Title: "Flying through the ages: rodent research for human health"

Abstract

As humans, we evolved, developed, grew, and now function in a continuous 1-gravity environment. Habitation in space poses unique challenges to human cells and organ systems. Biomedical research with rodents (primarily mice and rats) can help to both unravel molecular, cellular and physiologic mechanisms relevant to humans and test candidate interventions that mitigate adverse effects of space on humans, such as muscle atrophy, bone loss and cardiovascular deconditioning. One favored hypothesis that may explain the detrimental effects of spaceflight on humans is that reduced mechanical loading in microgravity accelerates aging. Rodents provide a relevant model system to study this problem as they age ~40 times faster than humans. Now scientists from both public and commercial sectors conduct rodent experiments on the ISS using a new capability developed primarily at ARC. Results from the maiden voyage of the Rodent Research project on the ISS reveal that long duration effects of spaceflight appear far different than short duration effects. Thus, Rodent Research missions on the ISS usher in a new era for exploration and biological discovery in space.

Biosketch

Dr. Globus is a space biologist studying how the space environment influences mammalian cell biology and physiology, with a focus on the skeleton and osteoporosis. She was awarded her undergraduate degrees in Biology and Sociology from the University of California, Santa Cruz and her doctorate degree in Endocrinology from the University of California, San Francisco and has worked at NASA Ames Research Center as a principal investigator in the Space Biosciences Division since 1993. She co-directs the Bone and Signaling Laboratory, where students, postdoctoral scholars and scientists work together as a team to perform hypothesis-driven research, working with mice and cultured cells as model systems. In addition to her activities as a research scientist, in the past she has led NASA project science activities, such as the ARC Centrifuge Facilities (Space Biology

Program) and the Artificial Gravity project (Human Research Program). Currently, she serves as lead Project Scientist for the Rodent Research project, providing scientific guidance for the development of a newly established capability to perform long duration, rodent experiments on the International Space Station.

Flying through the ages: rodent research for human health

Ruth Globus, Ph.D.
Space Biosciences Research Branch
NASA Ames Research Center

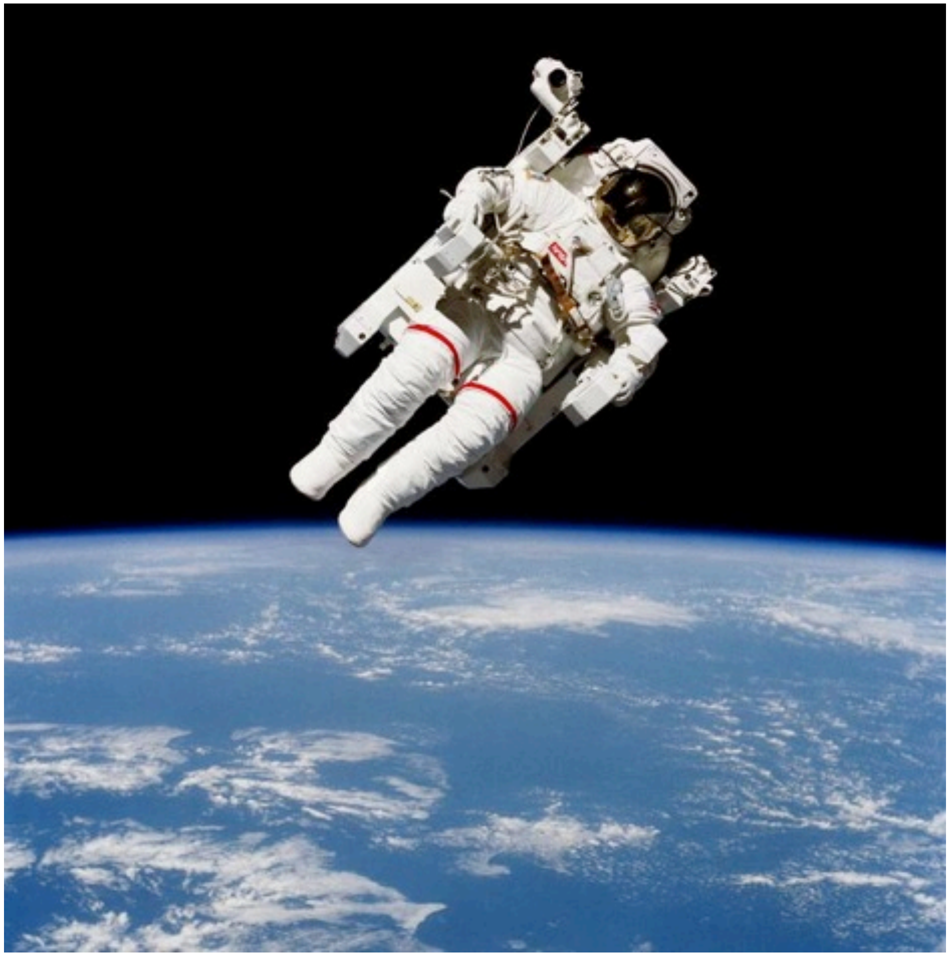
ARC Summer Seminar Series

June 16, 2015

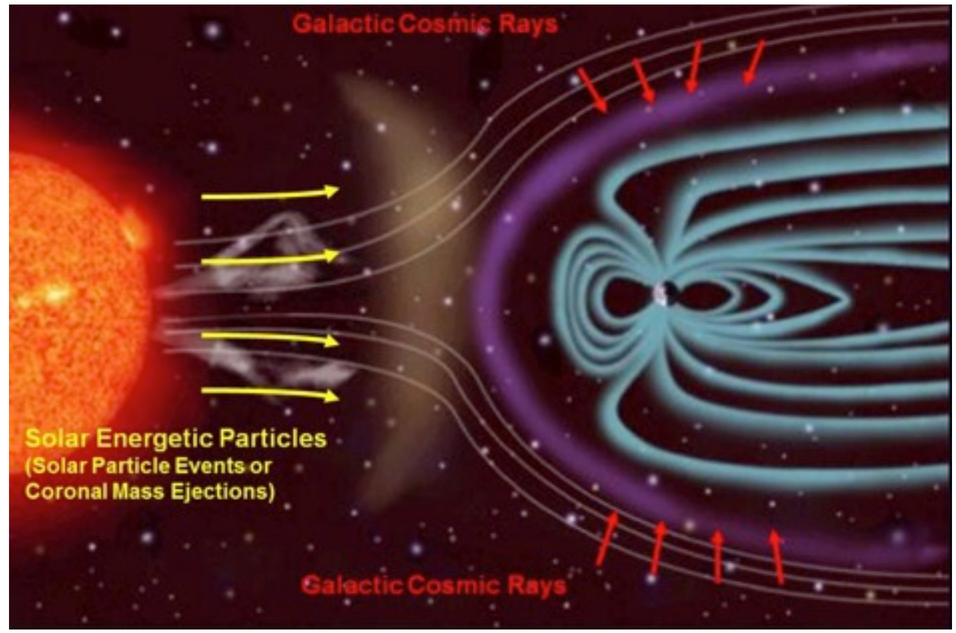
Outline

- Challenges of the space environment to mammalian biology
- The big questions: themes for long duration habitation in space
  - Why rodents?
- The past: exploration and discovery in mammalian biology
  - Previous platforms: rodent spaceflight experiments
    - Cosmos/Bion, Shuttle, and the ISS
  - Scientific/Technological advances
- Now and future: Rodent Research project on the ISS
  - Challenges
  - Capability (hardware, operations, science)
  - Rodent Research 1: New findings to date
  - Rodent Research 2 and beyond
- Acknowledgments
- Resources

Challenges of living in space to human biology



Microgravity



Space radiation

…but that's not all…

Other challenges to human biology of living on the space station

We understand some of these other factors better, but still not enough to predict long term consequences, especially when in combination:

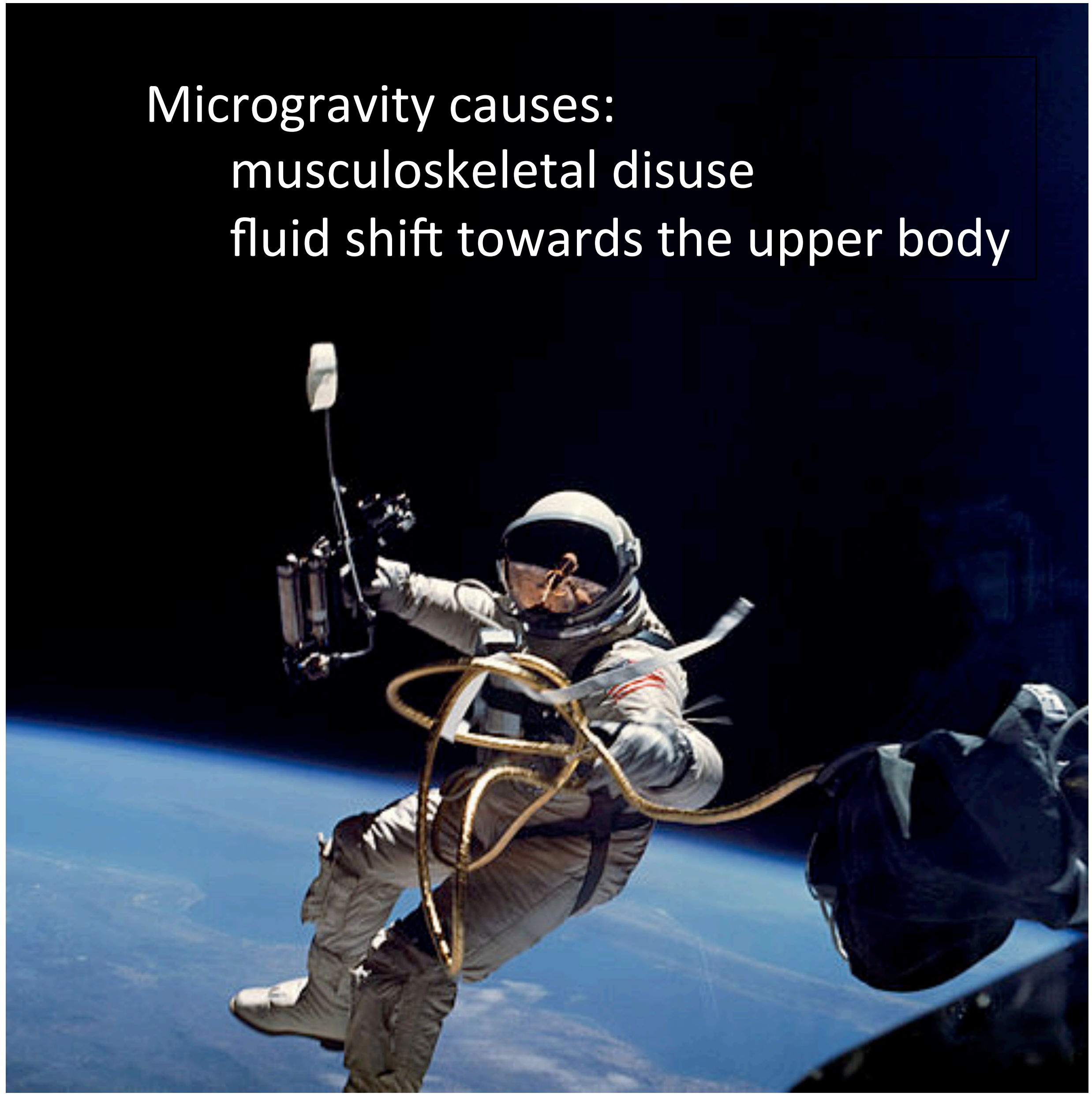
- Nutrition
- Demanding workload
- Sleep disruption/altered circadian rhythm
- Confined environment
- Elevated carbon dioxide

Changes in the body

Microgravity causes:

- musculoskeletal disuse
- fluid shift towards the upper body

Bone
Muscle
Fluid distribution
Vestibular system
Sensory changes
Blood
Immune
Vision
Hormones



Understanding gained from prior spaceflight studies

Human

- Bone decrements
  - ?
  - Cellular basis (limited)
  - ?
- Muscle
- Cardiovascular
- Vestibular/motor
- Vision changes
- Immune system

Rodent

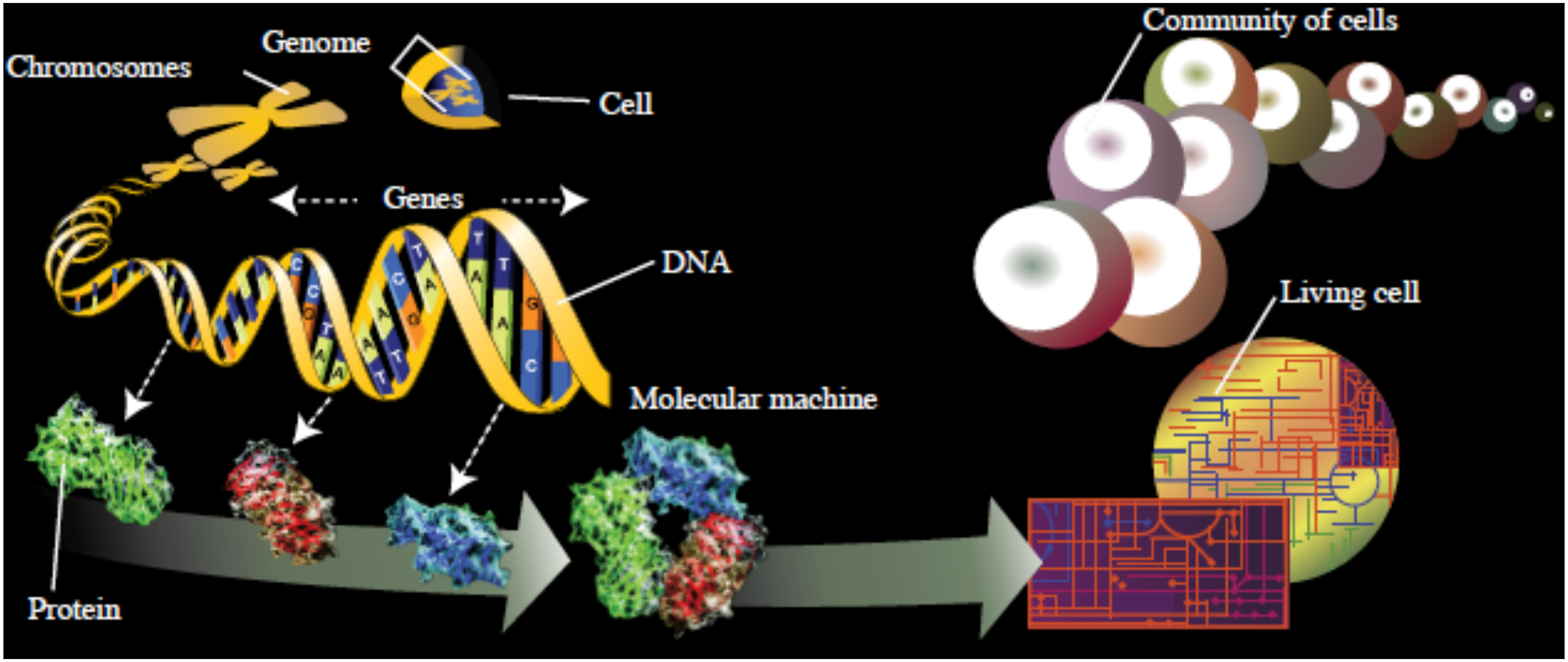
- Bone decrements
  - Fracture repair
  - Cellular basis (some)
  - Molecular mechanisms (some)
- Muscle
- Cardiovascular
- Vestibular/motor
- Eye: molecular changes
- Immune system

Big questions for long duration habitation in space

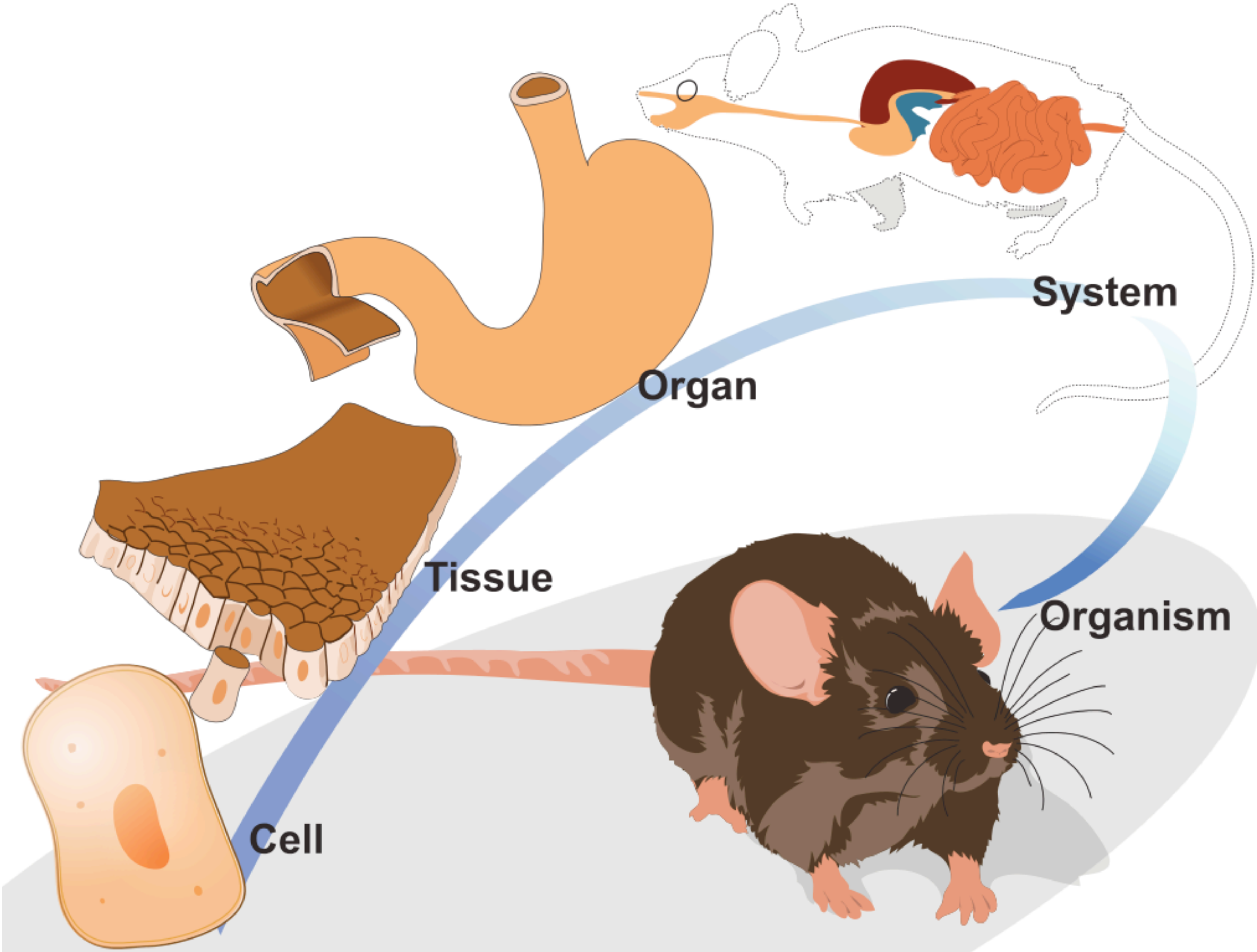
- **What** biological changes occur relevant to human health?
  - how far do adverse changes progress?
  - what (if anything) to do about them?

- **How** do these changes come about?
  - what are the mechanisms at the molecular, cellular and physiological, levels?
    - can we now better predict and identify interventions?
      - Taking the guess work out

Mechanisms at the molecular, cellular and physiological levels



Credit: the U.S. Department of Energy Human Genome Project (http://web.ornl.gov/sci/techresources/Human_Genome/publicat/index.shtml#science)


System
Organ
Tissue
Organism
Cell

Why rodents?

- Benefits that can be achieved:
  - Species most commonly used to better understand disease and potential treatments:
    - 71 of the Nobel Prizes for Medicine won in the last 103 years were awarded to scientists who used animals in their research
  - Scientific discoveries can be made that are simply not otherwise possible at this time

- Expert scientific advice for advancing space exploration:
  - *Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era (National Research Council, 2011)*
  - "The lack of an animal facility for rodents on the ISS suitable for long-duration studies on adult animals is a major research impediment that will hamper the ability to obtain information important for maintaining astronaut health and fitness for duty." p114

- Importantly, NASA animal research strictly adheres to all Federal regulations and requirements, and meets the highest standards for research animal care and welfare.

Rodents and Aging

Lifespan

Human: ~70-90yr   Mouse: ~2yr

Comparison of stages during aging:

AGE (HUMAN in YEARS)	20-30	38-47	56-69
AGE (MOUSE in MONTHS)	3-6	10-14	18-24

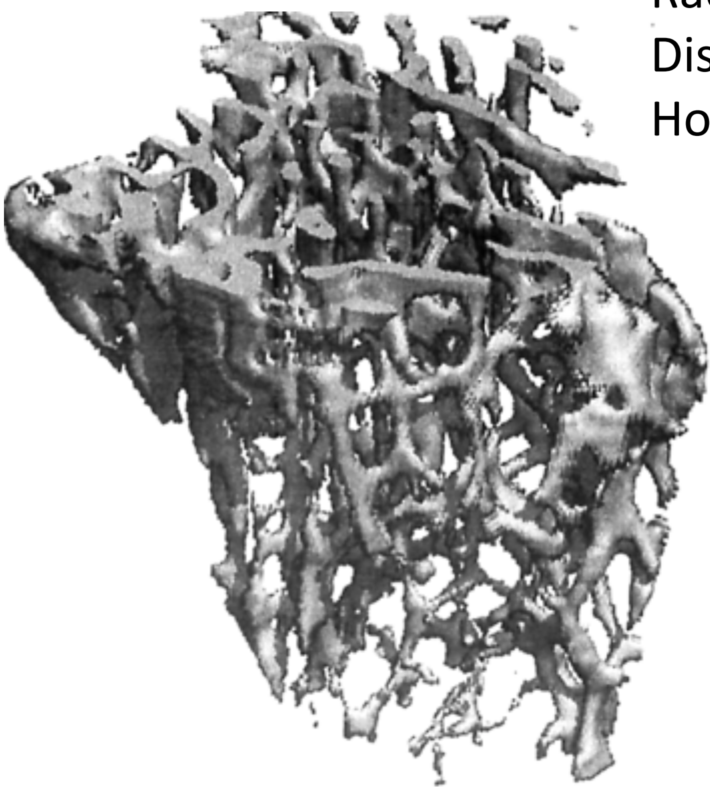
Comparison of rates maturation:

Ages	Mouse compared to human
Birth-1 month	150 X faster
1-6 months	45 X faster
6+ months	25 X faster

Source: http://research.jax.org/faculty/harrison/ger1vLifespan1.html#VD

Comparing rates of bone loss in humans and rodents

Aging
Microgravity
Radiation
Disuse
Hormonal changes



HUMANS:
Months-Decades

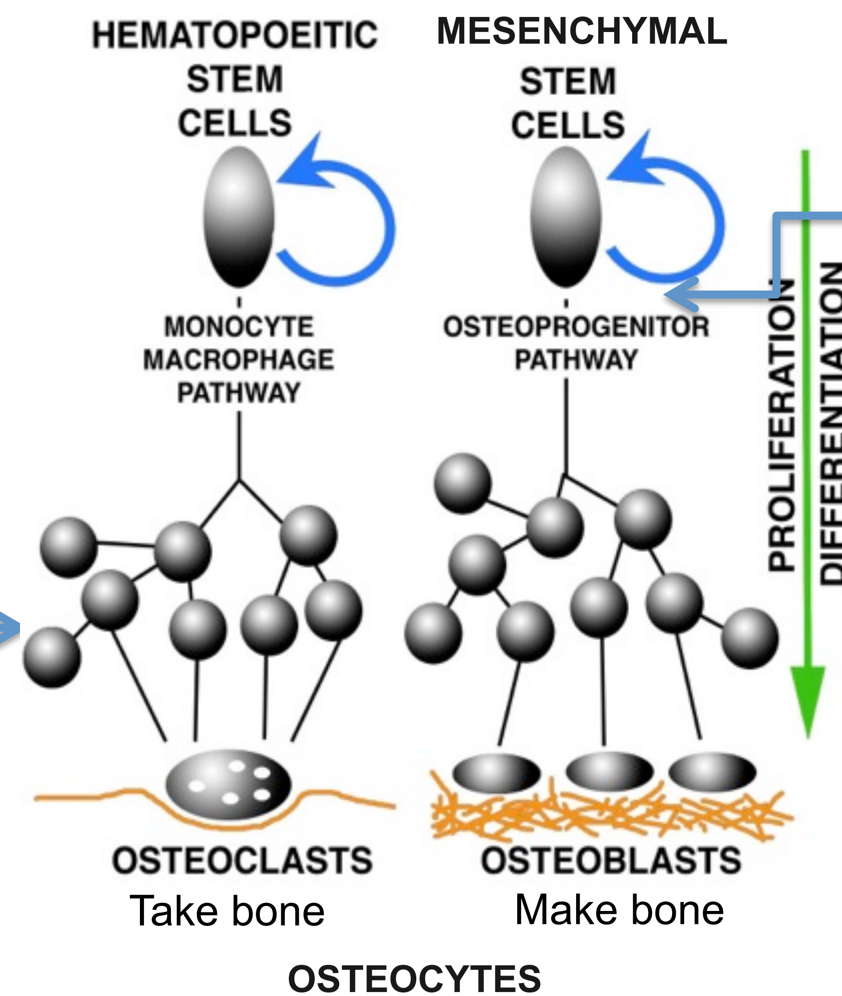
RODENTS:
Days-Weeks

Microcomputed tomography images of mouse tibia: Bone and Signaling Lab, ARC

Biological mechanisms: rodent spaceflight experiments, e.g. bone

Spaceflight affects cells at various stages during growth and maturation

Foton M3: Tamma et al.,: FASEB J. 2009 Aug; 23(8): 2549-54. Epub 2009 Mar 27.



STS131: Blaber et al. Stem Cell Res 2014. 12:181

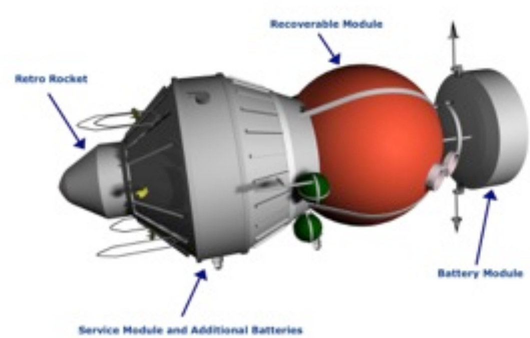
STS3: Roberts et al. Am J Physiol 1987; 252:R247-51.

BONE LOSS

RODENT RESEARCH EXPERIMENTS: EXPLORATION AND DISCOVERY IN SPACE

Spaceflight experiments: rodents

SPACE PLATFORMS



BIOCOSMOS 1971-present

SPACE SHUTTLE
1981-2011

INTERNATIONAL
SPACE STATION
2000-present

Rodent research: Shuttle Era

- Yielded new insight; growth of knowledge made possible by ability to perform many experiments
  - Responses to spaceflight
  - Treatments
- Relatively short duration: all <3 wk
- Mostly studied growing, not adult rats
  - What about adults?
- All but two flights entailed reentry and landing with several hour delay before tissue recovery
  - Variable of landing and delay may impact some (not all) outcomes
    - e.g. Muscle experiment showed a given measured response was due to landing, not microgravity (muscle micro-tears; Riley et al.)
  - This points to science value of on-orbit sample recovery

Moving beyond Shuttle: Recent long duration rodent studies

Bion-M1 (Russian)	**ISS: Mouse Drawer System**
• 30d on unmanned Biosatellite – Launch April 2013	• 91 days to and from ISS via Shuttle - Launch Aug 2009
• Hardware: – 4-5 mo old mice housed in groups of 3	• Hardware: Mouse Drawer System (MDS) (Italian) – Young mice individually housed
• Sample recovery 13 hours after landing	• Sample recovery after landing
• New results emerging: – Andreev-Andrievskiy et al. PlosOne 2014 – 13+ indexed papers to date	• Limited number of animals, but interesting new findings that invite further study – Cancedda et al. PlosOne 2012. – 5+ other indexed papers

Main objectives in development of Rodent Research project

- Provide reliable, long duration habitat for rodents (mice and rats) on the ISS
  - Group or individually housed; potential for future modifications for multiple generations
  - Low maintenance on orbit (minimize crew time)
- Capability to perform various on-orbit science procedures for sample recovery suitable for application of cutting-edge technologies to scientific problems
  - Avoiding the complication of return
- Opportunity to perform multiple missions
  - Current plan: two per year

Technical advances: usher in new opportunities for discovery

- Apply techniques to problems in space biology, for example
  - genetically modified animals: reveals mechanism, directs research for intervention/treatment
    - Flight Example: Rodent Research 1-Novartis experiment with MuRF-1 knock animals to study muscle wasting (in progress)
  - ‘omics
    - Wikipedia: “Omics aims at the collective characterization and quantification of pools of biological molecules that translate into the structure, function, and dynamics of an organism or organisms” e.g. genomics, proteomics, metabolomics.
    - Flight Example: Wilson et al. Space flight alters bacterial gene expression and virulence and reveals a role for global regulator Hfq.

      Proc Natl Acad Sci U S A. 2007 Oct 9;104(41):16299-304. Epub 2007 Sep 27.

Now and future: Rodent Research experiments on the ISS

Challenges

Challenges faced

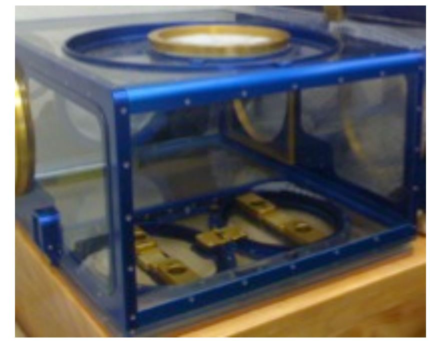
- Adapt legacy hardware to house the mice:
  - Animal Enclosure Modules:
    - 27 successful rodent experiments on the ISS
- Interface hardware with vehicles
  - SpaceX Dragon: unmanned vehicle, life support
  - ISS: 'Express racks', glove box to handle mice
- Taking care of animals (husbandry)
  - Training the crew
  - Monitoring animal welfare every day

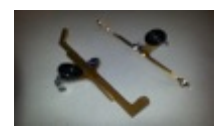
Basic equipment needed for conducting rodent experiments on Earth

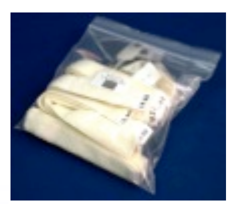
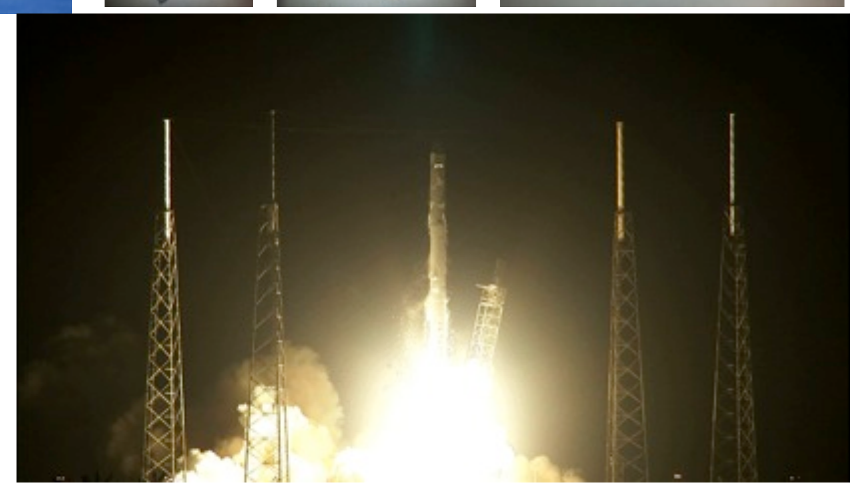
… pretty straightforward with the help of Earth's gravity…

Basic equipment needed for rodent experiment on orbit

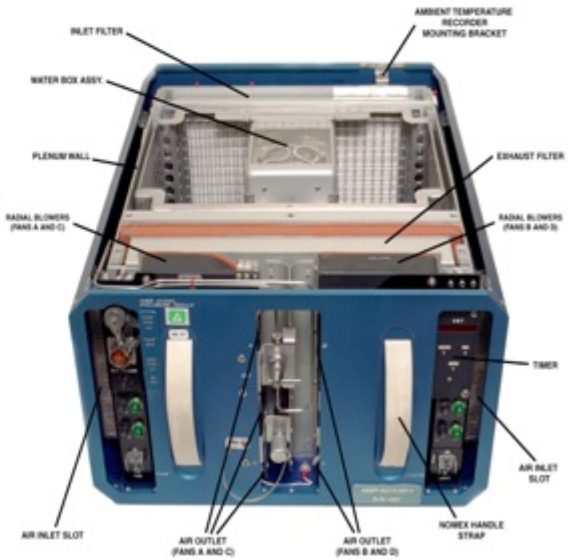




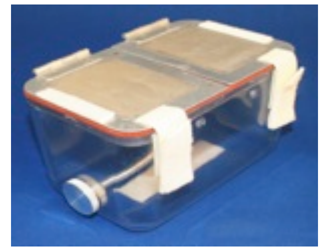




The RR Hardware



Transporter

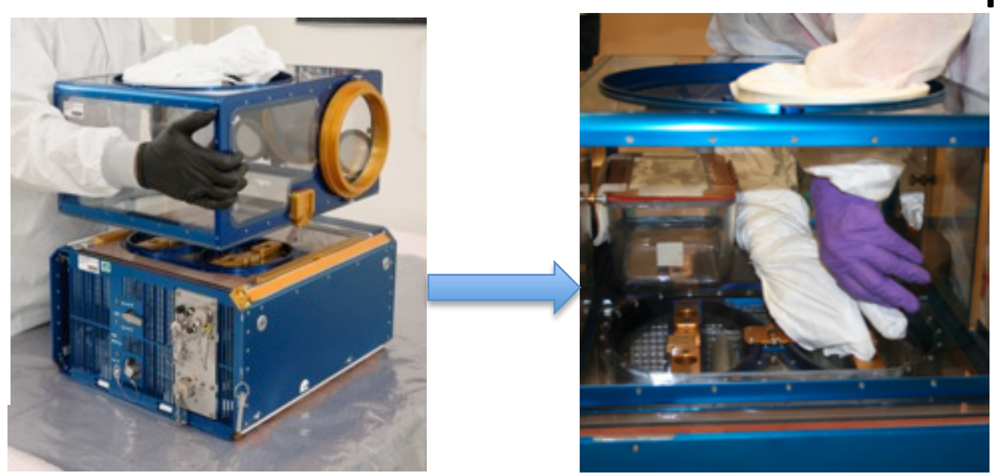
Animal Access Unit



Mouse Transfer Box

Kits (various)

Habitat



Rodents inside Habitat on Earth

Mice in Habitat on Earth

-5 mice per compartment, 10 per Habitat

-Grating on all sides

-Air flow thru to entrap waste in filters

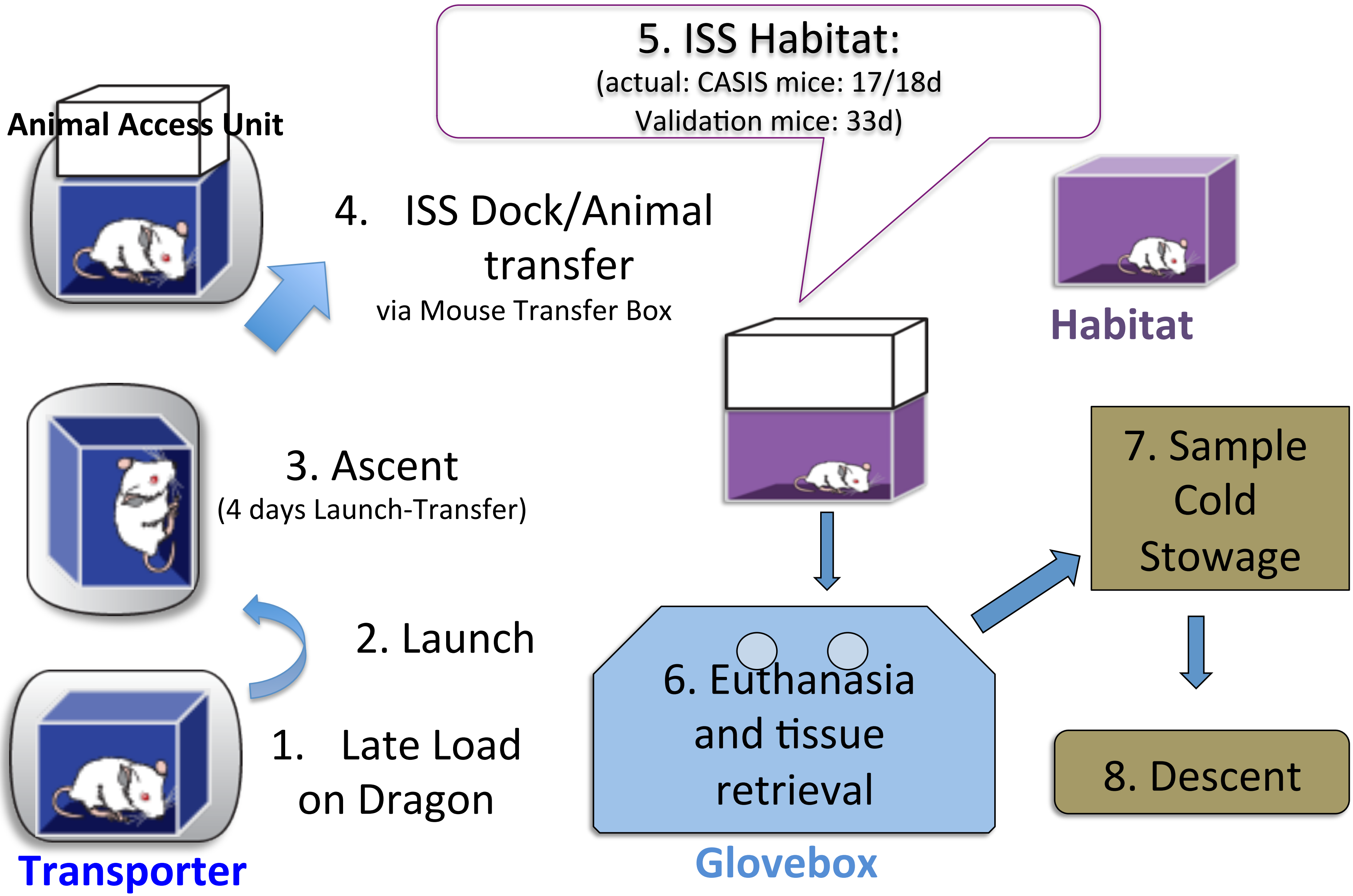
-Food supplied in form of bars

-Water supply (not in image)

-Lighting (dark-light cycle)

-Video cameras (infrared)

RR1: Concept of Operations



Ground based testing: Preparing for first flight

- Groundbased testing to ensure:
  - Animals thrive living in the hardware
  - All hardware and operational procedures work
  - Samples recovered according to the planned operations are of high quality so science outcome can be achieved
- Tests included

-Biocompatibility, launch simulation, tissue preservation and more

So, all systems go…

Now and future: Rodent Research project on the ISS

Rodent Research-1: Development and New findings

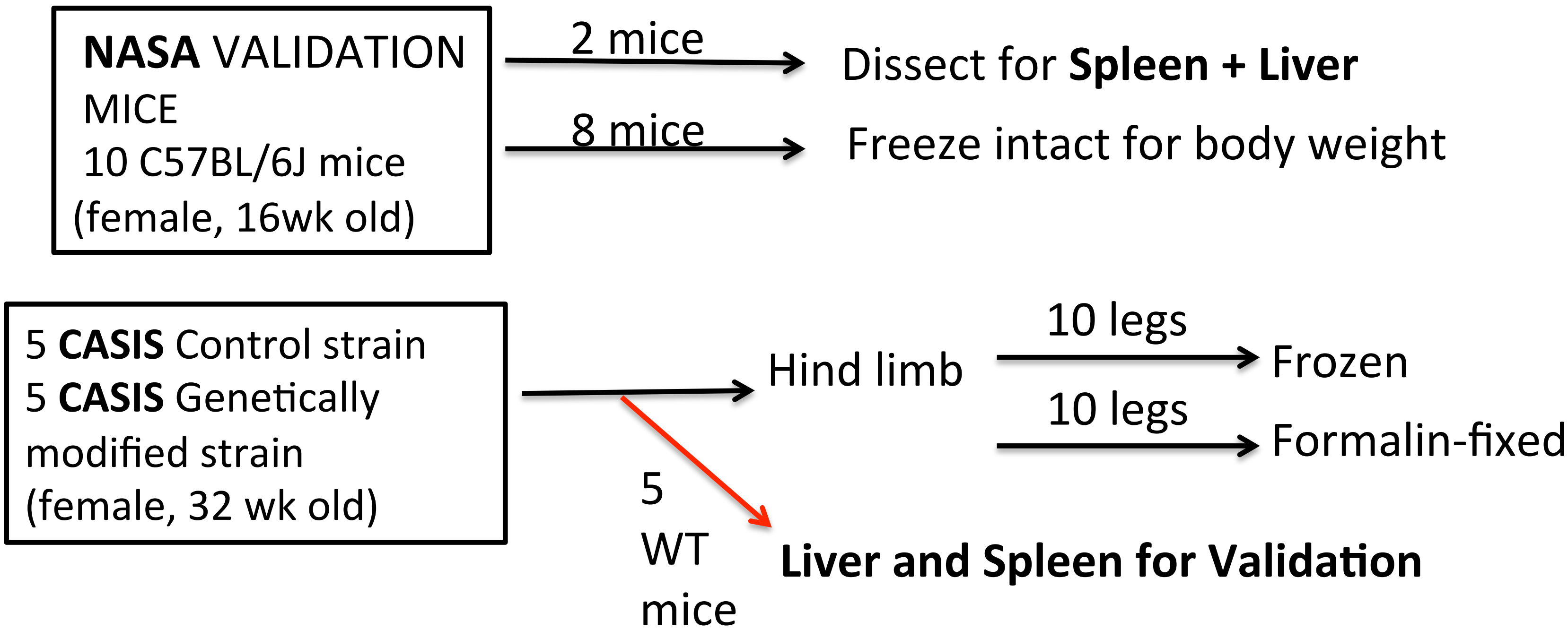
Rodent Research-1
Two main goals

1) Validation, demonstrating capability to support health animals: achieved.
   - Hardware
   - On-orbit operations
   - Science:
     - Animal health, behavior and tissue results
     - Samples retrieval high quality (Samples from both CASIS and Validation mice)

2) CASIS/Novartis science: Principal Investigator: Samuel Cadena

   - MuRF-1 Knockout mice and Control (wildtype) mice.
     -MuRF-1=Muscle RING-finger protein-1 to study muscle wasting

RR1 Detailed plan for on-orbit sample retrieval



Liver: fast frozen; RNA analysis and enzyme activity measurement
Spleen: preserved in RNAlater; RNA analysis

RR1: Validation mice behavior on ISS

Qualitative observations made during daily health checks:

- Upon initial introduction into the Habitat, mice actively explored the compartments
- Mice were observed eating, drinking, and grooming, both self and others, while in the Habitats

  All considered normal behaviors of healthy mice

- Mice propelled themselves about the compartment in more than one way
  - Mostly by ‘pulling’ along cage grate with their forelimbs
  - Also by ‘floating’ from one location to another

- As time went on, mice moved more quickly around the compartment, translating with ease thru open spaces, but also anchoring themselves using tails and/or paws

Quantitative validation analysis in progress : April Ronca, ARC

High RNA quality achieved from tissues dissected on orbit

Methods	Liver RNA (frozen)	Spleen RNA (preserved in RNA later)
qPCR (RIN≥6 preferred)	✔	✔
Gene Profiling Array (RIN≥7 preferred)	✔	✔
RNA-Seq (RIN≥8.0 preferred)	✔	✔

qPCR = quantitative Polymerase Chain Reaction
RNA-Seq = RNA Sequencing
RIN= RNA Integrity Number, index of quality/degradation of total RNA from value of 1 (lowest) to 10 (highest)

RR1: Validation
Experimental groups of mice

- 4 separate groups to better understand observed responses to this unique habitat and environment.

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

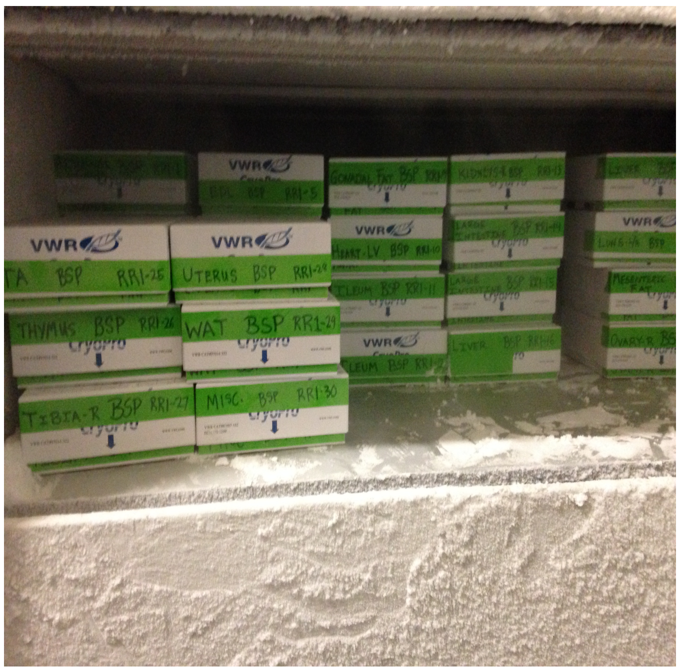
RR1: Validation

Changes:	Body	Liver	Adrenal	Thymus	Spleen	Soleus muscle*
Time (BAS to VIV)	No change	No change	No change	No change	↑	No change
Cage (VIV to GC)	No change	No change	No change	No change	No change	No change
Gravity (GC to FLT)	No change	↑	No change	↑	↓	↓

* No significant changes in masses of other muscles (gastrocnemius, tibialis anterior, quadriceps, EDL- extensor digitorum longus)

Expanding science return from RR

- RR science team recovered for distribution to scientists: 32 tissues from 40 RR1 Validation mice, yielding total of 3280 vials of tissues



Ames Life Science Data Archive (LSDA)

1) Biospecimen Sharing Program-Space Biology
   – to provide samples to various scientists, including Russian research colleagues at the Institute for Biomedical Problems (IMBP)

2) NASA Genelab project
   – large scale data set analyses ('omics) then provide open access

Space Biology:
Biospecimen sharing-enhanced science outcome

Examples:

Bion M1

Shuttle experiment: SLS-2

Muscle

Riley DA, et al. *In-flight and postflight changes in skeletal muscles of SLS-1 and SLS-2 spaceflown rats.* J Appl Physiol 1996. 81(1):133-44.

Neuro

Fagette S, et al., *Central and peripheral noradrenergic responses to 14 days of spaceflight (SLS-2) or hindlimb suspension in rats*. Aviat Space Environ Med. 1996;67(5): 458-62.

Immune

Lesnyak A, et al. *Effect of SLS-2 spaceflight on immunologic parameters of rats. J Appl Physio*l. 1996 ;81(1):178-82.

Ichiki AT,et al. *Effects of spaceflight on rat peripheral blood leukocytes and bone marrow progenitor cells.* J Leukoc Biol. 1996 ;60(1):37-43.

Bone

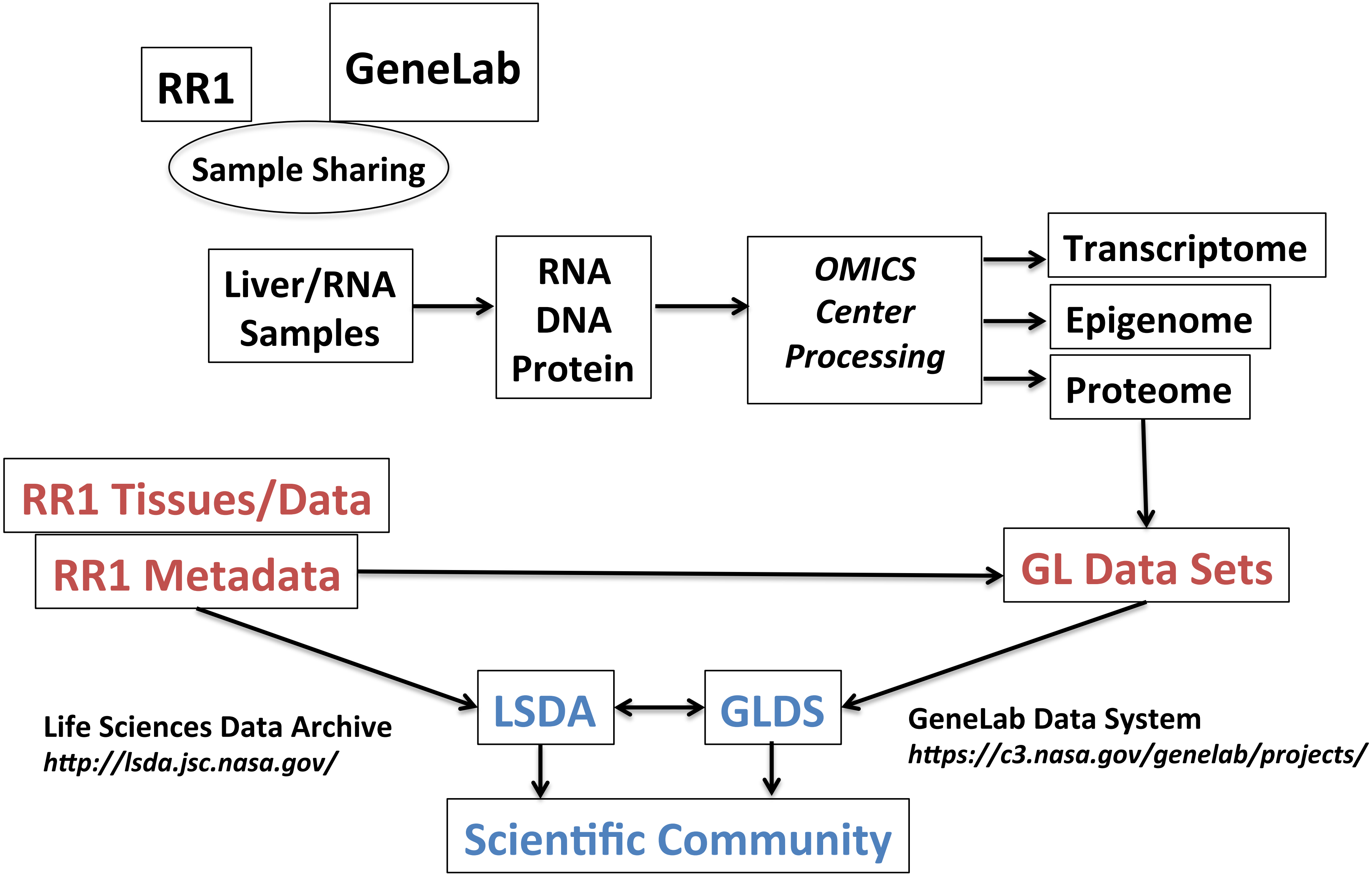
Evans, Glenda L, et al. *Spaceflight has compartment and gene-specific effects on mRNA levels for bone matrix proteins in rat femur.*
J. Appl. Physiol. 84(6): 2132–2137, 1998

Durnova G, et al. *Histomorphometric study of tibia of rats exposed aboard American Spacelab Life Sciences 2 Shuttle Mission.* J Gravit Physiol. 1996;3(2):80-1.

Blood

Allebban Z, et al. *Effects of spaceflight on rat erythroid parameters*. J Appl Physiol. 1996 Jul;81(1):117-22.

RR1- GeneLab sample analysis



RR1 Findings: Summary to date

- Planned operations were performed successfully on orbit, including animal transfer, euthanasia, dissection and sample retrieval and cold stowage.

- Although analyses are still in progress, findings to date show that mice thrived through 37 days in microgravity.

    -Body weights at end of flight were same as controls.

    -Common indicators of stress were <u>not</u> observed

        (e.g. body weight loss, adrenal gland hypertrophy)

    -Surprising differences observed compared to results obtained from short duration Shuttle experiments

Rodent Research-2 mission

- Launched on SpX-6
  - 20 mice for up to 60 days with on-orbit sample recovery.
    - 20 mice assigned to CASIS (Novartis) with objective to determine muscle and bone changes over time and development of medications to treat muscle wasting

CONCLUDING COMMENTS

Summary: to live in space

- Multiple challenges are posed by the space environment
- Multiple physiological systems are affected
- Resulting complexity is such that the consequences over a lifetime on human health and reproduction simply cannot be predicted at this time
  - Insight into consequences and mechanisms improves prediction and mitigation;
    - rodent research on the ISS can help get us there

Acknowledgements

- Space Biology Program
- Rodent Research Project
- ISS Program
- Bone and Signaling Laboratory

Links/Resources

- Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era (National Research Council, 2011)
  - http://www.nap.edu/catalog/13048/recapturing-a-future-for-space-exploration-life-and-physical-sciences
- NASA Life Sciences Data Archive
  - http://lsda.jsc.nasa.gov/
- NASA's Genelab project: Strategic Plan
  - http://genelab.nasa.gov/discovery-genelab-strategic-plan.html
- Novartis: Mice in Space video, RR1 study
  - https://www.youtube.com/watch?v=1L868FzjF2I