Alteration of the brain-to-bone axis in microgravity as a factor for bone loss for long-term spaceflight and a potential focus for prospective countermeasures

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Thomas Chretien[1]; S. Casarosa[2], L. Balasco[2]. V. Yotov[3], V. Turiyski[3]; Y. Bozzi[1]

[1]Center for Mind/Brain Sciences – CIMEC, University of Trento, Rovereto, Italy
[2]Cellular, Computational and Integrative Biology Departement – CIBIO, University of Trento, Trento, Italy
[3]Medical University-Plovdiv, Department of Medical Physics and Biophysics, Plovdiv, Bulgaria

Introduction & Background

The new objective of space exploration is **Mars.** The total time spent in microgravity will be about 14 months. Long-duration space travel can have significant effects on astronauts' bones due to the unique conditions of microgravity experienced in space. In microgravity, bones undergo significant density loss due to mechanical unloading, weakening skeletal structures and making them brittle and prone to breaking. Existing countermeasures are insufficient and will require more investigation for long-term spaceflight. Here, this line of research aims to provide insights on the brain-bone axis alteration in space.

Microgravity simulation

These investigations are designed to examine the long-term effects of microgravity on the brain-bone axis. For ground testing, a Random Positioning Machine (RPM) will be used to replicate as closely as possible microgravity conditions.

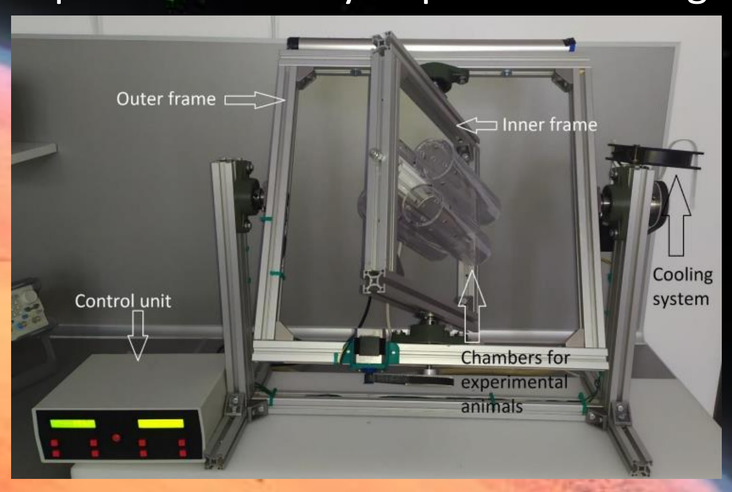


Figure 1: Modified Random Positioning Machine Setup

The RPM modification for mice (3-month-old, male, Albino ICR mice.) is ongoing. It is made of two squares that rotate independently and perpendicularly to each other.

Preliminary tests & Experimental design

First, mice stress levels will be measured to determine whether experimental conditions will affect results. Hair will be collected at different stages of the experiment. The first collection will occur in standard housing conditions (baseline).

- **Sham control** → Corticosterone levels will be measured in a non-operating RPM apparatus
- **Microgravity group** → Corticosterone levels will be measured in a operating RPM apparatus

For the main experiment, a between-subjects study design will be considered. Using the mice from the previous stress test if the stress difference is negligible, they will be separated into two groups. For this experiment 6 mice will put in the two apparatuses every four weeks (24 subjects total).

- **Control group** → 4 weeks in non-operating RPM apparatus
- **Microgravity group** → 4 weeks in operating RPM apparatus

Experimental Measures

1. Histological assessment

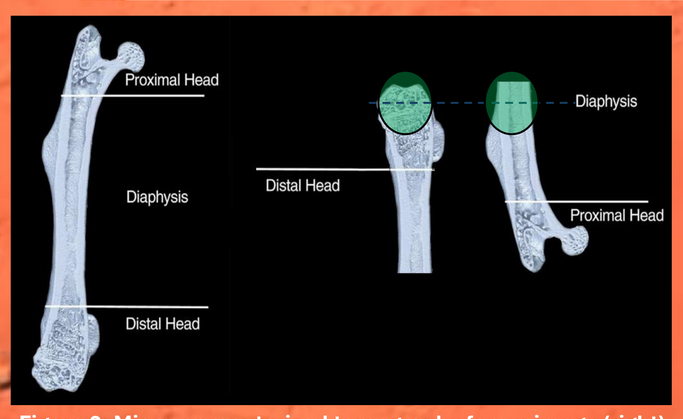


Figure 2: Micro-computerized tomography femur image (right) and paraffin-embedding femur for maximal innervation assessment (modified from Mach et al., 2002)

To assess bone innervation the femurs will be collected and embedded into paraffin for sectioning (10um cross sections every 100um from distal head).

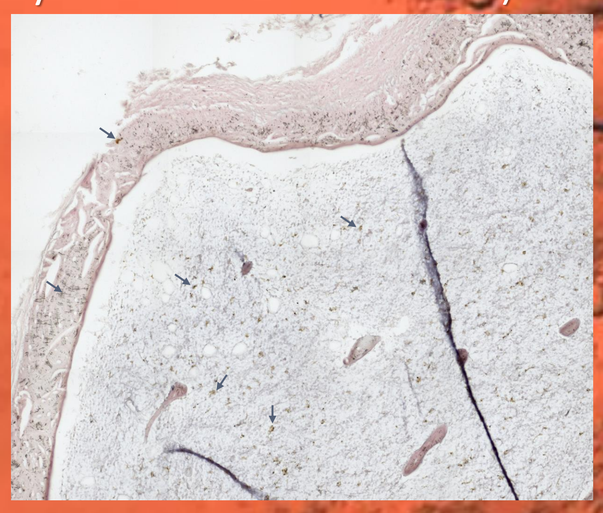


Figure 3: Cross-sectional part of the femur, lightly stained with the Luxol fast Blue stain. Arrows indicate bone nerves in different.

Each cross-section will be stained using a specific myelin stain. Quantification of nerve surface and total volume will be assessed (ZEN Microscopy software and Fiji) and compared between both conditions.

2. Neurotransmitter quantification

To quantify neurotransmitters (CGRP, VIP, etc…) in bone, femurs will be pulverized into small fragments, chemically treated and sonicated before being processed into a liquid phase chromatography.

Using Mass Spectrometry (MS) many neurotransmitters can be qualitatively separated and quantified.

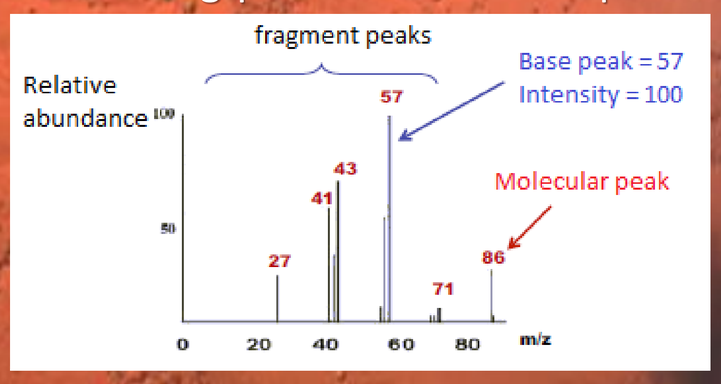
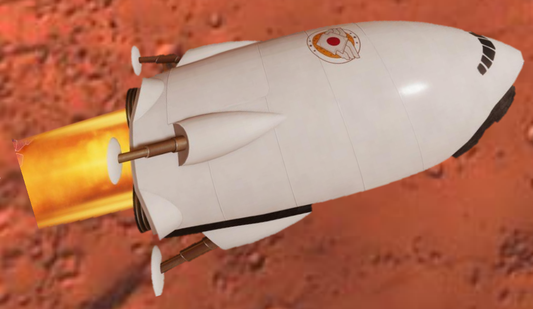


Figure 4: Typical Mass spectrometry ion fragmentation



Expected conclusion and Perspectives

The findings of this study will provide unprecedented empirical evidence on the effect of microgravity on bone terminal nerve and long bone synaptic exchange homeostasis. These findings may highlight the necessity to deploy viable countermeasures for long-term spaceflight to reduce nerve degeneration and regulate synaptic transmission in long bones, hence ensuring appropriate bone remodelling.

References

Acknowledgement

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