13-day mission (mice), in spleen, lower WBC, lymphocytes, and granulocytes. Lower numbers of T cells (both Th, and Tc) and B cells
- Spaceflight experiments are inconclusive with respect to response to actual *in vivo* immune challenge. Since spaceflight clearly disrupts innate population distributions and function *ex vivo*, it is likely the spaceflight environment is detrimental to an immune response essential for host resistance during flight.

Part I: Background – Pecaut

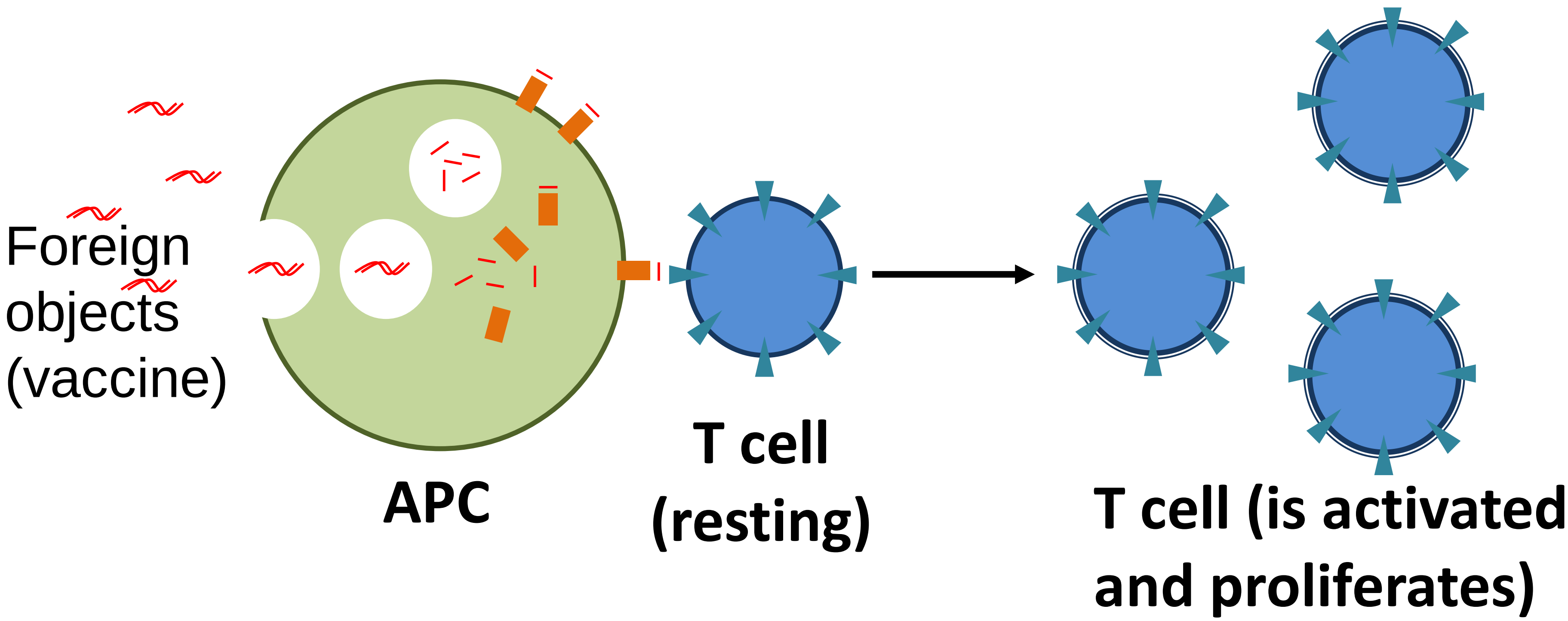
❖Previous animal research has shown that spaceflight has an adverse effect on immune populations.

❖These animal studies were performed after landing and may have been influenced by re-adaptation to Earth's gravity.

❖This study will look at one aspect of immunity, the generation of antibodies by B cells, in mice given a vaccination on-orbit.

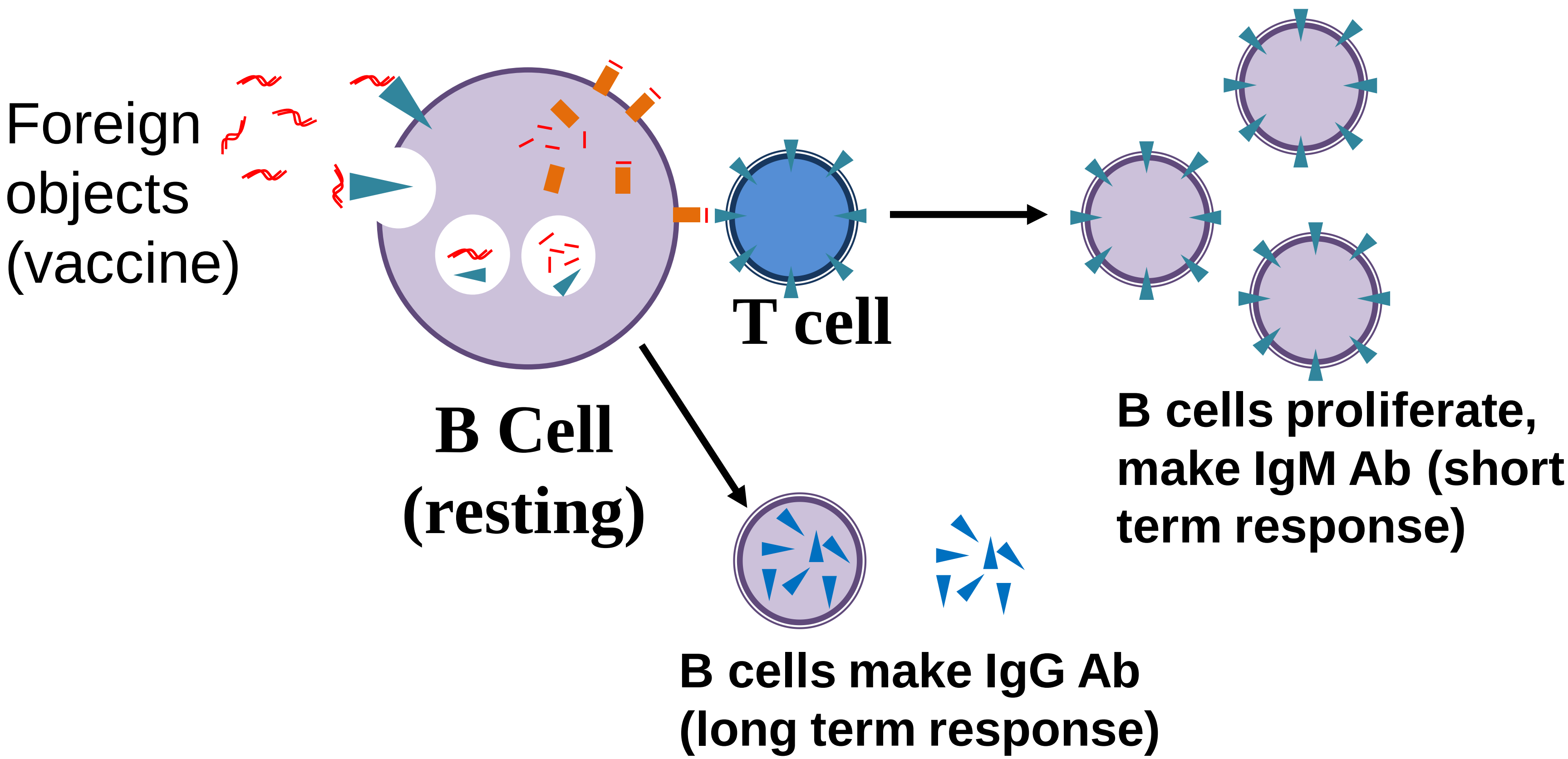
Part I: Background – Pecaut

Antibody Response: APC cells eat the vaccine, then bind to T cells specific to vaccine



Part I: Background – Pecaut

Antibody Response: B cells eat vaccine + are activated by T cells. Respond by making Ab



Part I: Objectives – Pecaut

❖**Aim 1**: Determine the impact of the spaceflight environment on primary antibody responses to vaccination.

❖**Aim #2**: Establish that CpG adjuvants (vaccine enhancers), which function through APC and B cell receptors, are effective during spaceflight.

Relevance/Impact: Previous research has shown that spaceflight can have an adverse effect on immune populations. However, while important, most of these studies have been performed after landing and may be influenced by re-adaptation to Earth's gravity. This study will look at one aspect of immunity, the generation of antibodies by B cells, in mice given a vaccination on-orbit. Through this research, we will not only help improve the health outcomes of astronauts, but increase our understanding of disease processes on the ground.

1. PI Experiment Objectives

Pecaut (primary PI) Loma Linda University

Impact of Spaceflight on Primary and Secondary Antibody Responses (NNX13AN34G)

- 20 female, 10-11 week old, wild-type C57BL/6J mice
- After 2 weeks (or more) in space, mice are injected with a vaccine or placebo to study immune response. Additionally they are injected with a vaccine enhancer (CpG Adjuvant) to see if the B-cell response can be improved
- At least 2 weeks after injection, mice are sacrificed, blood and spleen are collected

1. Chapes BSP Experiment Objectives

Chapes (BSP PI) Kansas State University

Collection of Immune/Stress-related Tissues from Mice Flown on ISS (NNX15AB45G)

- Collect liver, bone marrow, thymus and adrenal glands (as part of the kidney) from frozen carcasses after flight.
- Study impact of space flight on the B cell response to tetanus toxoid antigen immunization
  - **Specific Aim 1**: Collect thymus to weigh the tissue to compare relative thymic atrophy among treatment groups and adrenal glands to assess catecholamines. These tissues will be used to assess short- and long-term stress to the flight animals.
  - **Specific Aim 2**: Collect liver and bone marrow. These tissues will be used for the preparation of RNA to allow for a comparative analysis of B cell IgH usage between lymphoid compartments.

Relevance/Impact: assess if stress of space flight is evident and correlates with changes in antibody responses to uncover underlying mechanisms of stress and immune function in space

RR-2 Mission Science Overview in Ten Steps

Experiment Definition

1. Proposal reviewed by NASA or CASIS
2. Feasibility analysis and improvement
3. Integration, and baseline of requirements

Derivative requirements

4. Pre-Flight experiments
5. Hardware/EUE requirements
6. Operational requirements

Implementation

7. Procedures, documents, and safety
8. Operations
9. Kits / Ancilliary Hardware (Kits)
10. Pre- and post-Flight activities prep

2. Feasibility: Pecaut

❖**Following feasibility analysis, ARC science staff has worked with the PI to revise the proposed study plan as follows:**

1. Vaccinations can be delivered via intraperitoneal (belly) injection without loss of science; no longer requires intramuscular injection.
2. Injectable anesthesia is acceptable; originally requested isoflurane gas.
3. Achievement of all science objectives requires collection of blood and spleen only; all other tissues for other investigations of biospecimen sharing.
4. PI offered schedule flexibility allowing for pushing of dissections out of the dragon docked phase.
5. PI has agreed that injection of mice will occur on one day, and harvesting of tissue can be achieved over 5 days; reduces the number of dissections required per day.

❖**ARC concluded that the above modifications were sufficient to overcome all the obstacles to successful flight implementation that existed in the original NRA proposal.**

3. Integration of Space Biology Experiments

Adaptation — 2 Weeks

1 Primary Response — 2 or More Weeks

2 → **3** Process and storage of tissues

"Launch"

Injections

Euthanize, dissect

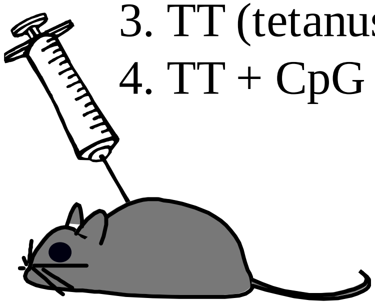
1 Injections (5 mice each):

1. PBS (salt water)
2. CpG (vaccine enhancer)
3. TT (tetanus vaccine)
4. TT + CpG

14 days

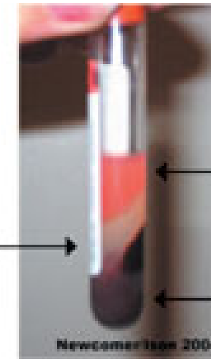
2 **Euthanize 4 mice/day for 5 days, harvest:**

1. Blood (for antibody levels)
2. Spleen (rich source of B cells)
3. Carcasses frozen (BSP, Chapes)



C57/BL6 female

Blood



Spleen

Carcass

Wrapped in Foil and Placed in Cold Bag

4. Pre-Flight Testing: 90 Day Biocompatibility Test

- How do we house, feed, and monitor mice for up to 90 days?

4. Pre-Flight Testing: 90 Day Biocompatibility Test

5. Hardware: Food bar plates

- Nutrient-upgraded Rodent Food Bars (NuRFB) consist primarily of cornmeal, milk protein, soybean oil, and added vitamins. Unlike standard food, moisture content is 25% - 30%. Potassium sorbate coating for preservation.

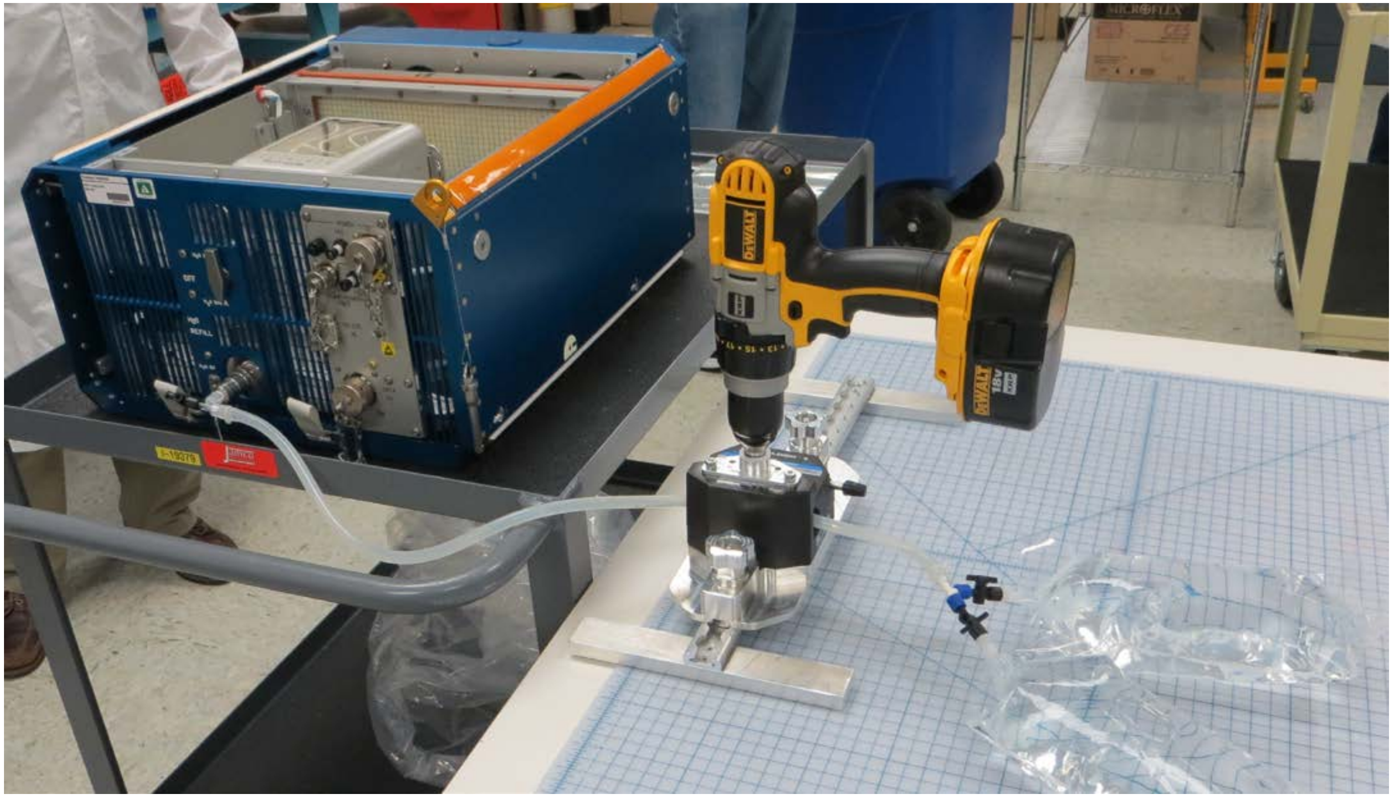
- Food mounted to aluminum plates and vacuum sealed

- Conducted food bar storage test – no mold growth observed on 12 plates stored at 4ºC for 56 days

- Water box used spring force to compress water in blood bags for delivery to pressurized lixits (~30 psi)

5. Hardware: Water Refill

- Consists of a peristaltic pump w/ counter and hose with fittings to interface with the Habitat.
- Crew drinking water “yesterday’s coffee” drawn from crew water bags
- Utilizes drill already on-orbit
- Pump mounted nearby Habitat but not directly to the same seat-track



90 Day Biocompatibility Test

4 weeks of acclimation to food bars, water lixits, cage mates, etc

0

30

45

60

75

90

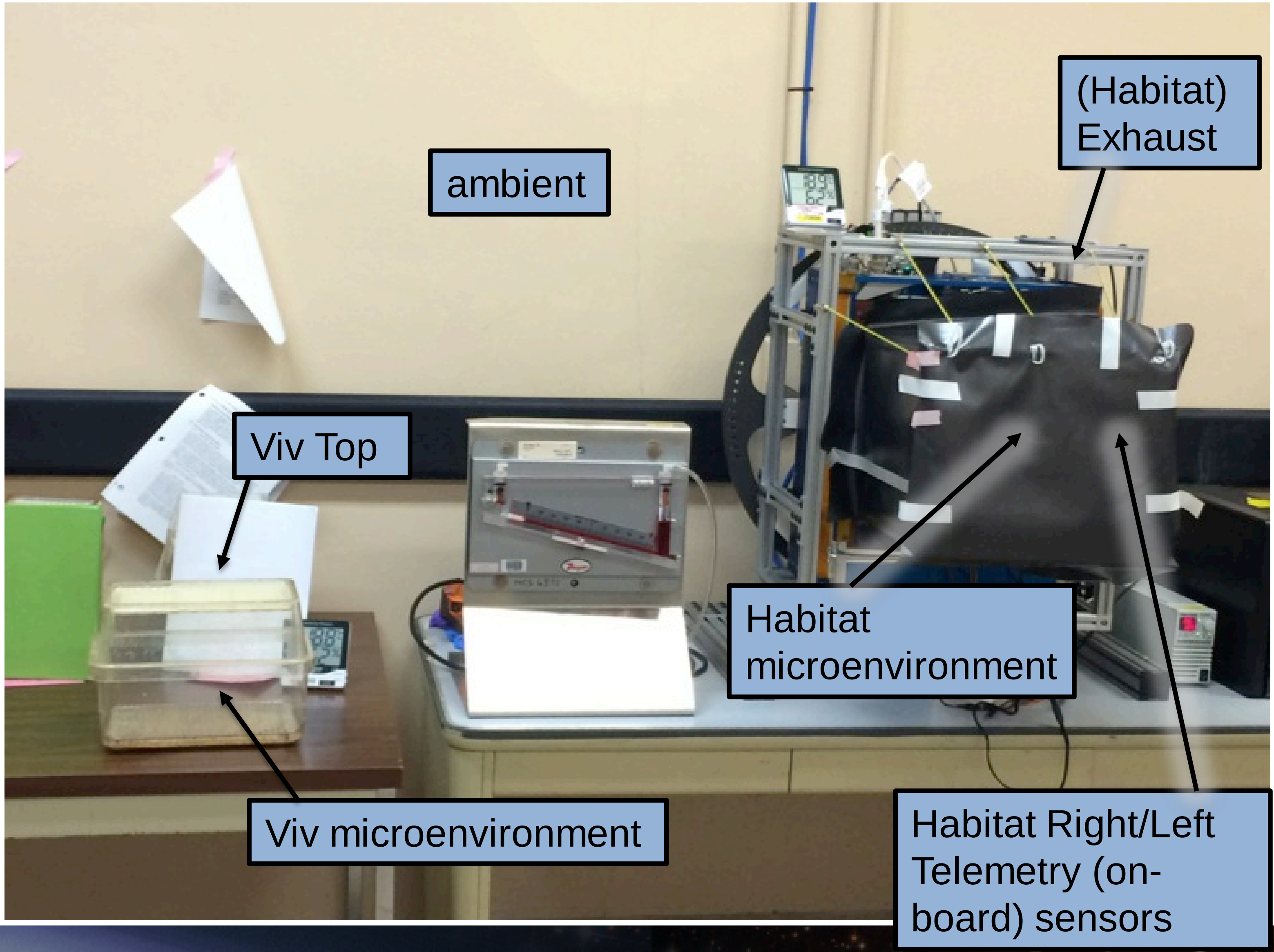
Days 30, 45, 60, 75, 90: mice weighed and checked by the vet

Day 0: Load 10 mice into the unit (5 per side)

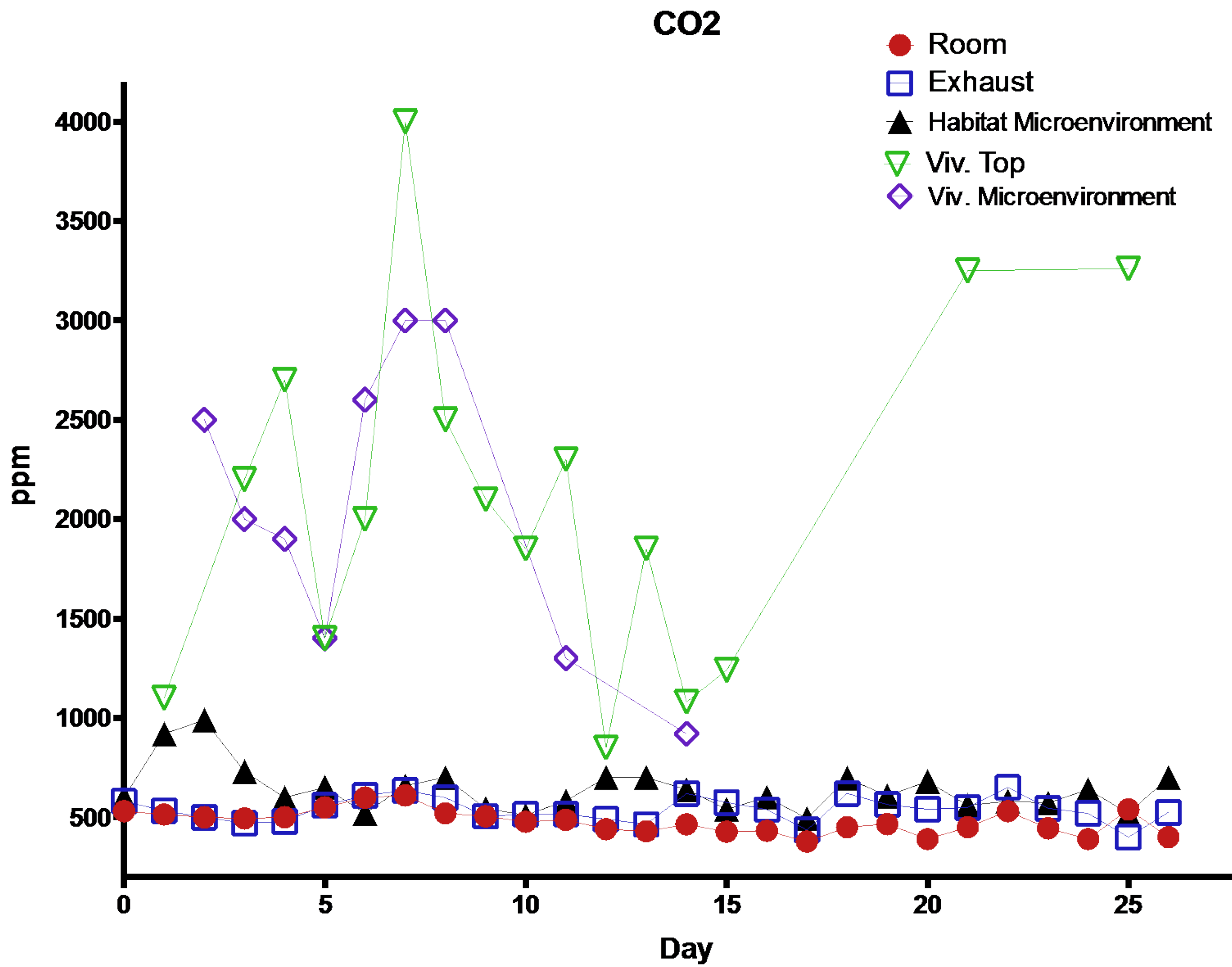
Day 90: Thymus, adrenal glands dissected and weighed. Blood stress hormones measured. Additional tissues taken and preserved.

Days 30 & 60: Food bars changed and water refilled

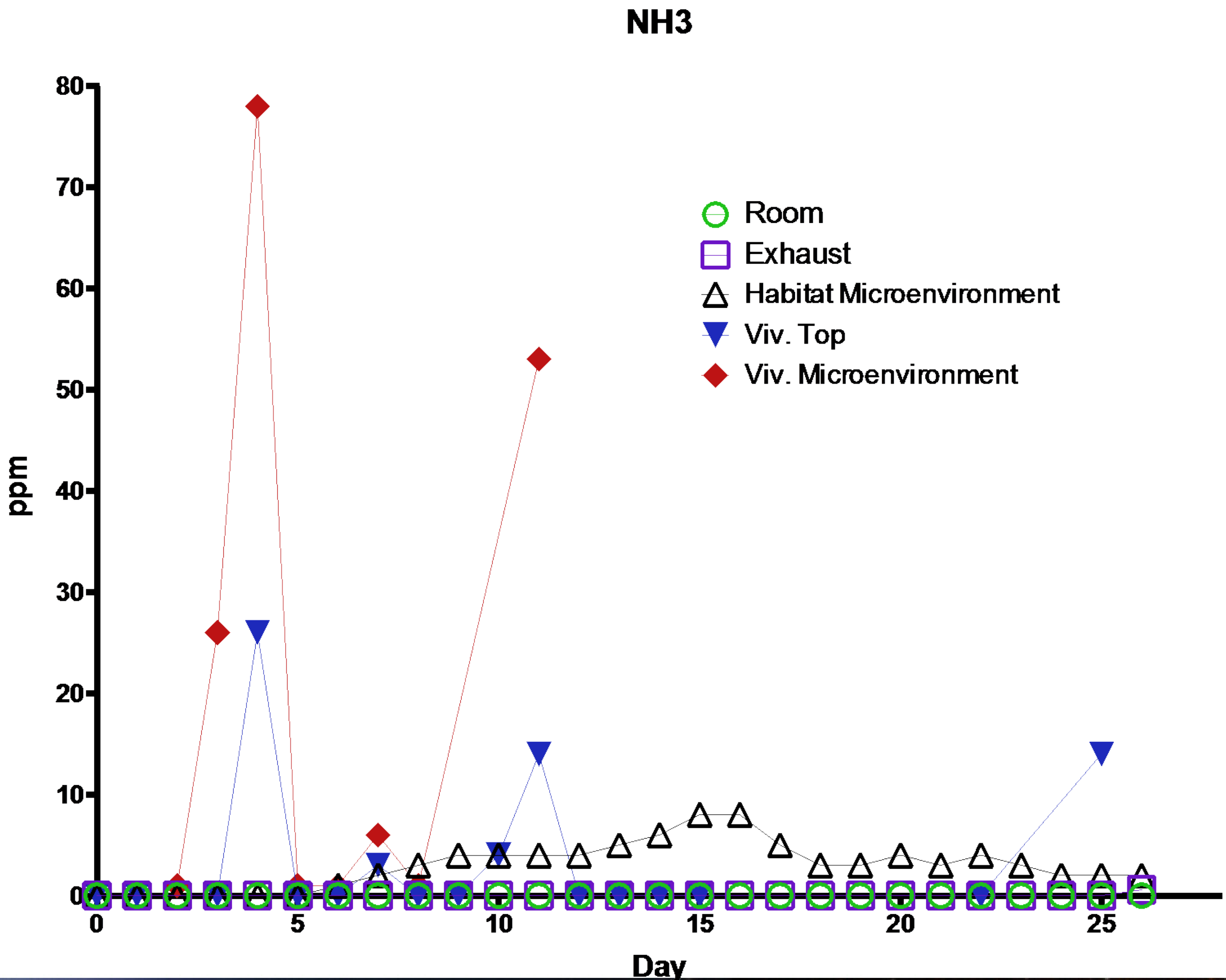
90 Day Test: Gas sampling locations



90 Day Test: CO_2 levels higher in vivarium control cages, remain near ambient (room) levels in Habitat

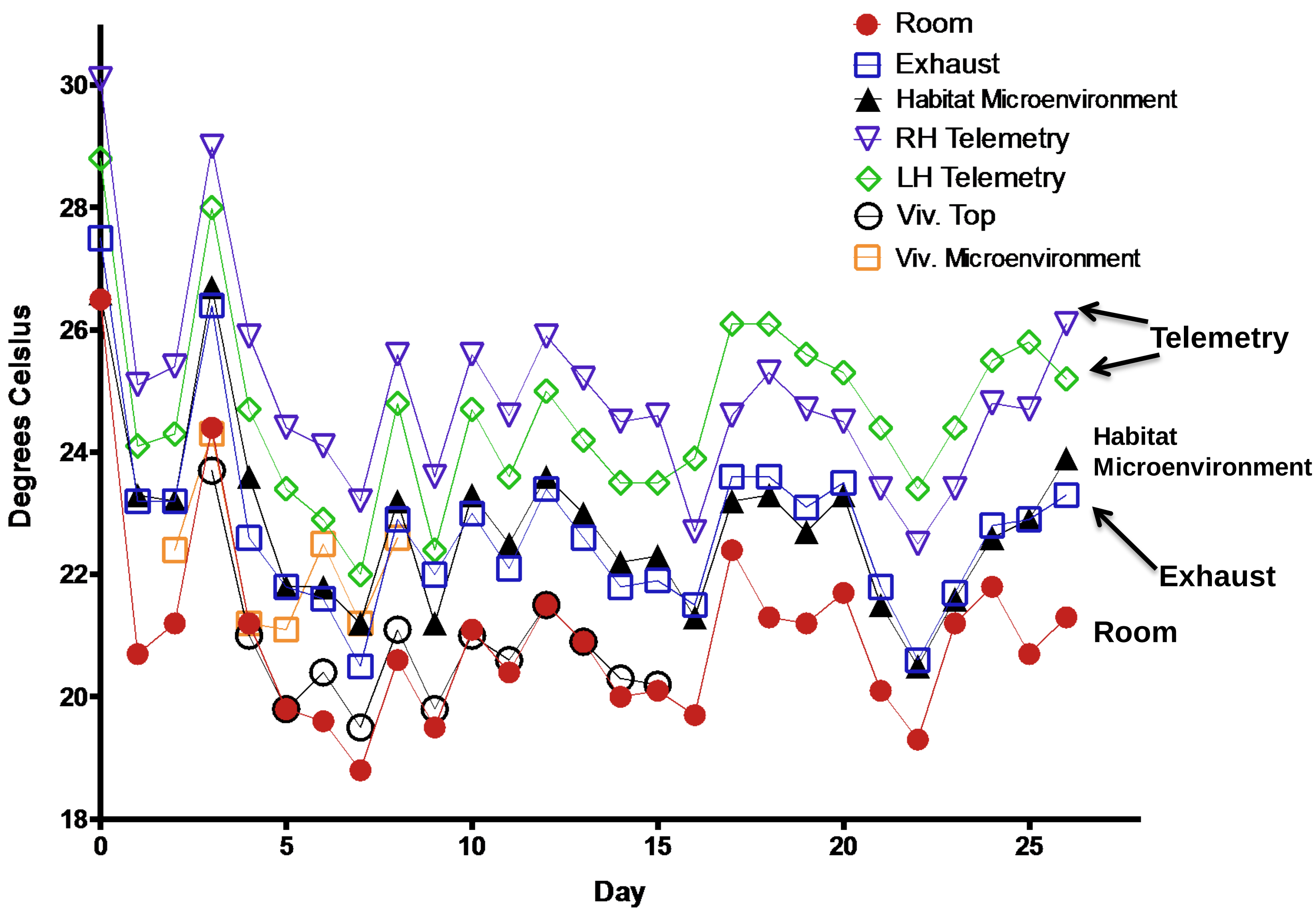


90 Day Test: NH_3 levels 0-3 ppm in Habitat, elevated in vivarium cages

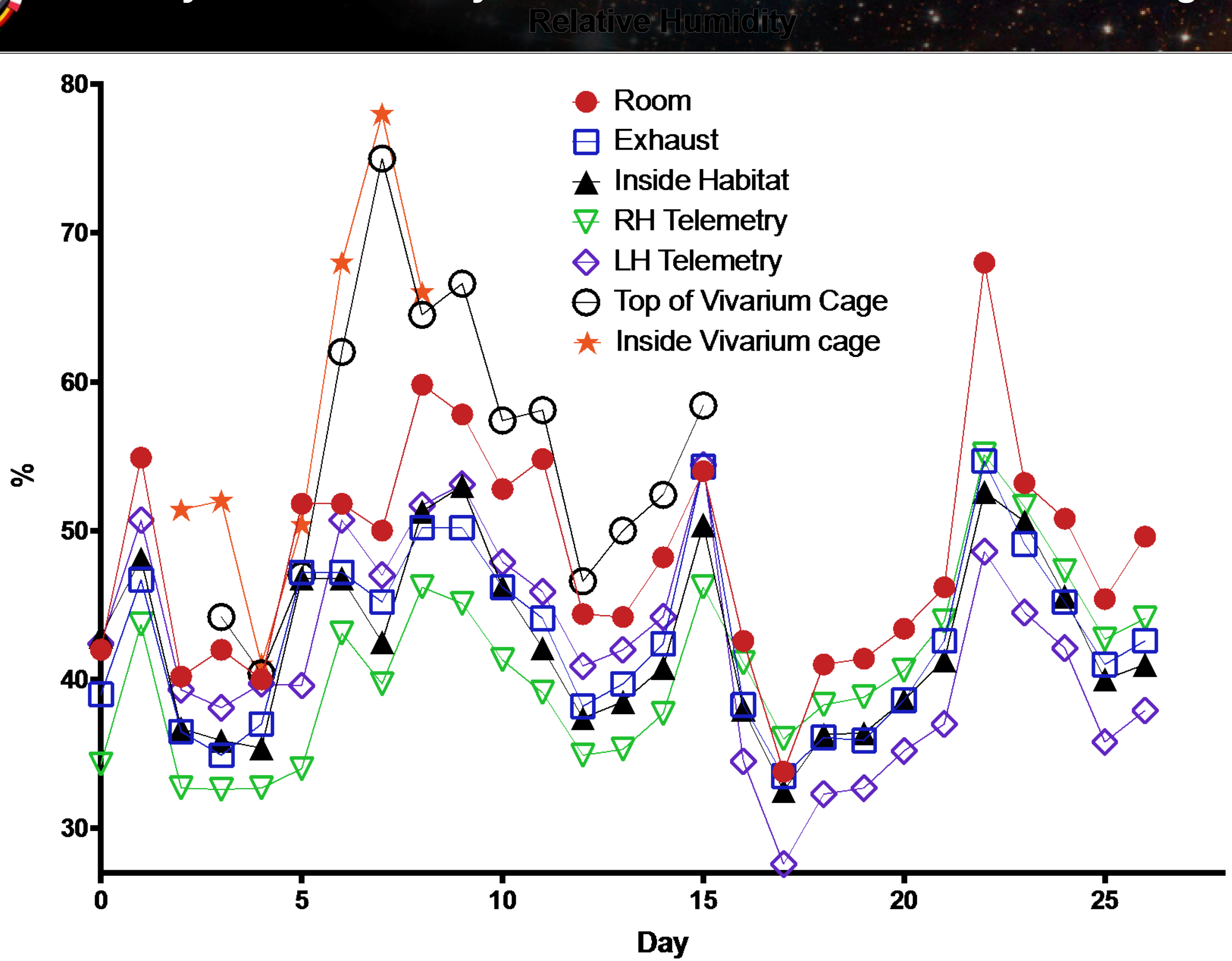


90 Day Test: Telemetry reads higher than the actual temperature, which is higher than ambient

Telemetry Temperature is Higher than Actual Temperature



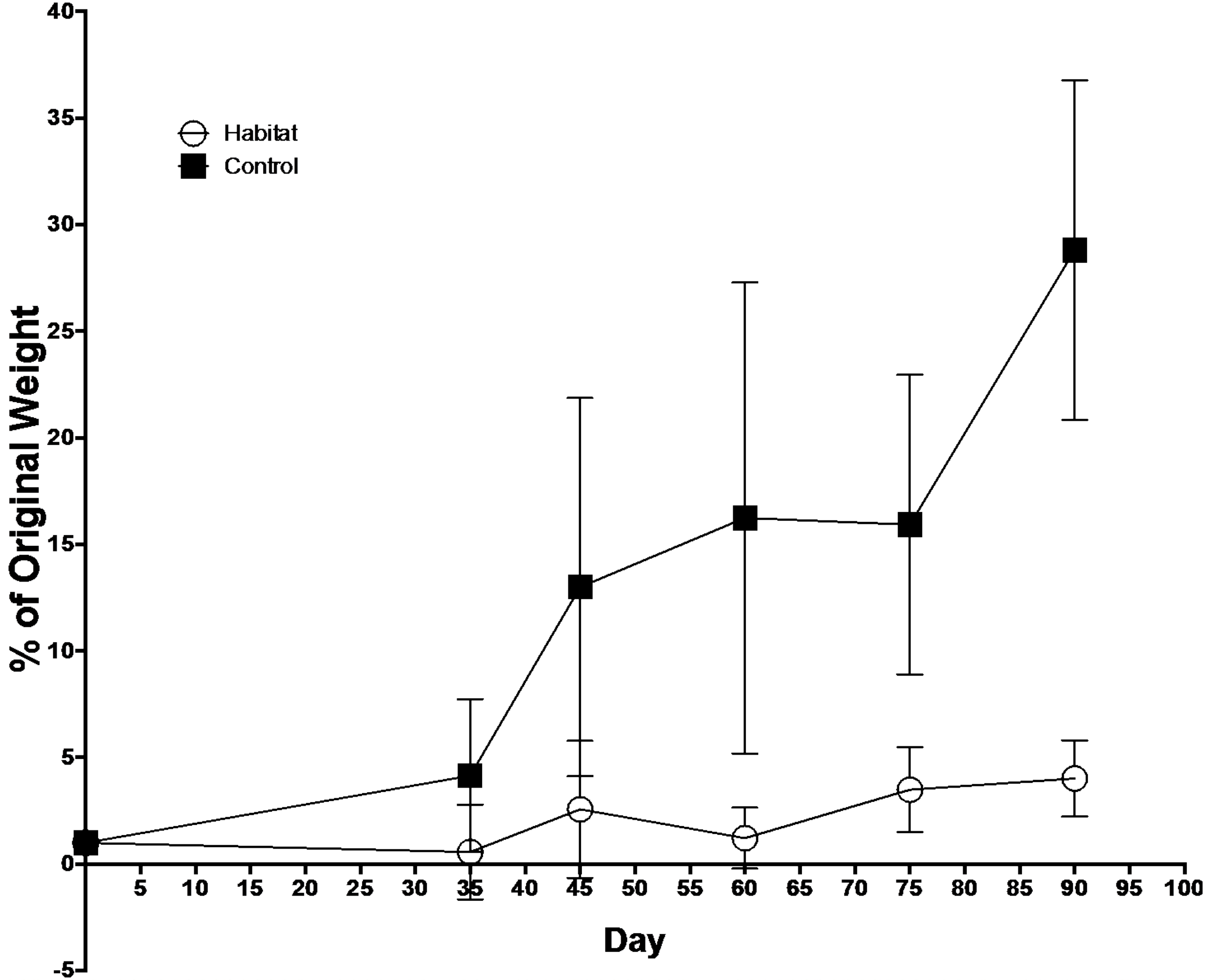
90 Day Test: Humidity in Habitat lower than in vivarium cages



90 Day Test: Environmental conditions summary

- Gas levels all within acceptable ranges
- Habitat CO_2, NH_3, and humidity, are lower than inside a standard mouse cage
- Temperature:
  - Habitat > room
  - Internal = exhaust
  - Right & Left integrated sensors > internal
- Results may be explained by high air exchange in the Habitat:
  - 1340 volume changes / hour
  - 22.3 volume changes / minute
  - 0.37 volume changes / second

90-day Test: Animals gain less weight than they do in vivarium cages



Results:

1. Mice were supported and remained healthy for the duration of the 90 day test.

2. Upon review of weight data and the soiling of the food bars, the Project Scientist, with the concurrence of Science Working Group, Attending Vet, and Chief Vet, implemented a requirement for food to be changed within 17 days.

3. Water was refilled at days 35 and 60 and water quality (fecal coliforms) was acceptable for the duration of the test

4. Analysis of indicators of stress was inconclusive (In Hab mice, no Δ in adrenal gland or thymus weight; but ↑ corticosterone in Hab mice versus Vivarium controls; Habitat mice had good bone density.

5. Mice exhibited ↑ in relative lean muscle mass and ↓ body fat in Habitat mice versus Controls

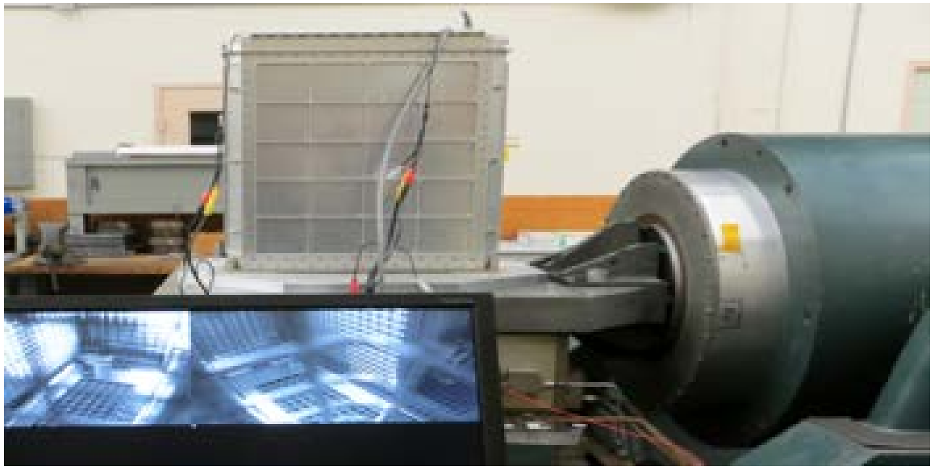
6. Multiple hypotheses exist for for weight Δ between Habitat and Control mice.

Launch Conditions

- How will the mice handle the launch environment?

Launch Conditions Evaluation

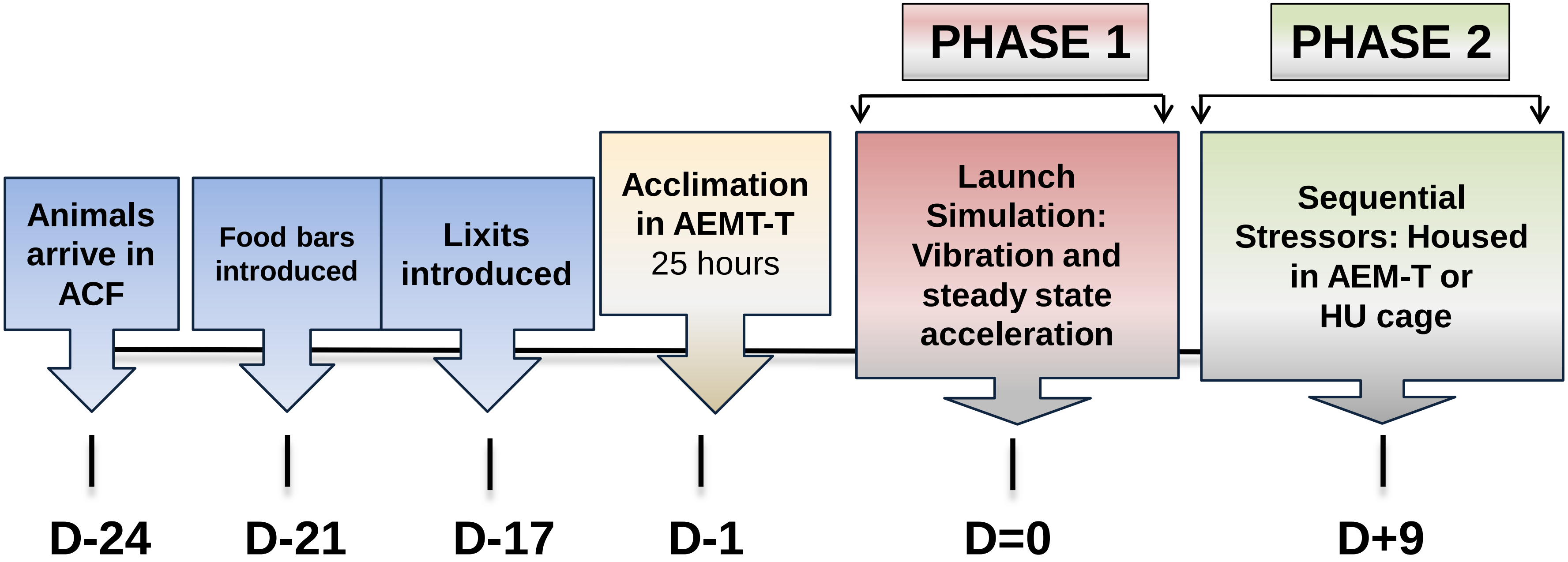
- Animals in Habitat exposed to sequential vibration and centrifugation
  - ARC Engineering Evaluation Lab shaker table produced **random vibration**



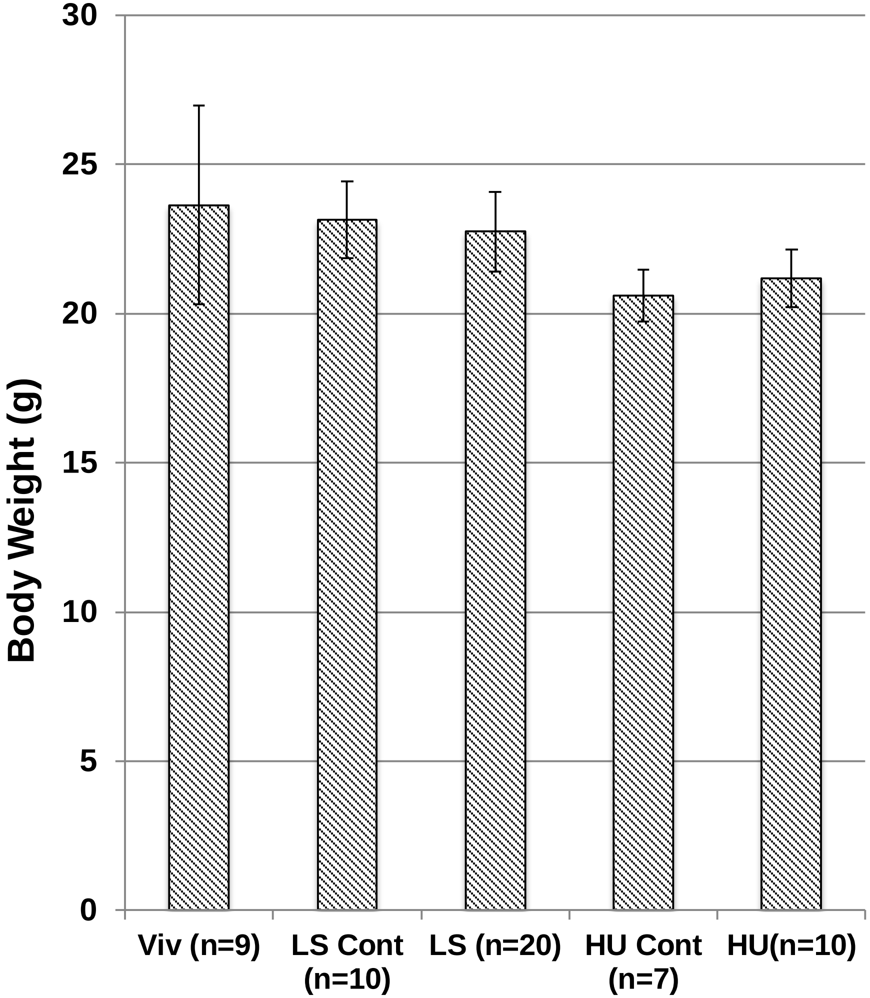
  - ARC 20-G centrifuge was used to produce **hypergravity:**

- Dragon launch profile ITAR controlled

Test Design and Timeline



Body weights of test groups were similar to controls



Mean+/- SD

Launch Conditions Evaluation

- **Animals tolerated simulated SpaceX Dragon launch**
- **No injuries occurred as a consequence of launch load, as determined by a veterinary health exam.**
- **At the end of the test, all animals were deemed healthy and suitable for research by the veterinarian.**
- **There were no significant differences in body weights between the test group mice and the matched control group mice.**
- **Acoustic levels deemed acceptable by analysis. Frequencies sub-sonic and non-auditory effects negligible (short duration)**

Pre-flight operations

- **Animals acclimated to cage-like environment (~ 3 weeks):**
  - **Lixit water**
  - **NuRFB**
  - **Raised cage floors**

- **The animals that have acclimated to flight conditions the best are chosen for flight and ground control groups**

- **Pre-flight treatments, baseline measurements done, as applicable**

- **Animals "flight certified" by testing for an extensive panel of zoonotic species to ensure they are specific pathogen free (SPF)**

Pre-flight operations – Animal Load to Capsule

Pre-flight: Crew Training

- Crew from diverse backgrounds, most of them have never handled a mouse
- Science input on all techniques trained to the crew
- Crew received hands-on training at JSC (~30 -> 40 hours) and are evaluated after each training session for proficiency. Additional training as required
- Rodent Research has started a stand-alone program, “Generic Rodent Skills” as pre-training. Astronauts eager to take this training as it increased their chances as being selected for a flight.

On-orbit operations

5. Hardware: Mouse Transfer Box (MTB)

- How do we move mice from the Habitat to the Microgravity Science Glovebox for experiment operations?

5. Hardware: Mouse Transfer Box (MTB)

- The Animal Access Unit (AAU) interfaces with the Habitat to maintain containment while the mice are being inserted or removed from the Habitat.

- Mouse Transfer Box (MTB) was designed for animal retrieval, temporary holding, and transferring between different units (Habitat/Transporter/ Microgravity Sciences Glovebox (MSG))

5. Hardware: Mouse Transfer Box (MTB)

waste & humidity ↑

Physical Activity Levels:
5-mouse MTB < 3-mouse MTB

All Active → **Reduced activity level, increased grooming** → Resumed activity

Time (Dark Cycle)

Hour 0

Added 3 or 5 mice to 2 MTBs (worst case scenario testing)

Hour 1.5

Hour 4

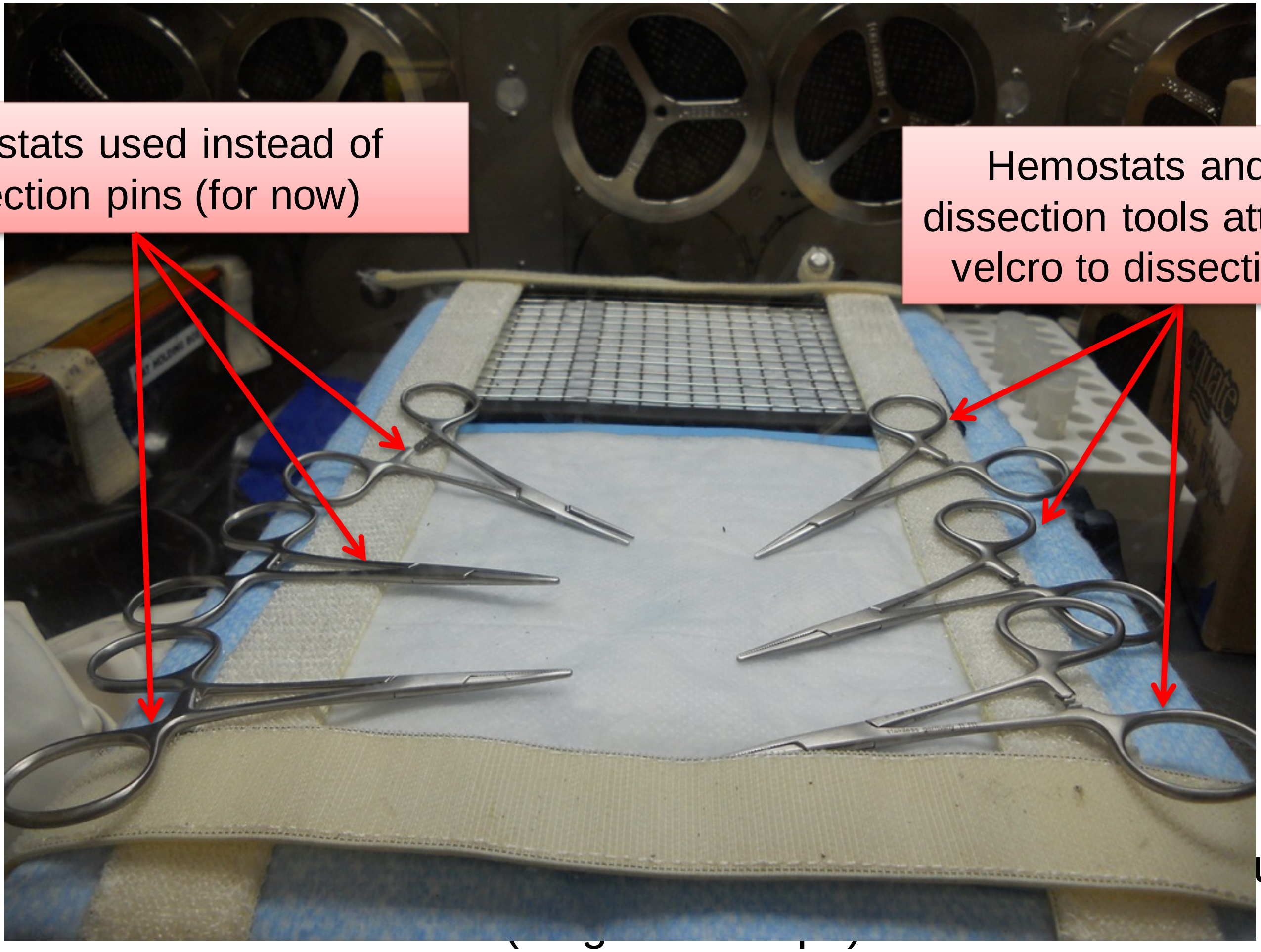
Test Ended: mice removed from the MTBs and placed into vivarium cages

- How do we perform injections and dissection on-orbit?

MSG (Ground Unit) with Rodent Research Hardware Setup
(single crew ops)

Microgravity Sciences Glovebox (MSG)

- How do we perform injections and dissection on-orbit?



up

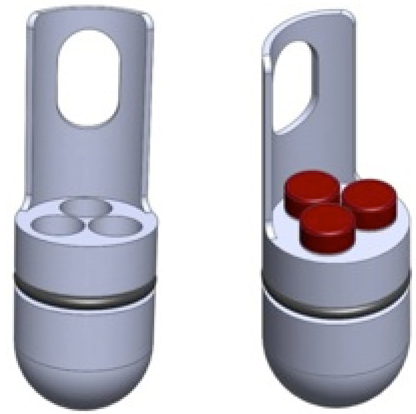
5. Hardware: **Rodent Research-2 Syringe Kits**

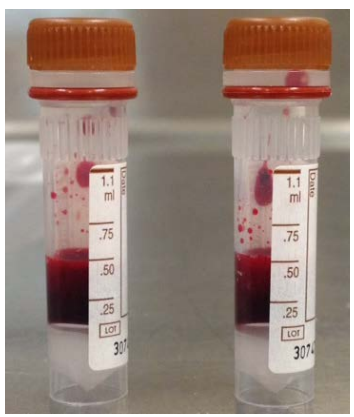
Syringe Kits

- ➢ Used for injections during flight and ground operations
- ➢ New procedures (ATOPs) were drafted to ensure the maintenance of cleanliness and sterility throughout manufacture
  - Testing was done to ensure that modified syringes could withstand possible press/depress
  - Since CpG must be frozen, testing performed at JSC confirmed that containment and syringe integrity was maintained during freezing to -80ºC

5. Hardware: **CPK fully validated for flight**

- Ames facilitated blood separation with new rotor adapter modification and longer centrifugation time

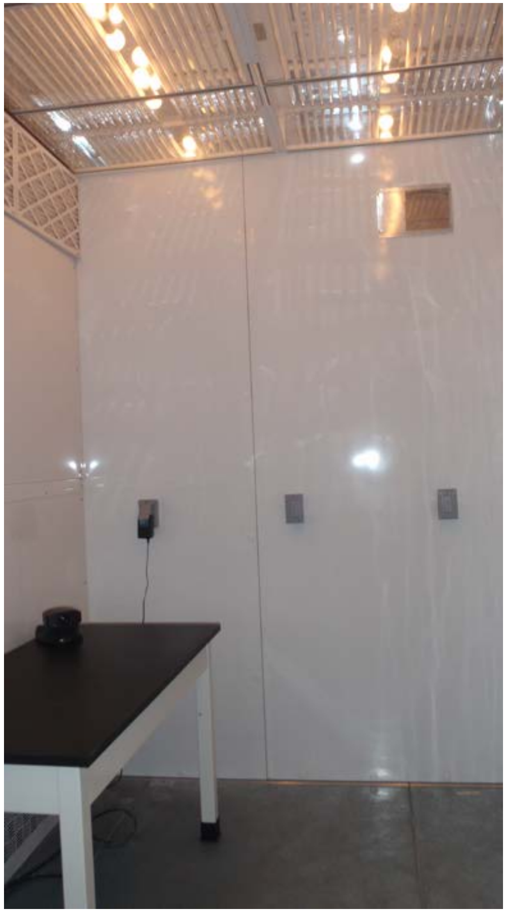




25 min

2,500 RCF

KSC ISS Environmental Simulator (ISSES; CO_2, O_2, Temp, RH)



Questions?