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CHARACTERIZATION OF SENSORY AND SHELTER ENRICHMENT IN THE RODENT RESEARCH HABITAT

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INTRODUCTION

The ISS provides a platform for conducting Rodent Research (RR) in microgravity and 9 missions have been successfully conducted. The results from these experiments have begun to provide new insights into the effects of spaceflight on mammalian physiological systems. After RR-1-4, the Flight IACUC required inclusion of additional cage enrichment into the Rodent Habitats (RH) to “enhance animal well-being by providing animals with sensory and motor stimulation, through structures and resources that facilitate the expression of species typical behaviors”. A Hut, in the form of a rigid, mesh igloo-like shelter was implemented beginning with RR-5. The potential influence of the Hut in the novel cage environment of RH on various spaceflight-sensitive physiological systems has not been fully explored.

OBJECTIVES

- Identify viable alternative enrichments to huts
 - Test hardware function
- Test enrichments side-by-side with plus-hut, no-hut, and vivarium groups
- Determine effects of various enrichments on animal health and welfare:
 - Body weights, plasma corticosterone, tissue weights (soleus, adrenal), immune cell profile, behavior, bone structure

EXPERIMENT DESIGN



Animals: Female, C57BL/6J, and 16 wks old
 Duration: 7 weeks in Flight-like Habitats.
 Husbandry: Weekly foodbar and enrichment changeout.
 Measurements:
 • Weekly environmental monitoring, enrichment soiling, NH₃, airflow, CO₂, Food, and Water.
 • 3 weekly 24-hour video recording sessions and daily health checks via video recording (1h/habitat).
 • Weekly body mass measurements.
 • Behavioral analysis of animals' interaction with enrichment, open field/novel object test.
 Tissue analysis: Bones (femur and tibia) for micro-CT analysis, adrenal glands for mass measurements, blood for corticosterone and immune cell phenotype

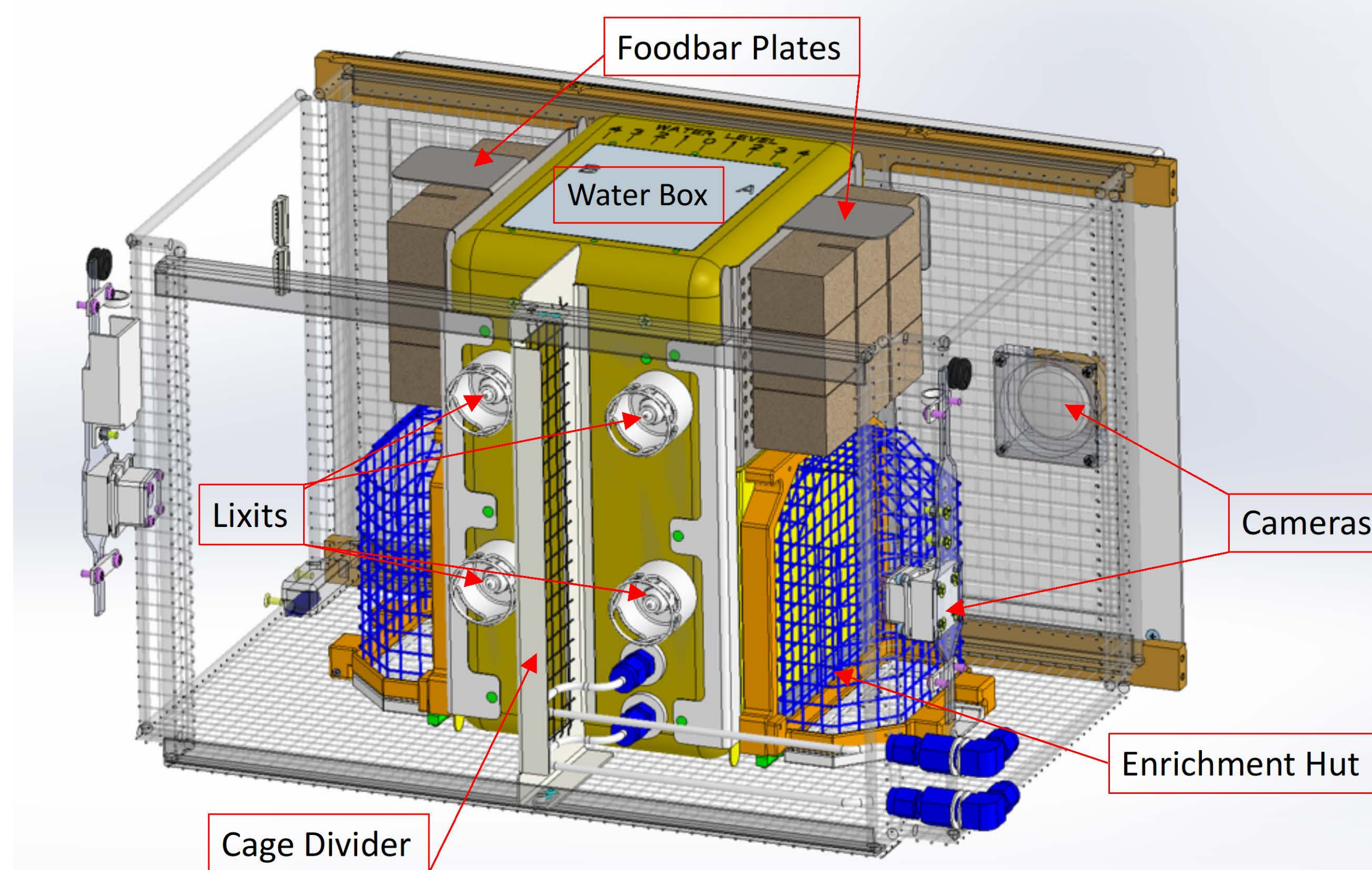
RODENT RESEARCH (RR) MISSION SUMMARY

Mission	Date	Institution	Sponsor	Objective	Strain	Age (wks)	Gender	Duration (days)
RR-1	Sep2014	NASA CASIS	NASA CASIS	Validation Mission	C57Bl/6J C57Bl/6T	16 32	Female	37 21
RR-2	Apr 2015	Novartis	CASIS	Muscle Atrophy & Development of Novel Therapies for Muscle Disease	C57Bl/6T	16	Female	7, 15, 27, 54
RR-3	Apr 2016	Eli Lilly	CASIS	Muscle Atrophy Mitigation Using Anti-Myostatin	BALB/c	12	Female	42
RR-4	Feb 2017	DoD	US Army	Fracture Healing & Tissue Regeneration	C57Bl/6J	10	Male	26
RR-5	Jun 2017	UCLA	CASIS	Systemic Therapy of NELL1 for Osteoporosis	BALB/c	30	Female	30 (LAR) & 60
RR-9	Aug 2017	FSU Loma Linda U. Wake Forest U.	SLPS	Effects of Elevated Intracranial Pressure, Characterization of Musculoskeletal and Vascular Systems	C57Bl/6J	10	Male	30 (LAR)
RR-6	Dec 2017	HMRI Novartis	CASIS	Efficacy of Sustained Delivery of Formoterol using Nanochannel Implants	C57Bl/6T	36	Female	30 (LAR) & 60
RR-7	Jun 2018	Northwestern Univ.	SLPS	Effects of Genotype Interaction to Impact Composition of Gut Microbiota and Circadian Rhythm	C57Bl/6J C3H/HeJ	11	Female	30 & 90
RR-8	Dec 2018	CASIS	CASIS	Reference Mission-1: Applications for Spaceflight Biospecimens	BALB/cAnT ac	10-11 31	Female	21 & 28 (LAR)
RR-12	Apr 2019	Loma Linda University	SLPS	Effects of Spaceflight on Primary and Secondary Immune Response	C57Bl/6J	10-15	Female	28
RR-17	July 2019	CASIS	CASIS	Reference Mission-2: Applications for Spaceflight Biospecimens	C57Bl/6Tac	10-16 30-52	Female	30 (LAR) & 60
RR-14	Nov 2019	Baylor College of Medicine	CASIS	Microgravity as a Disruptor of the 12-hour Circadian Clock	C57Bl/6	8-12	Male	20-60

RODENT RESEARCH HABITAT CONFIGURATION

Husbandry

- Animals are group housed similar to standard animal facilities
- Animals are provided with an enrichment hut to enable nesting and huddling
- Animals have access to food and water *ad libitum*
- Foodbars are changed weekly
- Waterbox is refilled monthly
- Lights are adjusted on a 12:12hr dark/light cycle



Telemetry

- Habitats are equipped with 4 cameras
- Video health checks are performed daily (1hr) during the dark cycle using infrared red lights
- Extended (48hrs) video collection for behavioral analysis
- Live telemetry feed available to monitor temperature and relative humidity
- Real time alerts for monitoring environmental conditions

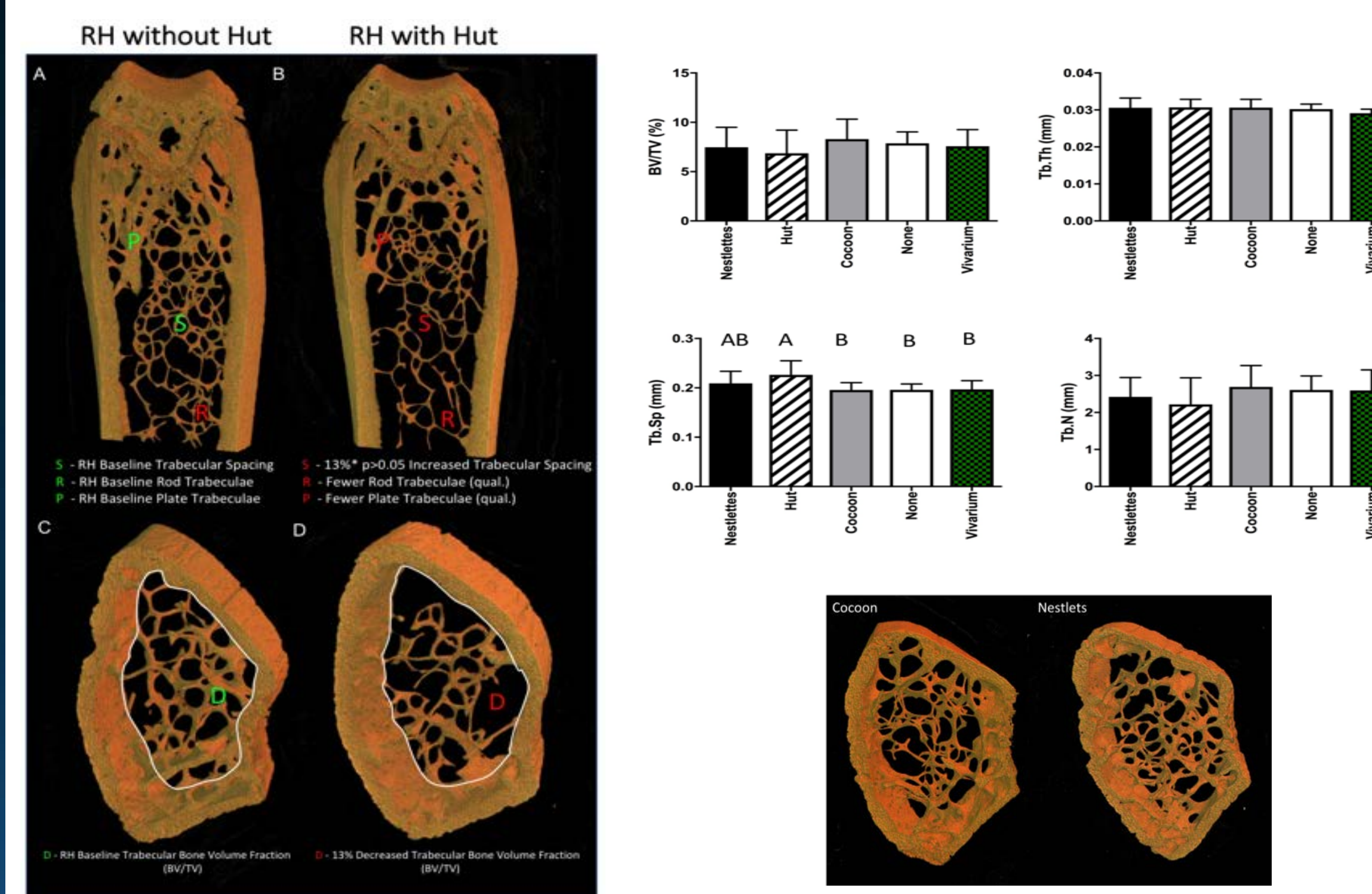
EXPERIMENTAL RESULTS

Weekly Measurements

- Body Mass – primary indicative of animals' health and well being
- No differences across groups indicating no adverse or beneficial effect of enrichment types
- Food Consumption
- No differences across group
- Enrichment Usage
- Mice interacted with and slept in both nestlets and cocoons
 - Mice pushed the shredded enrichment through the divider
 - Nestlets fell through the cage grid more than cocoons
- Hardware performance:
- No differences in Air Flow, CO₂, and Ammonia Measurements
 - observed across habitats, all measurements met requirements

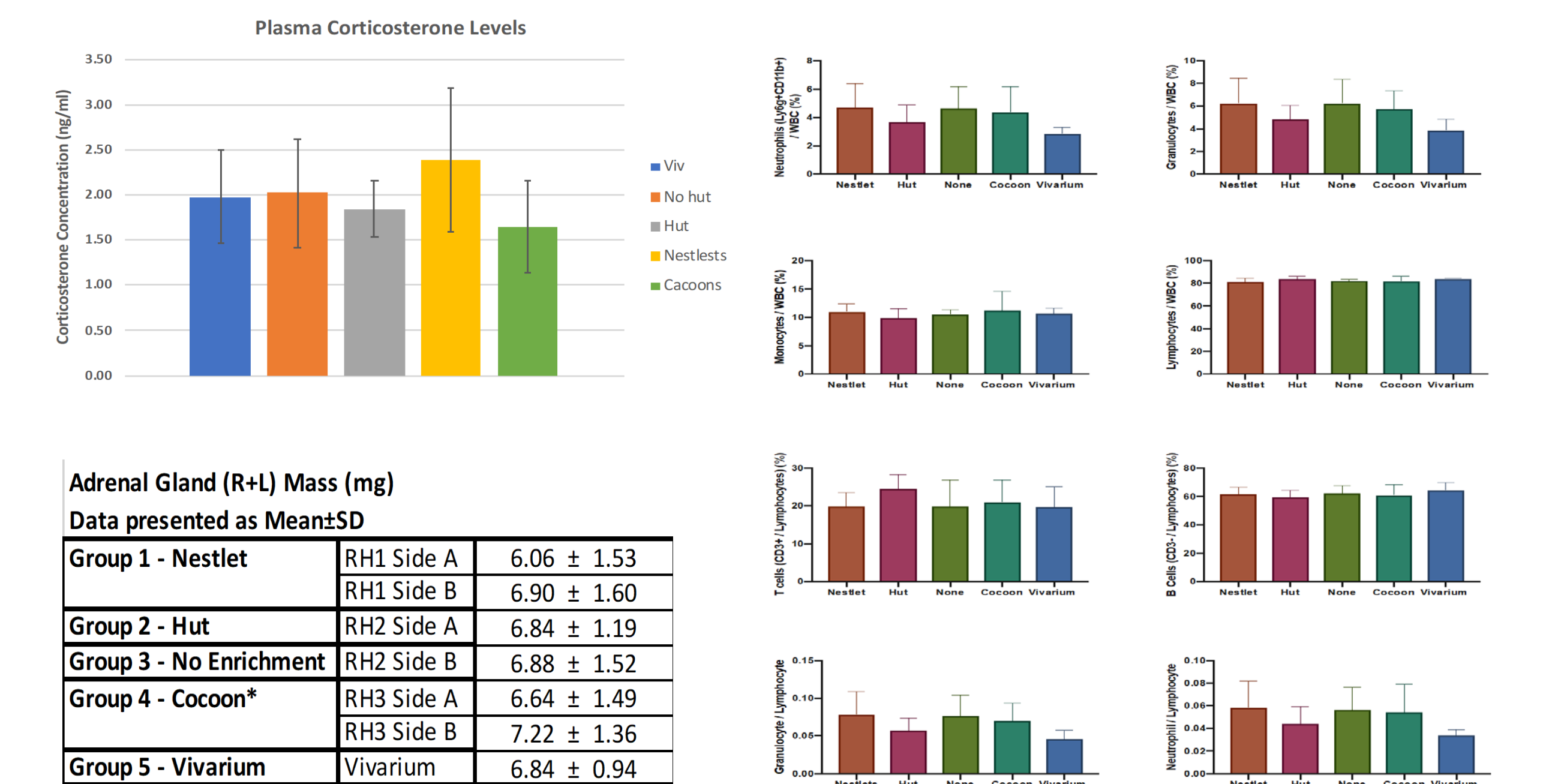
Muscle Mass & Cancellous Bone Microarchitecture

- Soleus Muscle Mass** - Significant atrophy of this slow-twitch hindlimb muscle occurs due to both actual and simulated weightlessness
- No statistically significant differences were observed across groups.
 - This was expected given the animals were in normal gravity and normal ambulation conditions.
- Distal Femur Cancellous Bone Microarchitecture**
- No statistical differences were observed in bone volume fraction (BV/TV), however a trend towards lower BV/TV lower BV/TV in Hut compared to No Hut (13%) or Cocoon group (13%).
 - Trabecular spacing was significantly increased in the Hut group compared to no hut, cocoon or vivarium (t-test).



Indices of Stress & Immune Response

- Plasma Corticosterone** – Plasma corticosterone reflects acute distress; (responses can be amplified in animals that are reactive due to prior stressors)
- Adrenal gland mass**– adrenal hypertrophy can reflect chronic stress
- Select Immune Cell Profile** - Characteristic changes in immune cell types and IgA levels can occur in response to environmental stressors and changes in social groups



No statistically significant differences between any of the groups (P>.05 by 1-factor ANOVA)

Novel Object Test & Behavioral Patterns

- Novel Object Test** –for understanding how the Habitat and various enrichment types influence stress-related behaviors.
- Test was performed 24hrs prior to euthanasia. Mice were individually transferred to test room and allowed to acclimate (10 min). Each mouse was placed in the center of an Open Field (OF) to explore (5 min). Novel Object was placed in the field (2.5 min) and then removed (2.5 min)
 - Results:** No differences between groups noted across groups. All mice engaged in thigmotaxis (arena wall proximity) in the OF over 50% of the recorded time. Thigmotaxis declined when a NO was introduced.

Video Behavioral Analyses: Preliminary results indicate:

- Plus-hut: physical activity was absent when animals were inside the hut (dark cycle).
- Cocoons & nestlets: mice were physically active while in their enrichments (dark cycle)
- During the **dark phase** (active) of the cycle, we observed:
 - Nestlet: 31% activity inside and 69% activity outside of enrichment
 - Hut: 0% activity in and 100% activity outside of the enrichment
 - Cocoon: 35% activity in and 65% outside of enrichment.