A REVIEW AND COMPARISON OF MOUSE AND RAT RESPONSES TO MICRO GRAVITY, HYPER GRAVITY AND SIMULATED MODELS OF PARTIAL GRAVITY; SPECIES DIFFERENCES, GAPS IN THE AVAILABLE DATA, AND CONSIDERATION OF THE ADVANTAGES AND CAVEATS OF EACH MODEL FOR SPACEFLIGHT RESEARCH.

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Laboratory strains of mice and rat are widely used to study mammalian responses to stimulus, and both have been studied under a variety of gravity conditions, including space flight. We compared results obtained from exposure to spaceflight and microgravity, hyper gravity via centrifugation, earth gravity, and models of simulated partial gravity (hind-limb unloading and partial weight bearing treatments). We examined the reported changes in survival, body mass, circadian rhythm (body temperature and activity levels), behavior, bone, muscle, immune, cardio-vasculature, vestibular, reproduction and neonate survival, microbiome, and the visual system. Not all categories have published data for both species, some have limited data, and there are variations in experiment design that allow for only relative comparisons to be considered. The data reveal species differences in both the level of gravity required to obtain a response, degree of response, and in temporal expression of responses. Examination of the data across the gravity levels allows consideration of the hypothesis that gravitational responses follow a continuum, and organ specific differences are noted. In summary, we present advantages and caveats of each model system as pertains to gravitational biology research and identify gaps in our knowledge of how these mammals respond to gravity.

Introduction:
Rodents have been used for decades in gravitational biology research. A review of spaceflight rodent missions reveals that early flight missions focused on the rat, with a change to the mouse by 2000. Table 1 lists the missions that resulted in publications used in this analysis. Until the 1998 Neurolab mission, only rats, Wistar and Sprague-Dawley, were flown for any significant duration in space. Early studies using centrifugation to induce increased load did examine both species, and show the rat was more sensitive to gravity forces than the mouse when assessing endpoints such as survival and reproduction (Figure 1). The rat also showed greater sensitivity to increased load when it came to body mass reduction and circadian rhythm changes. This fit with studies using other species in which the response to increased gravity scales with the mass of the animal – the larger the mass of the animal, the greater the response to the increased load (reviewed in Wade, C. 2005). In prior reviews, data on the mouse responses was still limited, especially from flight studies, so comparisons with rat data on how additional systems – vestibular, bone, muscle, cardiovascular, and the immune system, could not be made with certainty, although a trend toward the rat as having a stronger response was noted. Considerable data on the mouse has now been published, and a comparison with the rat data and consideration of these two models is the subject of this review.

Methods: For this analysis, PubMed, Google internet, and NASA task book searches were conducted to gather data on rodent responses to gravity conditions. Data was gathered on effects

of gravity loads on animal survival, reproduction, body mass, circadian rhythm, vestibular, bone, muscle, cardiovascular, immune, visual system and microbiome, and research using centrifugation as a counter measure or simulated versions of "artificial gravity" as treatments in the unloading models. Data was summarized for each species, and grouped by short duration flight mission, longer duration (30+ days) flight mission, hyper gravity studies, and simulated µg models - hind limb unloading and partial weight bearing.

Results:

Summary of available data and gap analysis: The summarized results of the literature query are in figure 1. To allow an assessment of the gaps in the data on each species and topic, and to distinguish research topics that have multiple publications or missions with relatively similar results from which we can derive some confidence in the results, from areas in which few studies or no studies have been conducted and hence consensus is not yet reached, color coding of the topic areas was done. Research areas for which three or more publications with relatively consistent data are colored darker green, research areas with two studies with similar results are light green, research areas with 2 studies with conflicting results or a single study are colored yellow, and areas that have no studies are light grey. The written summaries and references table are available as an appendix to this poster presentation but are too lengthy for display in the limited space provided.

Table 1. Summary of the missions that resulted in publication of results that contributed to this report.

Mission	**Year**	**Days in orbit**	**Rodent species and housing conditions**
Cosmos 605, Bion 1	1973	21	Rat, male, ~200-250g
Cosmos 782, Bion 3	1975	19.5	Rat, male Wistar, 63 days old, ~200g, plus and minus centrifugation
Cosmos 936, Bion 4	1977	19.5	Rat, male Wistar, 62 days, ~215 g, plus and minus centrifugation
Cosmos 1129, Bion 5	1979	18.5	Adult male and female rats, 85 days, ~300g, bone muscle and reproduction
Cosmos 1514, Bion 6	1982	4.5	Pregnant and fetal rats (G day 13-18), Wistar
Cosmos 2044, Bion 9	1989	14	Rat, male Wistar, 109 days, ~330 g, delayed retrieval of returned vehicle.
STS 40	1991	9	AEM and RAF housed male Sprague-Dawley 58 day old, ~285g rats. RAF temperature 25 C+/-2, AEM was 27 – 32C.
STS 62	1994	14	Female Twelve-week-old Fischer 344 rats, ovx 14 days prior to launch
STS 66 R1	1994	11	Pregnant and fetal rats (G day 13-18)
STS 70 R2	1995	9	Pregnant and fetal rats (G day 13-18)
STS 72 R3	1996	9	Nursing rat litters (P day 5,8,14)
STS 90 Neurolab	1998	16	Nursing rat litters (P day 8,14) pregnant mice (G 3,5,8)
STS 108	2001	11	Female C57BL/6J mice, 9 weeks

Mouse Drawer System	2009	91	6 male apx 8 weeks at launch: Pleiotrophin transgenic mice (PTN-Tg) +/-, 3 died in flight: Wt3 at day 6 and Wt1 at day 44, PTN-Tg3 at day 24, thus limiting results to more observational
STS 131 Mouse Immunology	2010	15	Female C57BL/6 mice,16–23 wk old
STS 133 Mouse Immunology II	2011	13	Female BALB/cJ mice (12–13 wk old)
STS 135	2011	13	Female C57BL/6 mice (9 wk old)
Bion M-1	2013	30	Male mice, C57/BL6 4-5 months old, grouped in threes,
Rodent Research-1	2015	30	20 group housed female C57BL/6 mice, 14 weeks
Mouse Habitat Unit 1	2016	30	12 individually housed male C57bl/6j mice, 6 centrifuged 1g, 6 at micro g

Table 2. Summary of data:

Response system	Species	Microgravity Short duration – shuttle missions	Microgravity 30+ days	Hyper gravity	Simulated (Hind limb unloaded or partial weight bearing)
Survival	Rat	Neonatal pups - 15 Days Neurolab	N/A	Up to 4.5g survived, also over 4 months at	Up to 160 days (Zhang 2005), 120 days (Wang
	Mouse	STS 108 11.5 days, STS 131 15days, STS 135	91 days MDS (Italian Mouse drawer)	Up to 7g (Wunder C., 1962)	90 days (Canciani 2015), 30++ days HUL
Reproductio nneonate survival	Rat	viable pups can be produced in microgravity	N/A	1.87 g stopped mating (Ishay 1977)	N/A
	Mouse	n/a -pregnant mice were flown on STS 90, brain	MHU-1 sperm from mice 30 days µg gave	3.5g stops mating, weight and crown	N/A
Body mass	Rat	STS 62 - Generally no change (Wade	N/A	Overall decrease in mass reported due to decrease	Animals whose body mass changes by
	Mouse	N/A	MHU-1: The µg mice either gained some	3% reduction after 4 weeks at 2g, (Morita	Animals whose body mass changes by
Circadian rhythm	Rat	Embryo development shows no	N/A	Dose dependent response temp decreasing from	N/A
	Mouse	N/A	MHU-1: Initial decrease in activity levels	2g takes less time (6-13 days) to recover	N/A
Vestibular	Rat	The size of otoconia is increased in	N/A	Vestibular-mediated motor coordination is	120 days unloading: otoconia had
	mouse	Otoconia -For shorter duration exposures to	MDS: Preliminary results of inner	Mice subjected to 91-days of 1.24g	HUL, HUL + radiation: 0.01 cGy/h for a total
bone	Rat	Bion 4: Some expected bone loss but results	N/A	Increased bone mass (Smith 1975, Jaekel	HUL, strong response with readily measured

	Mouse	STS 131, 15 days: weight bearing bone	MDS: bone loss during spaceflight in	Reviewed in Wade 2005, adolescent mice	PWB lead to 9-7-5% dose dependent bone
muscle	Rat	Bion 4, 18 day; Bion 9, 4 day: decrease in the	N/A	2*g* 14 days: expressed relative to body	Obvious strong response, muscle mass
	Mouse	STS 108: decreased cross sectional area,	JAXA MHU-, 30 days: soleus and	3*g* 4 weeks increased 16 or 45% (around	PWB: dose dependent wet weight loss of
Cardio vascular	Rat	Reviewed as limited and conflicting by	N/A	Reviewed as limited and conflicting by	Microvaculature remodeling seen in skeletal muscle
	Mouse	STS 131: Spaceflight reduces	N/A	Female ICR mice, from 4 weeks of age,	14 week old, male, implanted, HUL for 2 weeks
Immune	Rat	depressed immune responses	N/A	No changes seen in rat (Oyama and	HUL show depressed immune
	mouse	STS 131: tolerance induction	Bion M-1, In response to flight	7 week old, male C57BL/6, Immune	HUL plus radiation: At 21 days
Microbiome	Rat	N/A	N/A	N/A	Radiation + HUL: exposure to low LET plus iron
	Mouse	STS 135 13 days, some	N/A planned in	N/A	1/6g PWB no change detected,
Visiual	Rat	Bion 4: Retinal damage seen in both centrifuged	N/A	Loss of rod photo receptor cells due to 2	N/A
	mouse	N/A	N/A -- MHU-1,2: tissues under examination	N/A	N/A
Artificial Gravity	Rat	Bion 4: Some expected bone loss but results	N/A	N/A	"simulated AG" – unloaded animals treated with
	Mouse	N/A	MHU1, 2: 30 days micro g and 1 g , Initial	N/A	N/A
notes	*Full text with references in appendix to presentation.*				

Comparison of rat and mouse data: Direct comparison between the species and even within species studies is not possible. These studies have variables that largely make each one unique – duration at a gravity level, sex and strain differences, and age/development period of the specimens, as well as considerable differences in assessment methodologies and exact biomarkers or physiological changes being tracked– making direct analysis of the results impossible. Some data endpoints, such as viability or reproduction, are endpoints that can be grossly compared, and for other systems, the trends in responses can be compared. A detailed review of the comparative results is in preparation.

Figure 1. The Rat is more sensitive to increases in gravity: The mouse requires greater force than rat to achieve similar responses to hyper gravity.

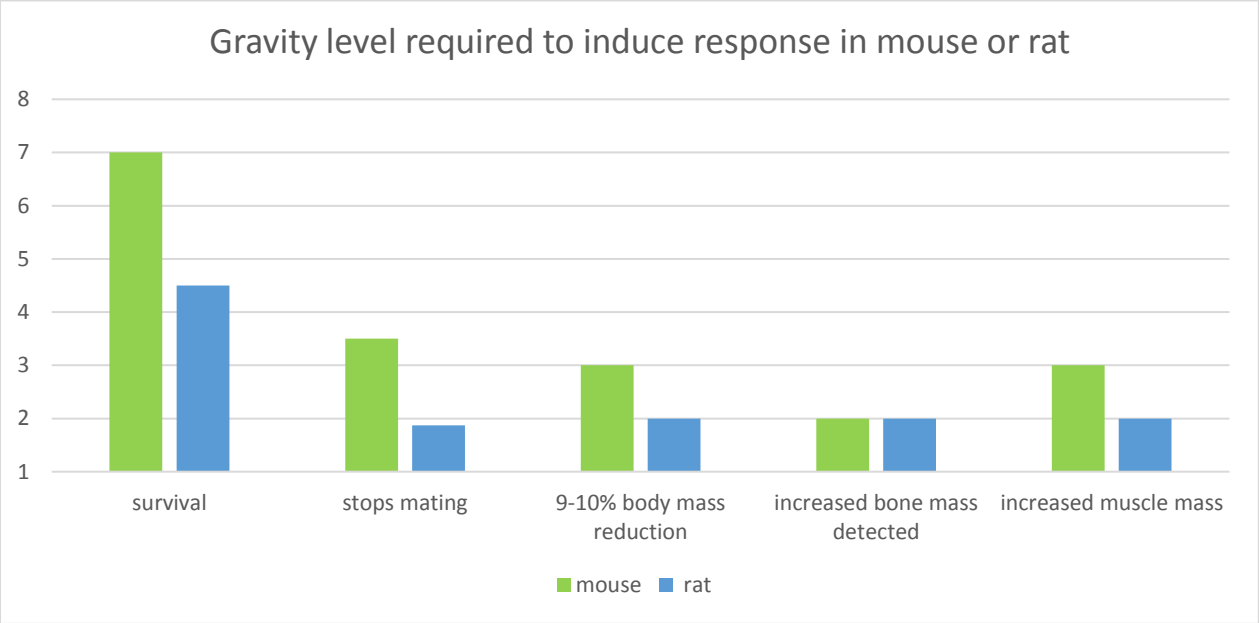

Figure 2: Both Rat and Mouse have measurable responses and similar trends to their responses across the gravity spectrum for vestibular, bone and muscle.

Figure 2a: Both rat and mouse vestibular system respond similarly to gravity, increasing otoconia or type II hair cell synapses in response to lowered gravity and decreasing otoconia or type II hair cell synapses in response to increased force.

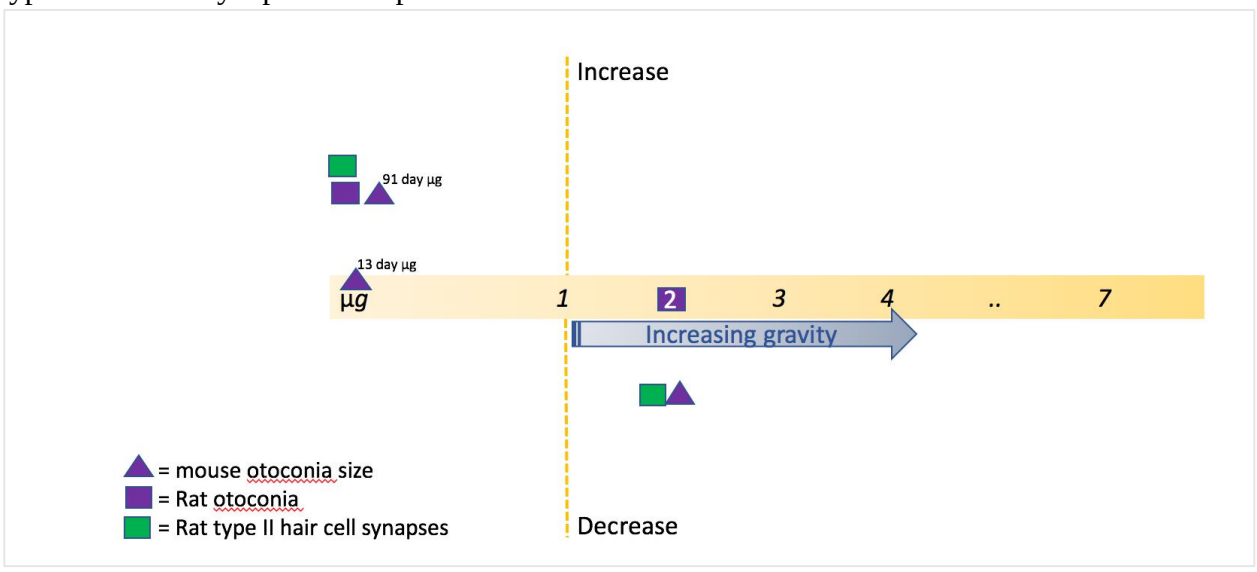

Figure 2b: Both rat and mouse skeletal muscular systems respond similarly to gravity, increasing with increasing gravity. Mouse data reveals that at higher loads, the response changes to a destructive response. *This is a notation of relative trends only, not a depiction of percent change or even relative change of endpoint values.*

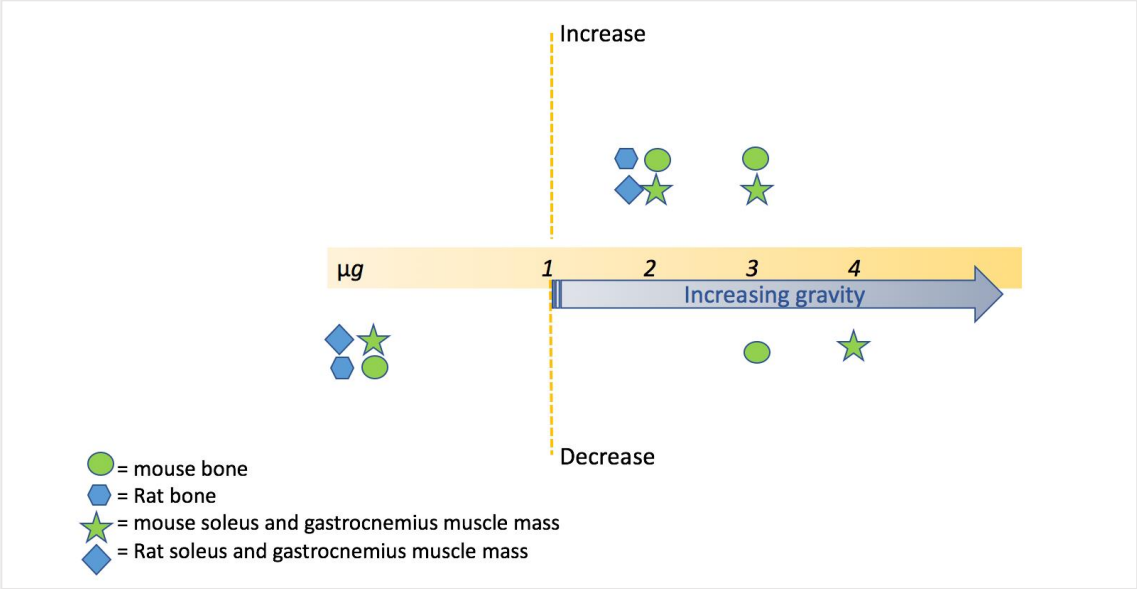

Figure 2c. Not enough data to assess all systems. Systems that respond in in an opposing manner to micro gravity as compared to hyper gravity (does the response fall along a continuum of responses):

System	Trend shows response in µ*g* is in opposition to hyper *g* response
Vestibular	√
Bone	√
Muscle	√
Body mass	√
Cardiovascular	Not enough data, responses of vascular system to µg detected in mouse.
Immune	Reported as depressed in µg for rat. Pro inflammatory cytokines reported as increased in µg and decreased in hyper g in one mouse study.
Microbiome	Not enough data
Visual system	Not enough data

Threshold vs Continuous responses: It should be noted that there is not enough data to determine if any of these systems has a linear response to gravity, or if there are thresholds for responses. Other than upper thresholds for bone and muscle increases and animal viability, there are not enough data points to determine if the responses are continuous or if there are thresholds for gravity responses. There have been no partial gravity studies with rodents to date.

Consideration of the rat and mouse as translational models for human disease:

The rodent lineage, which gave rise to the rat and mouse, and the primate lineage, which gave rise to humans, diverged about 80 million years ago. Humans have 23 pairs of chromosomes, while rats have 21 and mice have 20. Chromosomes from all three organisms are related to each other by about 280 large regions of sequence similarity called syntenic blocks. Both species are routinely used in biotech and pharmaceutical industries to elucidate mechanisms and treatments for human pathologies. There is a general trend to utilize the power of genetics with the mouse to search for genetic underpinnings to disease, and to utilize the rat for pharmacokinetics and drug development prior to clinical trial.

Genetics: the mouse is far and away the more characterized species with more genetic strains and transgenic models than the rat, but the rat is catching up.

- A draft of the human genome sequence was published in February 2001, and the completed human sequence was announced in April 2003. A draft of the mouse genome sequence was published in December 2002, and a draft of the rat was published in 2004.
- Rat transgenic models – over 400 as documented in 2016.
- Two major rat model repositories, the Rat Resource & Research Center (http://www.rrrc.us) in Missouri and the National Bio Resource Project –Rat in Kyoto, Japan (http://www.anim.med.kyoto-u.ac.jp/NBR/) provide the community with options for long-term preservation and distribution of transgenic rats.
- From Systems Genetics studies:
    - The first fully integrated systems genetics study was carried out in rats, and the results, which revealed conserved *trans*-acting genetic regulation of a pro-inflammatory network relevant to type 1 diabetes, were translated to humans.
- Synteny to human genome
    - Osteocalcin as example: mouse has three copies, the rat and human only one allele, making the rat closer to the human. More comparisons of synteny are being examined and a better sense of the relativity of rat and mouse to human is growing.

Results from the pharmaceutical industry (reviewed in Aitman 2016) indicated rat as the better model for human diseases including:

- inflammation and inflammatory disease processes/ the immune system
- neuro and cognitive disorders
- cardiovascular
- breast cancer
- diabetes

Important considerations for this review:

- *Differences in the degree or amplitude of response between the mouse and rat cannot be made due to the number of variables that are different between the studies*. For example, in Vasques et al., 1998, 7 muscle types of the rat species were shown to be decreased in microgravity and increased in hyper gravity, whereas mouse studies have only seen a

difference in at most two muscle types (soleus and gastrocnemius). This could be due to the mouse being a less sensitive species to gravity or it could be due to a number of other factors including the age/remaining growth potential of the animals used in the rat studies and cage effects, most notably single housed vs. group housing (reviewed in Morey-Holton 2000).

- Requirements for space, food, and water for a cohort of rats is greater than mice, and can result in limited opportunities for space flight research.
- Additional potential trends to be examined further:
    - *Remaining growth potential as factor in amplitude of response in rodents*. Although most of the studies, rat or mouse, used sexually mature animals, may of the rat studies used animals that had significant remaining growth potential. For example, table 1 indicates many of the male rats were ~200- 300 g in size. The strains used in these studies generally grow to over 400g. The male mice flown in the JAXA MHU experiments were typically younger (8 weeks), and had more remaining growth potential than the female 16 week old mice flown in the RR-1 mission. In both cases, the sexually mature, but younger, smaller mass male animals tended to have a larger response to the altered gravity conditions than the older, larger counterparts. Further investigation is warranted.
    - *Housing/social affect* (individual vs group) in the mouse will be ascertainable in the near future with data from recent NASA and JAXA missions in the Rodent Habitat and MARS facilities respectively. Prior comparisons of the RAF vs the AEM (single housed rats vs group housed) demonstrated a correlation to housing condition.

Conclusions:

- **Both Rat and Mouse have measurable responses to gravity changes in all systems of interest to human exploration.**
- **There is evidence that the rat is more sensitive to gravity**
    - E.g. Short duration µg for Rat but not mouse show effect on vestibular system. With longer exposure, similar results detected with the mouse.
- **Different systems respond to gravity at different levels** (the gravity load required to see a vestibular system response is lower and extends to higher loads before reversal or attenuation than either muscle or bone as one example)
- **Limited opportunities for spaceflight have left many gaps in the data sets:**
    - Cardiovascular, immune system, the microbiome, and the visual system have only initial and at times conflicting data reports.
    - No in-flight cognitive testing has been performed.
    - No long-term spaceflight mission to the international Space Station has involved rats.
- **Limited opportunities to study rodent centrifugation in space**:
    - Partial gravity has not been researched.
    - Artificial gravity – use of a centrifuge to supply loading during a spaceflight – has only 2 published results.
    - Intermittent exposures to centrifugation/increased loading during space flight has not been studied.