Impact of the ISS Environment on CNS in Drosophila Melanogaster

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Abstract

Altered gravity and elevated carbon dioxide (CO_2) levels as experienced on the ISS can adversely affect human health across various organ systems, especially the Central Nervous System (CNS). Investigating these changes is essential for understanding the long-term effects of spaceflight on human physiology to ensure crew health. Ground-based analogs provide an efficient method to evaluate alterations induced by chronic spaceflight on a larger scale.

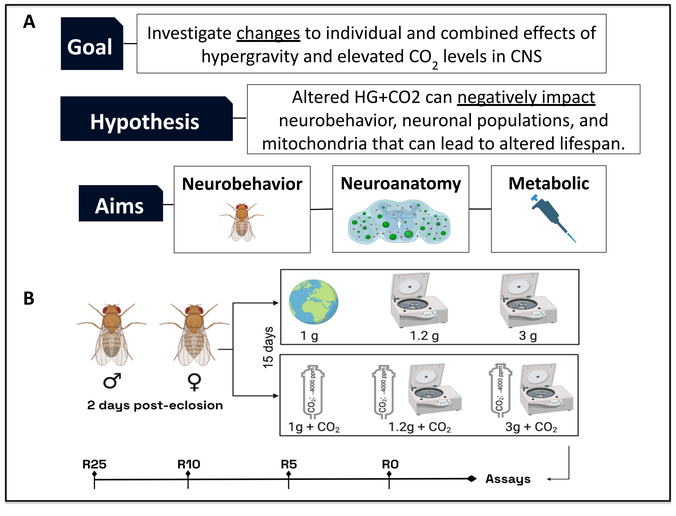
This study focuses on CNS changes in response to hypergravity (HG) and elevated CO_2 levels via a ground-based analog using the well-established model organism, *Drosophila melanogaster*. We hypothesize behavior and physiological changes immediately post exposure to HG+CO_2, along with chronic effects up to 25 days post-exposure. Adult male and female flies were exposed to varying gravity loads (1g, 1.2g, and 3g) and elevated CO_2 levels (~4000 ppm, mimicking CO_2 levels on the ISS) for 15 days and were assessed immediately and at 5-, 10-, and 25-days post-exposure, mirroring astronauts' post-return profiling. The flies were assessed for neurobehavioral changes, including longevity and negative geotaxis; brain morphological changes, such as dopaminergic neuron count, apoptosis, and glial cell density; and bioenergetic changes in the brain, including mitochondrial abundance and membrane potential.

Longevity remained unchanged under hypergravity, even with the addition of elevated CO_2 as a stressor. However, despite the stable lifespan, quality of life appeared to be affected, as shown by negative geotaxis and neuroanatomical changes. Negative geotaxis testing revealed a reduction in motor ability at R0 across all conditions, which correlated with a decrease in dopaminergic (DA) neuron count. Additional observations suggested further systemic alterations, including a decrease in glial cell count, an increase in apoptosis, and notable bioenergetic changes. Collectively, these findings contribute to our understanding of the long-term effects of spaceflight on the CNS.

Background and Introduction

- NASA has identified five primary stressors in long-term space missions that may impact astronaut health: altered gravity, radiation, isolation, distance from Earth, and hostile/closed environments (NASA, 2024).
- Additional stressors, like CO_2 buildup, also pose health risks to astronauts (Beard, 2020).
- In low Earth orbit (LEO) on the ISS, altered gravity and CO_2 buildup are key concerns, while other hazards increase as distance from Earth grows (Chancellor et al., 2014).
- *Drosophila melanogaster* serves as an ideal model for studying these stressors due to its genetic similarities to humans, and a well-documented genome.
- *Drosophila*'s simple nervous system (DA Circuit), short lifespan, and extensive previous literature make it useful for multigenerational and aging studies in space research.
- Previous spaceflight studies have shown CNS changes in *Drosophila* under combined spaceflight conditions, affecting behavior and brain profiles (Mhatre et al., 2022). However, data on long-term effects on the CNS after spaceflight remain limited (Clément et al., 2020).
- This project aims to study hypergravity (HG) and elevated CO_2 stressors on *Drosophila* CNS, hypothesizing that these conditions may negatively affect neurobehavior, neuronal populations, mitochondria, and lifespan.

Materials and Methods



Results

Longevity is not altered by HG or CO2 stressors

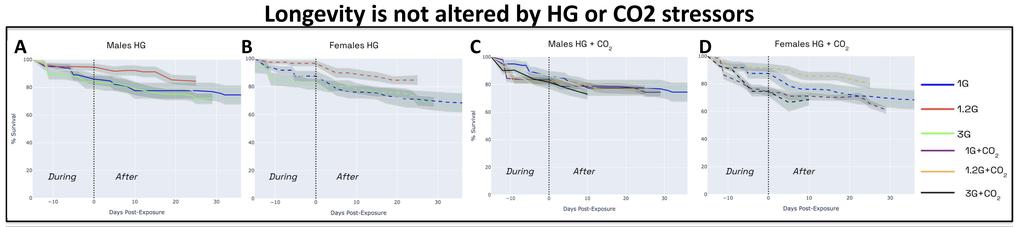


Figure 1: Longevity of different stressors over time for both sexes. The dotted line in each graph depicts when flies re-acclimate to 1g post exposure to the stressor conditions for 15 days (0 Days Post-Exposure). A: HG stressors males, B: HG stressors females, C: HG+CO_2 stressors males, D: HG+CO_2 stressors females.

Negative Geotaxis Reveals Motor Deficits

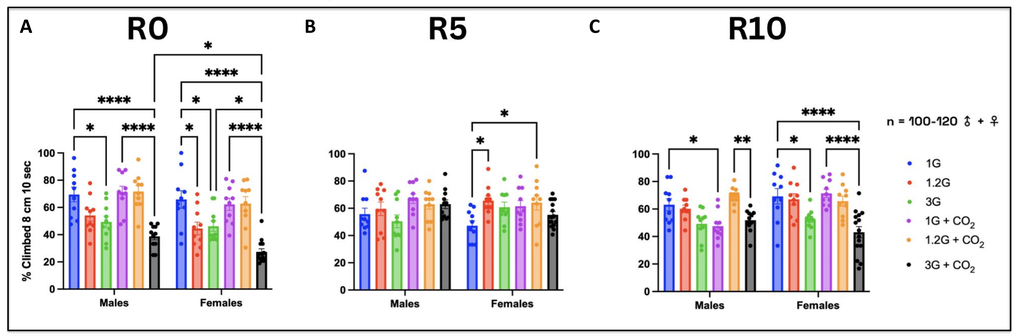


Figure 2: Negative geotaxis of different stressors tested over time post-exposure to stressors. A: R0 days, B: R5 days, C: R10 days. For all significance values, *: p-value <0.05, **: p-value <0.01, ***: p-value <0.001, ****: p-value <0.0001.

Neuroanatomy Markers of Show Reduced Glia and Elevated Apoptosis to HG

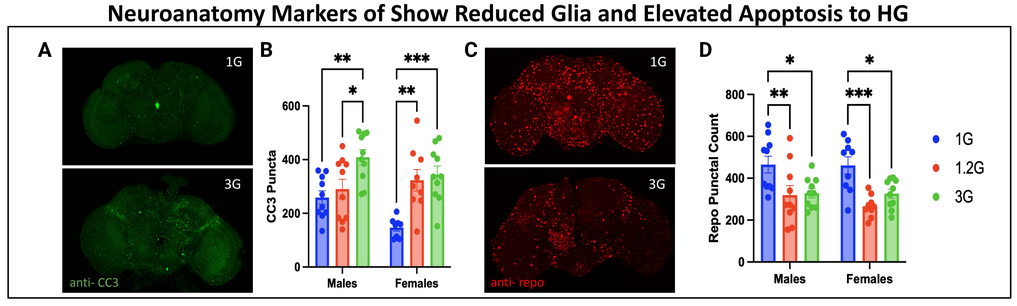


Figure 3: Representative image of adult brain stained with anti-CC3, apoptotic marker (A), and anti-Repo, glial marker (C), Increased CC3 positive puncta in HG adult flies as compared control (B), decreased glial counts in HG adult flies as compared control (D). *: p-value <0.05, **: p-value <0.01, ***: p-value <0.001

Key correlations between Negative Geotaxis and DA Neuronal Count

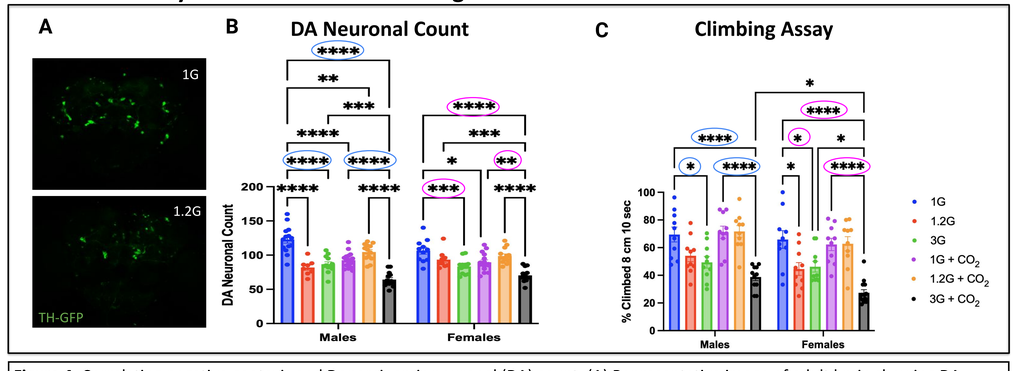


Figure 4: Correlating negative geotaxis and Dopaminergic neuronal (DA) count. (A) Representative image of adult brain showing DA neurons (B) Significant decrease in dopaminergic neurons across HG/+CO_2 conditions at the R0 timepoint. (C) Bar graphs of %Climbing Ability across HG/+CO_2 condition at R0 shows correlation with dopaminergic neuron loss. Key correlations circled in blue for males and magenta for females.*: p-value <0.05, **: p-value <0.01, ***: p-value <0.001.

Results Continued

Bioenergetics Indicate Mitochondrial Dysfunction

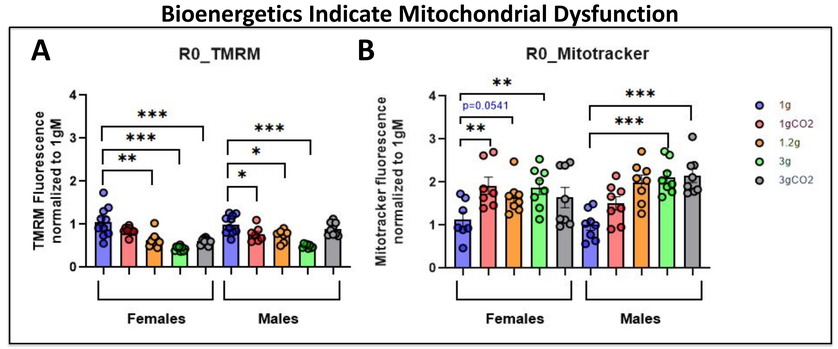


Figure 5: Bioenergetics quantified post exposure at the R0 timepoint. (A) Quantification of mitochondrial membrane potential using TMRM stain shows depolarization of the mitochondrial membrane (B) Quantification of mitochondrial abundance in the brain using Mitotracker shows significant increase in mitochondrial abundance in HG/+CO_2 conditions *: p-value <0.05, **: p-value <0.01, ***: p-value <0.001, ****: p-value <0.0001.

Conclusion

Lifespan
- No change observed in HG/CO_2 conditions (**Figure 1 A-D**)
- Lifespan alone is not indicative of quality of life

Acute Effects of Spaceflight: R0
- Decrease in climbing ability was observe in 1.2g, 3g, and 3g + CO_2 conditions (**Figure 2A**)
- This decrease correlates with a decrease in DA neurons for both males and females (**Figure 4 A/B**)

Long-term Effects of Spaceflight: Climbing Ability
- R5 recovery to baseline in all conditions (**Figure 2B**)
- Differential reduction in climbing ability observed at R10, where only 3g and 3g + CO2 conditions reduced climbing once again (**Figure 2C**)

Neuroanatomy
- Apoptosis ↑ and Glia ↓ with HG (**Figure 3**)
- Overall ↓ in DA neurons under HG and HG/+CO_2 conditions (**Figure 4B**)

Bioenergetics
- Increased mitochondrial abundance observed, potentially as compensation for ↑ oxidative stress under HG/+CO_2 conditions (**Figure 5B**)
- ↓ TMRM, a measure of mitochondrial membrane potential, indicates ↑mitochondrial depolarization, suggesting potential mitochondrial dysfunction or stress (**Figure 5A**)

Future Directions

- Neuromorphological analyses at R5, R10, and R25 time points.
- Bioenergetic evaluation including ATP assay at all time points.
- Quantification of DA Neuron deficits to specific clusters.
- Correlation studies to examine the relationship between Climbing ability and DA Neurons across different time points.

Key References

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- NASA Space Biosciences SLSTP: https://www.nasa.gov/ames/research/space-life-sciences-training-program
- NASA Space Biology Program: https://science.nasa.gov/biological-physical/programs/space-biology