National Aeronautics and Space Administration

Potential NASA Rodent Centrifuge

Artificial Gravity Workshop

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Overview

- Presentation describes options for Artificial Gravity research using rodents (rat and mouse).

- Radius is a major limiting constraint for a centrifuge on the ISS, and the ISS is the best platform for this research at this point.
  - Space Biology commissioned a trade study into available options and funded ground based research into short radius centrifugation.
  - New facilities for short radius centrifugation are planned at NASA ARC, to allow testing at the radii of spaceflight centrifuges, and to complement the larger (4 foot, 20 foot) centrifuge facilities.
  - Space Biology and Human Research Program are now jointly supporting research into short radius centrifugation with rats, and supporting review of proposed centrifuge facilities.
  - Prior results and ongoing effort are outlined.

Justification for a Rodent Centrifuge

National Research Council (NRC) 2011, *Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era Life and Physical Sciences*

- High Priority Recommendations: AH3 (bone loss studies on mice), AH4 (animal testing of osteoporosis drugs), AH5 (muscular-skeletal protein balance and turnover), AH10 (orthostatic intolerance), AH14 (immune system changes), AH15 (mouse immune studies), CC2 (artificial gravity studies)
- "**...the AHB panel would be remiss by not strongly recommending the need for an animal centrifuge capable of accommodating rats/mice at variable gravity levels.** This would enhance the research potential of the ISS laboratory by creating both partial gravity and hyper-gravity stimuli in order to dissect the gravity vector in modulating functional capacity across many organ systems, especially the bone, muscle, neurosensory, and cardiovascular systems." (p. 6-70)
- "**In collaboration with international partners, NASA should ...develop small animal AG experimental facilities for the ISS...** for eventual countermeasure evaluation experiments aboard the ISS." (p. 7-6)

1988 National Research Council report *Space Science in the Twenty-First Century* recommended:

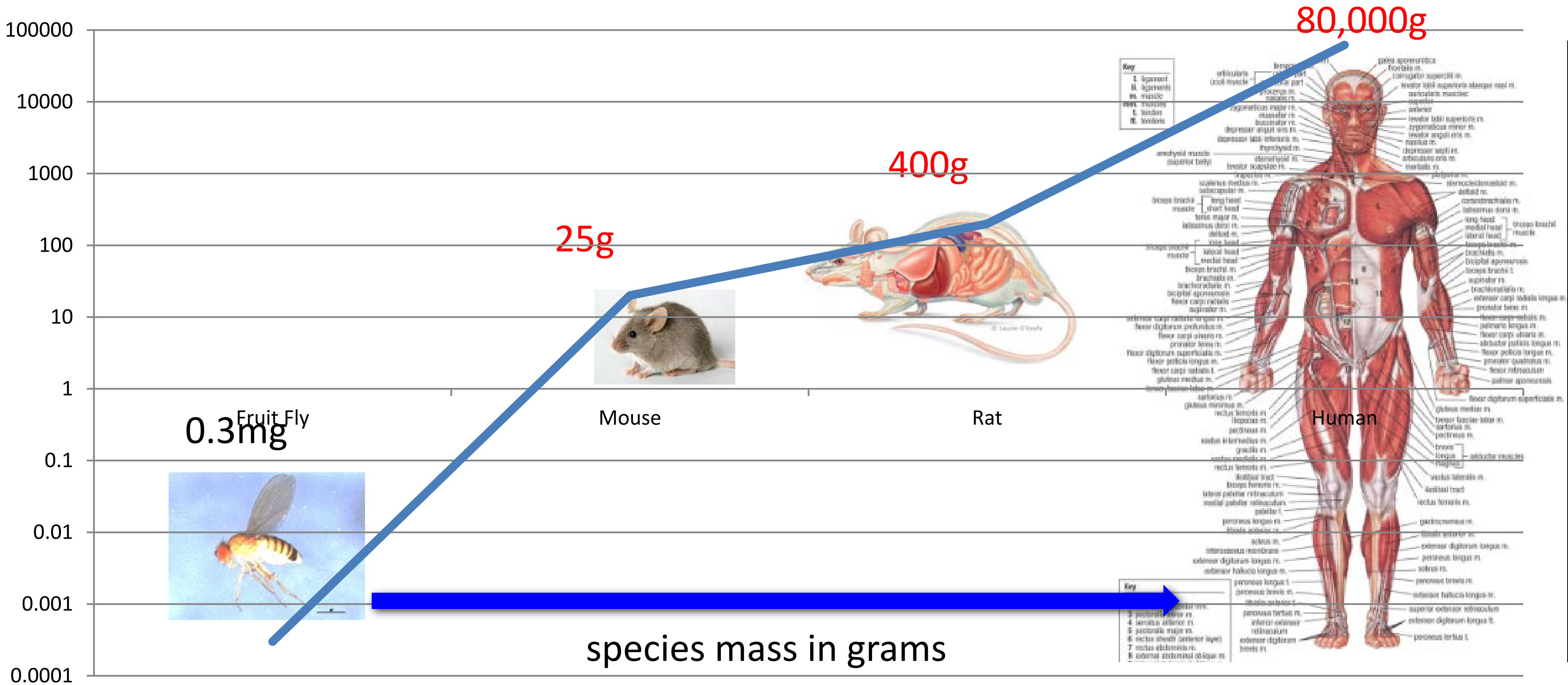
- "**Construct a dedicated life sciences laboratory with a large variable-speed centrifuge to hold plants and animals and to provide one-g controls.** Without adequate controls, most of the experiments at micro-g will be of limited value."

1987 COSPAR: ***A Strategy for Space Biology and Medical Science for the 1980s and 1990s*****,** Committee on Space Biology and Medicine of the National Research Council, Washington, D.C., National Academy Press:

- **"A (Centrifuge) is an essential instrument for the future of space biology and medicine."**

Mammalian research into AG is accessible now

- Size and scale of model organisms provide opportunities for artificial gravity (AG) research on the ISS and beyond through use of smaller radius centrifuges.
- Rapid lifespan, greater sample number, complete analysis, and translational systems should allow definition of countermeasure markers and characterization of the potential of AG.
- *JAXA MHU (and Techshot RCF if built) provide ISS facilities for AG research with rodents*



Previous results: Cosmos -936
Rat centrifugation flight, 1977

N.N. Gurovsky, et.al., Study of physiological effects of weightlessness and artificial gravity in the flight of the biosatellite Cosmos-936. 1980 *Acta Astonautica,* Vol. 7, pp. 113-121

- 18.5 day mission, 30 male Wister-SPF rats, initial weights 210-230g
- 10 animals at 1*g*, 34cm radius, 53.5+/-0.3 RPM
- Migrogravity animals displayed skeleto-muscular, myocardium, and excretory system changes that were generally mitigated by the 1*g* centrifugation
- Centrifuged animals showed fewer behavioral changes and more rapid weight recovery following flight

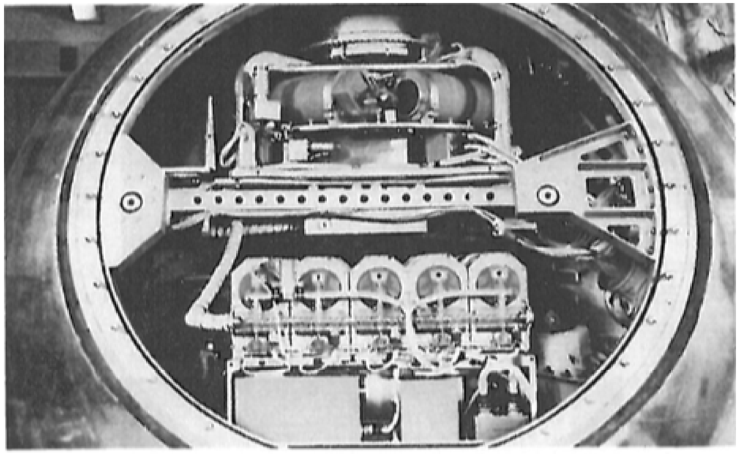


Fig. 2. General view of the biosatellite mock-up.

Vasques, M. et. al., Comparison of hyper- and microgravity on rat muscle, organ weights and selected plasma constituents. 1998 *Aviat Space Environ Med.* Jun;69(6 Suppl):A2-8.

- 14 day, male rat hypergravity centrifugation study at 2*g* to compare with the 14 day micro-*g* Cosmos 2044 rats.
- Muscle and organ weights were greater in centrifuge animals than control or when compared to Cosmos 2044 microgravity animals.
- Plasma thyroxine and testosterone levels were significantly reduced following flight, whereas only thyroxine was decreased after centrifugation.
- Centrifugation resulted in a decrease in most other plasma chemistry measurements, whereas flight rats showed no change or an elevation in these measures.
- Data indicate that the physiological responses to micro- and hypergravity are often in the opposite direction, suggesting that in general there is a continuum of physiological and morphological effects from microgravity to 1G to hyper-gravity.

Other hyper-*g* studies and hind limb unloading studies have demonstrated the rodent as a model for characterization and study of the adaptive and deleterious changes seen during spaceflight. Most studies were not using the current relatively short radii.

Mouse Centrifuge Project (2013-2015)

Approach

- **Form SWG, Draft requirements**: A Science Working Group made up of U.S. and international members of the space biology community, CASIS and the Human Research Program was convened to draft a Science Requirements Envelope Document (SRED) for rodent centrifugation on the ISS. Draft was based on former rodent habitat and rodent centrifuge requirements. Document is a living document and continues to be worked.

- **Conduct Trade Study**: Level one requirements from this SRED were used to assess potential centrifuge capabilities as part of an engineering trade study.

- **Perform ground based testing to determine minimum radius that will accommodate rodents (mice)**: The project commissioned a ground based study of short radius centrifugation of mice to assess the animal welfare and systemic responses, e.g heart rate, and to determine a minimum radius for rodent centrifuges.

- **Collaborate with International partners:**
  - JAXA has shared its Mouse Habitat Unit design and test data throughout its development.
  - NASA ARC and JAXA have shared three technical exchange meetings and shared data from ground based studies.
  - ESA has shared documentation from MISS project and EMCS facilities.
  - ESA and JAXA are invited members of the Science Working Group.
  - JAXA has visited KSC and JSC to discuss flight implementation and operations plans.
  - ARC has shared details of Rodent Habitat design solutions and composition of Rodent Food Bars.
  - ARC IACUC has worked with JAXA to work animal welfare issues and facilitate IACUC approvals for flight animals.
  - ARC has extended information on animal ordering in the US and equipment and facilities support for post flight animal processing for the JAXA MHU mice.

DRAFT Science Requirements Envelope Document

The Draft Science Requirements Envelope Document (SRED) for Rodent Centrifugation Research on the ISS is intended to provide top-level requirements that define the envelope for rodent centrifugation research likely to be conducted on the ISS. It includes science requirements to meet anticipated research needs of the NASA Space Biology and Human Research programs, commercial research (as represented by the Center for the Advancement of Science in Space - CASIS), as well as from the international scientific community.

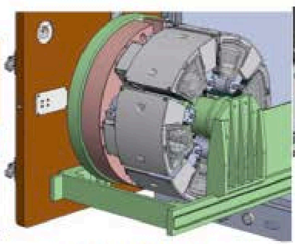
REQUIREMENTS COVER:

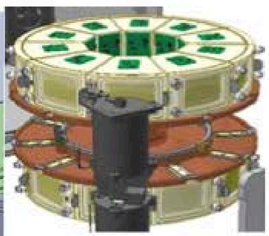
1.01 Numbers
1.02 Species
1.03 Gender
1.04 Strain
1.05 Animal Age
1.06 Duration
1.07 Live Animal Return
1.08 Gravitational Force
1.09 Gravitational Force Gradient
1.10 Radius
1.11 Ramp Up/Down Acceleration Phase
1.12 Desired Gravitational Force
1.13 Duration of Operation of Centrifuge
1.14 Animal Access
1.15 Video Monitoring
1.16 Telemetry
1.17 Day/Night Cycle
1.18 Cage Exchange
1.19 Visual Monitoring By Crew
1.20 Food and Water
1.21 Animal Health Maintenance Requirements

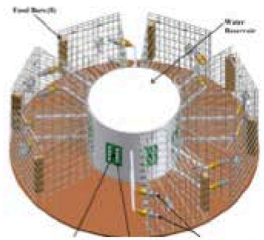
Engineering Trade Study

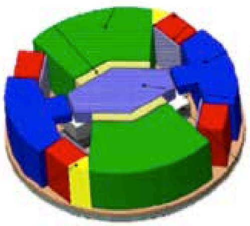
- 8 current, previous or proposed centrifuge facilities available for consideration were included in this analysis.
- Level one requirements derived from SRED, as specific to centrifuges as opposed to overall centrifugation studies, were used to assess and rate the facilities.
- For purposes of our study, we used the older IACUC guidelines to evaluate the likelihood of success in the proposed designs. Current IACUC guidelines have replaced the previous size dimensions with a *requirement for cage designers to demonstrate normal animal behavior of test subjects in the cage design. This allows for greater freedom of design, but puts the burden of proof on the final design.*
- Multiple options for a Small Rodent Centrifuge that meet minimum requirements are presented.

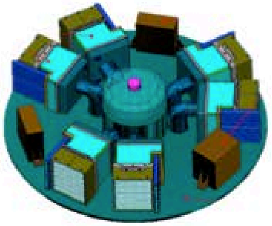

NanoRacks (ISS)


JAXA (ISS)

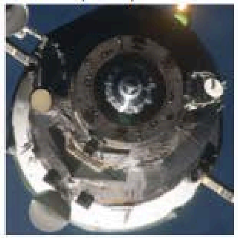

Techshot MVP (ISS)

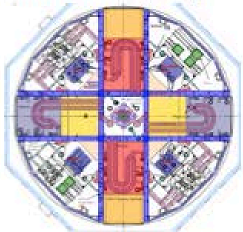

Techshot Rodent (ISS)


EMCS (ISS)


MISS (ISS)


MIS (Bion)


Spinning Bion


SSBRP (ISS)

Assessment:

- Facilities capabilities were compared to the SRED draft requirements.
- Requirements were “envelope” requirements, in which often a minimum requirement and a maximum were defined
- Each capability was rated as resulting in a strong advantage, moderate advantage, neutral, moderate disadvantage or strong disadvantage.
- TRL level of rotor and facility were also considered.
- Assessment was reviewed with SWG, project scientist and chief engineer, and ARC management.

JAXA MHU supports individually housed mice, Techshot RCF supports single or group housed mice or rats

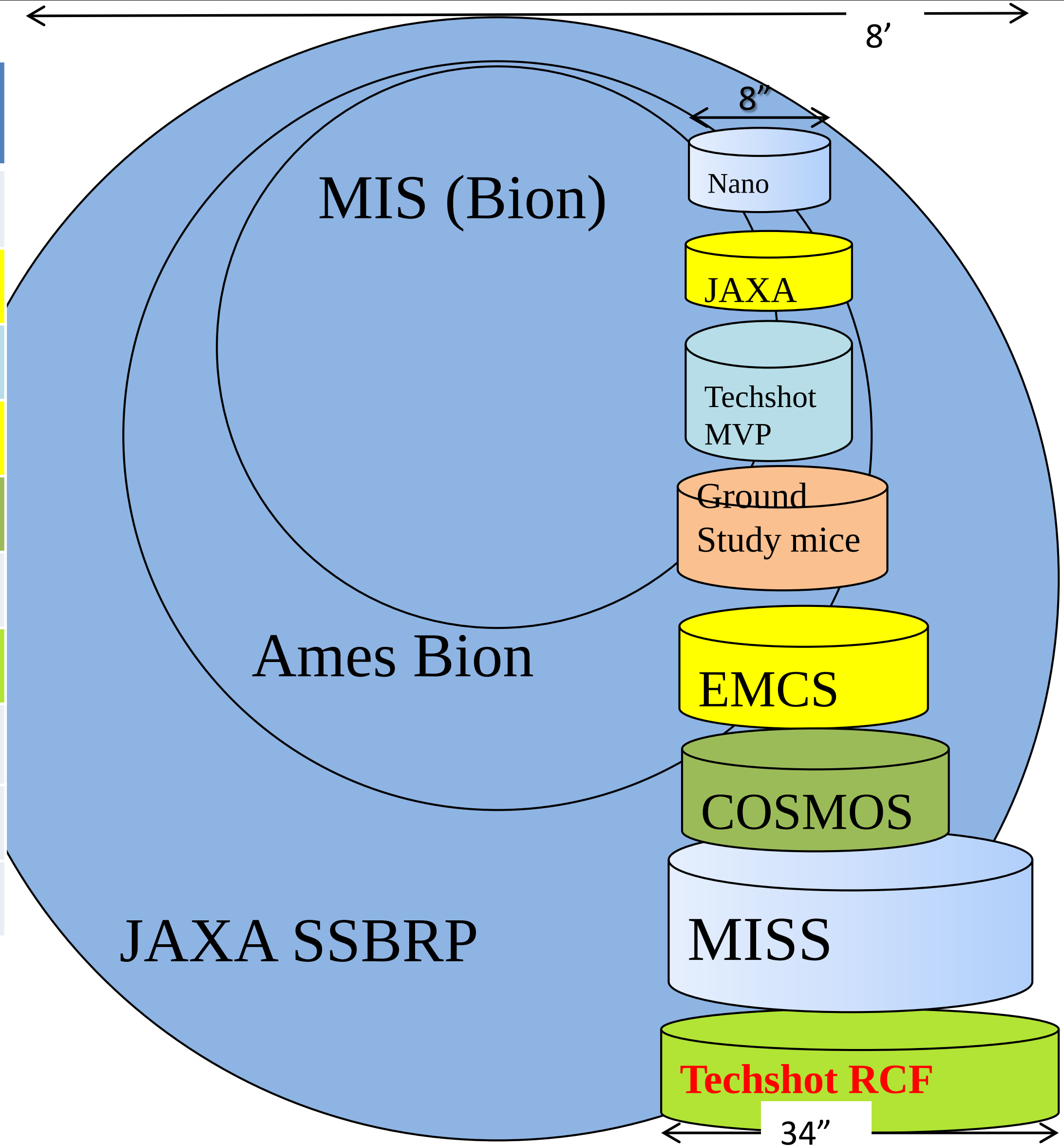
Centrifuge Comparisons	Nano	JAXA MHU	Techshot MVP	EMCS	Cosmos	MISS	Techshot RCF	MIS Bion	Ames Bion	JAXA SSBRP
Radius in/cm	4/10	6/15	7.6/19.5	12/30	13.4/34	16/40	16.3/41.4	22/55	31/80	48/122
Mice #	Not developed for rodents	6	Not developed for rodents	Not developed for rodents	N/A	12	42	12	45	96
Rat #	0	0	0	TBD, n=2?	10/5 per rotor	TBD	28 juvenile 14 adult	TBD	Yes, #tbd	48
Group house	no	no	YES	yes	?	yes	YES	yes	yes	yes
gravity gradient across animal @ RPM of 1g	25%	17%	15%	8%	7%	6%	6%	5%	3%	2-4%
Gravity levels	µg-1g	µg-2g	µg-2g	µg-1g	µg-1g	µg-1g	µg-1g	µg-1g	µg-2g	µg-2g
Environmental control	ambient	CO_2, T, RH, NH_3	CO_2, O_2 T, RH,+	T, RH, CO_2?, +	CO_2, O_2 T, RH	CO_2, O_2 T, RH,+	CO_2, O_2 T, RH	CO_2, O_2 T, RH,+	CO_2, O_2 T, RH	yes
Video and Day/ night cycle light	yes +	yes	yes	yes	No	yes	yes	yes	yes	yes
On-orbit access	yes	yes	yes	yes	No	yes	yes	no	no	yes
Cage volume / animal	176cc – does not meet IACUC	2,500cc, cage height is 4"	2,700cc cage height is 3"	5,700cc cage height is 4.2"	? – but fits rats	765 cc - 1,564 cc	2,202cc/ variable sizes	842 cc – 1,717 cc	TBD	AAH
Rotor status/TRL	On-orbit/9	On-orbit/ 9	TRL 4	On-orbit/9	1977 18.5 days in flight/9	TRL 5	TRL 4	TRL 4	TRL 2	Needs shuttle
Other Considerations	No ramp speeds available very small	Mice only, small n,	Not designed for rodent	Heavily used by multiple payloads	Decommissioned	larger n	Rats and mice, larger n	No crew	Significant Develo p-ment Risk	Included as reference

Space Biology P

Key: Strong Advantage | Moderate Advantage | Neutral | Moderate Disadvantage | Strong Disadvantage

Comparison of Spaceflight Centrifuge Radii

Centrifuge	Volume (whole support system)	Radius (in/cm)
NanoRacks Biorack*	Standard Drawer	4/10
JAXA MHU	CBEF rack	6/15
Techshot MVP*	Single locker	6/15
Astrium EMCS*	EMCS rack	12/30
COSMOS	Bion	13.4/34
ESA's MISS	Double locker	16/40
Techshot RCF	Quad locker	16.3/43
ESA's MIS (Bion)	Bion	22/55
Ames' Bion	Bion	31/80
JAXA's SSBRP	ISS module	48/1219



* Needs modifications to accommodate rodents

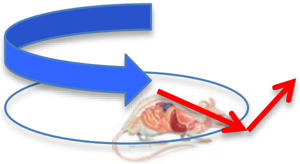
On ISS now

RAT capable

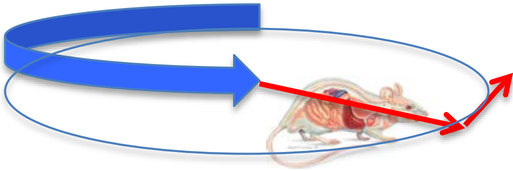
Radius size: Coriolis and gravity gradients are confounding factors

- Coriolis Effect – “an effect whereby a body moving in a rotating frame of reference experiences the Coriolis force acting perpendicular to the direction of motion and to the axis of rotation”.
- From studies with humans -this force not only causes visual disorientation but also adversely effects motion sensation from the inner ear structures causing disequilibrium and creating a sense of vertigo.
- Gradient of force across the subject increases with shorter radius
  - Head experiences slower rotational rate than feet
  - Gradient of force across subject is substantial in short radii facilities
  - Increases likelihood of difficult data interpretation

JAXA -15cm– **77RPM**



RCF-43 cm – **45RPM**



Shorter Radius = Faster rotation = Greater Coriolis Force

Shorter Radius = Greater Gradient

The Coriolis effect is an unavoidable result of artificial gravity produced by rotation. The effect is especially noticeable when the rotational radius is small as in this Skylab simulation.

Credit-Racetomars.ca skylab simulation

Mouse Centrifugation Study Design: Dr. C. Fuller

2 studies were performed at 25cm radius:

- Adaptation to max RPM in 4 2-week increments (stepped ramp up)
  (Lunar G (25 RPM) -> Martian G (37 RPM) -> 0.68G (48 RPM) -> 1G (60 RPM) *Note: G-levels refer to centripetal acceleration and not resultant vector.
- Adaptation to max RPM in 1 8-week increment (rapid ramp)
  - 0-60 RPM in 90 seconds

Subjects:

- Female mice, housed at 3/cage and 4 cages per group (total n = 12/group), and 4 groups per study,

Measurements:

- Animals instrumented with IP data logger recording body temperature.
- Body Mass & Food intake measured bi-weekly.
- Cage video recording recorded daily with 4 2-hour recording increments (around LD transitions, mid- day, and mid-night).

Analysis:

- Body mass, body temperature, behavior, veterinary inspection, and proteomics on tissues post study

Mice Adapt to Short Radius Centrifugation

- A significant amount of data are yet to be analyzed, but based on the data thus far, the mice appear to be readily adapting with little distinguishing responses to chronic acceleration (1.4G for 60 days) with a short radius (at 60 RPM).
- Veterinary inspection determined animals are suitable for scientific research.
- Rate of G-onset profile affects rate of adaptation to chronic rotation.
- The movement up/down the cage walls identifies the requirement to understand the role of gravity gradients, both in cage design and spaceflight operation.
- Habitat design and testing critical for understanding inflight science data. For experiment design capabilities, to meet IACUC requirements and to understand adaptation profile of animal to facility.

Techshot RCF -

- Quad sized Rodent centrifuge, uses 8 locker spaces in total
  - Meets Decadal Survey requirement for rat and mouse capability
  - Meets SWG recommendation that the largest radius feasible be utilized to limit the adverse effects of Coriolis force and gradient of force across specimen

Design features on-orbit change out of major systems using simple tools – allowing for replacement of failed subsystems without return of the primary hardware

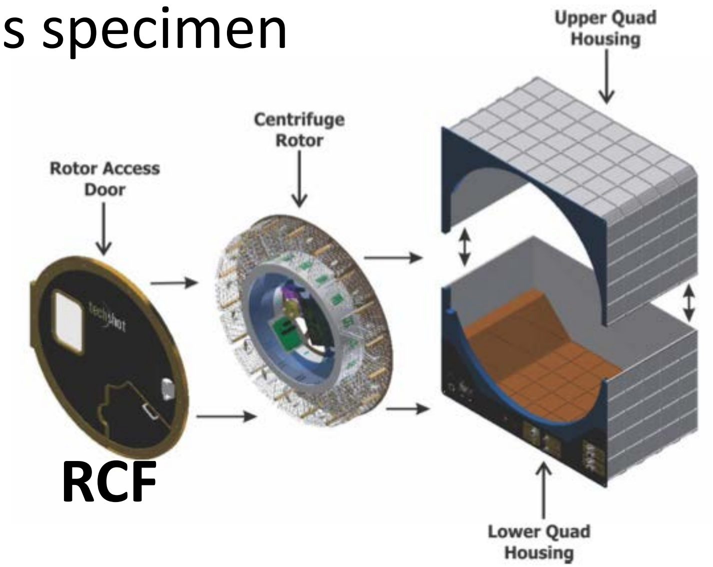


Figure 12. The RCF will be transported to ISS in separate pieces and assembled on-orbit by first attaching the two quad housing units together and then installing the centrifuge rotor and rotor access door.

Techshot RCF –flexible cage size, active rotor balance

- **Modular design** –e.g. the door can be removed and replaced and all the electronics are in the door. The rotors can be replaced also if something breaks. Modularization lowers risk.
- Up to 14 cages, **collapsible** for smaller up-volume, **replaceable** on orbit for cage change out.
- **houses rats and mice**– can do individual or group housing
- **Uses standard RFB** – can access and change food out.
- Lixit design from AAH - water filled from pressurized double lines, 2 line redundancy, can measure amount dispensed based on how many times pump into lixit reservoir.
- Waste – similar to that developed for AAH, and cages are replaceable. Cages are removable, replaceable module, fold flat
- **Counterbalance system** – approximately 2 lbs accommodated – prototyped in MVP version. Meets station requirements for disruption/forces (MVP, RCF under review).
- Working with MSFC express rack group on changes that free up center of the express rack so a quad locker sized centrifuge can be accommodated- **new express rack available 2018**.

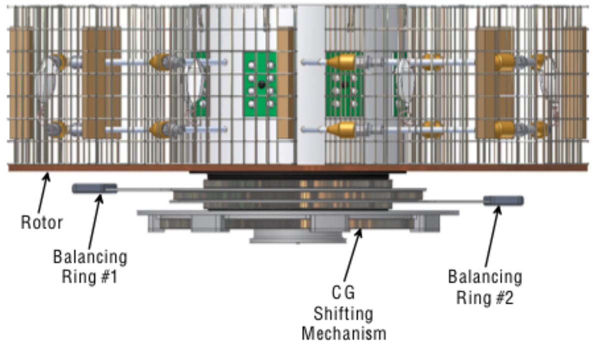


Figure 9. The centrifuge system provides real-time rotor balance as the rodents and fluids change positions inside the cage. This system is adapted from the proven Techshot Multi-specimen Variable-G Platform (MVP) auto-balance system.

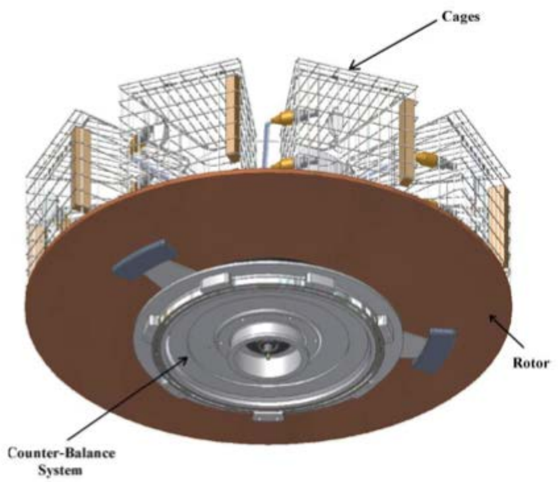
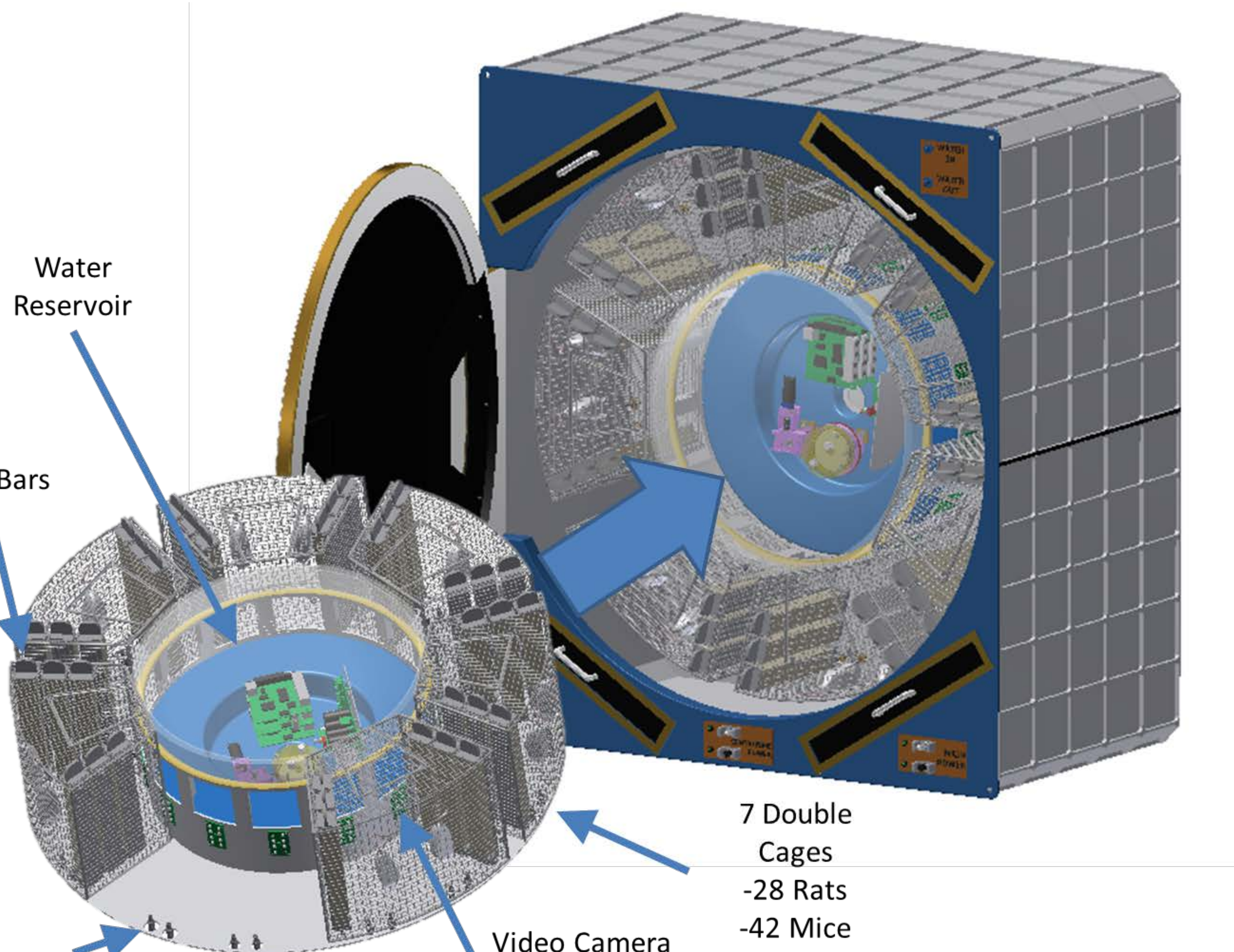


Figure 10. The centrifuge electronics control card receives data from the encoder and re-aligns the weights to counter any out-of-balance condition.

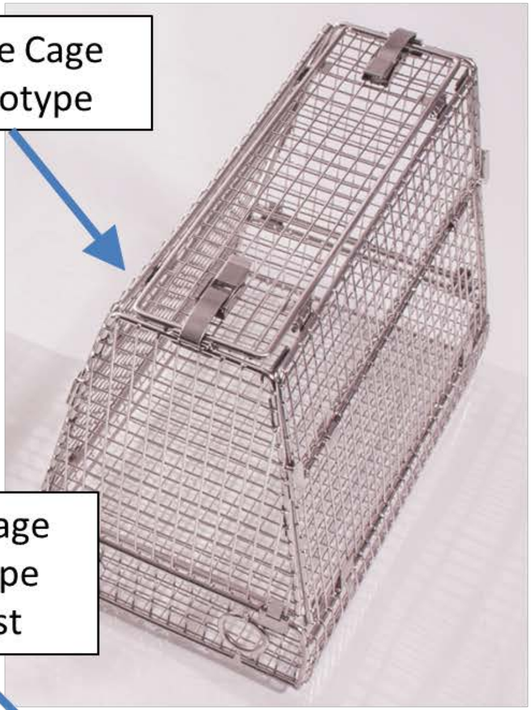
Images courtesy of Techshot



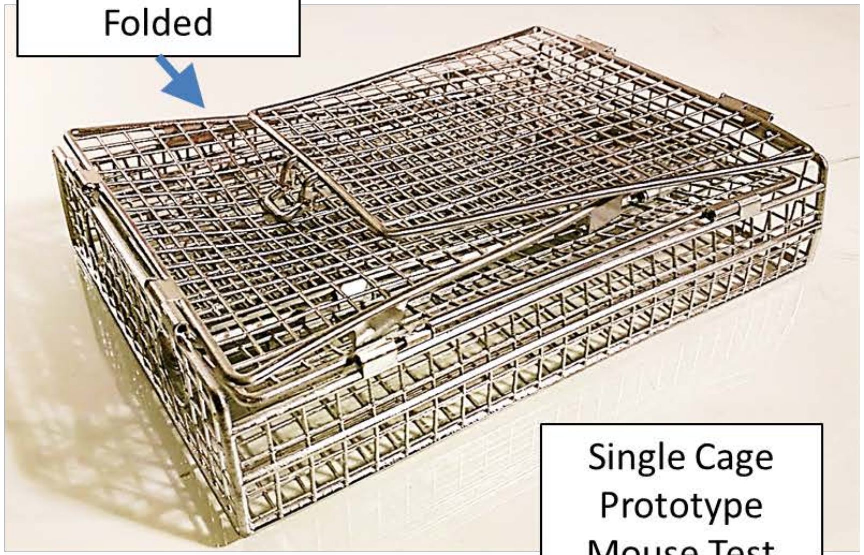
Cage design: foldable, variable sizes, replaceable

Images courtesy of Techshot

Single Cage Prototype



Single Cage Prototype Folded



Single Cage Prototype Rat Test

Single Cage Prototype Mouse Test

ARC Centrifuge Facilities to be Used for Hypergravity Studies

Facility to be managed as a core resource for NASA

24-Foot Centrifuge

- Acute or chronic exposures (with stops for animal husbandry) to 3 G
- Small animals, plants and hardware payloads
- 10 radial arms, each supporting two opaque enclosures (2.2ftx3.2ftx1.8ft)
- radii (variable from 4 feet to 12 feet at 6-inch intervals) and gravity levels. Multiple mounting positions on each arm.
- Continuous data and video acquisition

8-Foot Diameter Centrifuge

- Acute or chronic exposures (with stops for animal husbandry) to 4 G Small animals and plant payloads
- Temperature controlled
- Continuous data and video acquisition
- Rotational and stationary controls
- Designed to allow for ISS habitat compatibility
- Minimum 53 inch radius

Short to very short radius Centrifuge

- Acute or chronic exposures (with stops for animal husbandry) from 0- 2G applied force to small animals and plant payloads
- Temperature controlled
- Continuous data and video acquisition
- Rotational and stationary controls
- Minimum On-Center radius to maximum 1m

Designed from COTS rate table/ controllers similar to Academic facilities

Conclusion

- A rodent centrifuge is a critical link to countermeasure development, and the only capability that allows study of partial gravity and intermittent exposures in mammals.
- There is a recognized risk in limiting research to mice only.
- There is recognized risk in using short radius centrifugation –confounding factors of increased Coriolis and gradient of force across test subjects.
- The short radius rodent centrifuge - Techshot RCF - is under consideration with NASA

Back up slides

Centrifuge Comparisons	Nano	JAXA MHU	Techshot MVP	EMCS	Cosmos	MISS	Techshot RCF	MIS Bion	Ames Bion	JAXA SSBRP
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Rat #	0	0	0	TBD, n=2?	**10/5 per rotor**	TBD	**28 juvenile 14 adult**	TBD	Yes, #tbd	48
Group house	no	no	YES	yes	?	yes	YES	yes	yes	yes
gravity gradient across animal @ RPM of 1g	25%	17%	15%	8%	7%	6%	6%	5%	3%	2-4%
Gravity levels	*µg-1g*	*µg-2g*	*µg-2g*	*µg-1g*	*µg-1g*	*µg-1g*	*µg-1g*	*µg-1g*	*µg-2g*	*µg-2g*
Environmental control	ambient	CO_2, T, RH, NH_3	CO_2, O_2 T, RH,+	T, RH, CO_2?, +	CO_2, O_2 T, RH	CO_2, O_2 T, RH,+	CO_2, O_2 T, RH	CO_2, O_2 T, RH,+	CO_2, O_2 T, RH	yes
Video and Day/night cycle light	yes +	yes	yes	yes	No	yes	yes	yes	yes	yes
On-orbit access	yes	yes	yes	yes	No	yes	yes	no	no	yes
Cage volume / animal	176cc – does not meet IACUC	2,500cc, cage height is 4”	2,700cc cage height is 3”	5,700cc cage height is 4.2”	? – but fits rats	765 cc - 1,564 cc	2,202cc/variable sizes	842 cc – 1,717 cc	TBD	AAH
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Other Considerations	No ramp speeds available very ...	Mice only, small n,	Not designed for rodent	Heavily used by multiple payloads	Decommissioned	larger n	Rats and mice, larger n	No crew	Significant Develo-pment ...	Included as reference

Behavioral Observations, Dr. Jeff Alberts, U. Indiana (analysis ongoing)

- Absence of heightened aggressive behavior - a good sign of positive adaptation to housing conditions and relatively short-radius centrifugation.
- Maintenance of periodicity in activity.
- Displays of species-typical amicable behavior during centrifugation.
- Climbing behavior during centrifugation suggests relative indifference to +G.
- Barbering of fur is evident, though it may be that the most extreme instances are related to the fur-dying (ID marking) procedure; analysis in process.
- More general social interactions being quantified and compared across conditions - these pertain directly to dominance, social stress, immune function, and potential epigenetic effects.
- Cage size and configuration may compress behavioral repertoires; careful comparisons with control conditions in process