The ABCs of Spaceflight

Evaluating the Impact of Spaceflight on ABC Proteins (P-Glycoprotein) in the Blood Brain Barrier

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INTRODUCTION

Both astronauts and other model organisms experience negative effects from spaceflight, such as **Blood-Brain Barrier (BBB) leakage**—this vital barrier protects the brain from harmful substances. This leakage results from oxidative stress and neuroinflammation caused by proinflammatory cytokines which damage surrounding tissue. Thus, our proposal uses *Drosophila Melanogaster* (Fruit fly) transcriptomics data from OSD-588 to investigate the role of microgravity on the BBB and methods to mitigate these implications,

BACKGROUND

Exposure to Microgravity increases the production of Reactive Oxygen Species (ROS) causing oxidative stress→ **destruction of Blood Brain barrier integrity**

- In response, proinflammatory cytokines/ chemokines are secreted leading to tissue and endothelial cell damage leading to BBB leakage.

Proteins involved in BBB semi-permeability:

- **P Glycoprotein, PGP**: ATP-binding cassette (ABC) transporters (protect BBB from harmful substances in body
- **TNF-alpha**: inflammatory cytokine involved in cascade that downregulates Pgp

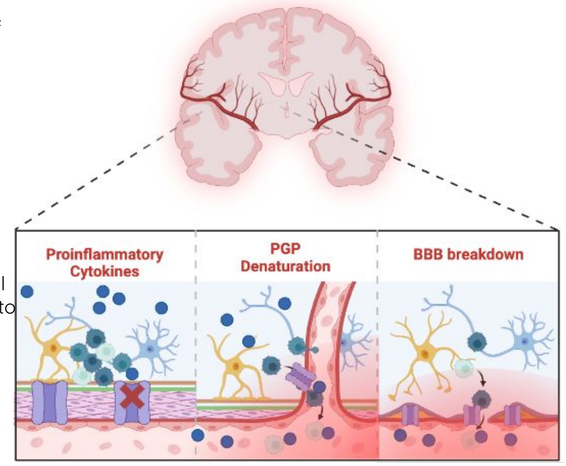


Figure 1: Blood Barrier Barrier damaged by proinflammatory cytokines, seen in blue irregularly shaped molecules, causing substance leakage, seen as blue vesicles, between the capillary vessels and the endothelial and glial cells that support the BBB semipermeable membrane. Figure Accredited to BioRender.

Genes involved in PGP Reduction:

- CG10226 (PGP)
- CG7497 (TNF alpha & inflammatory cytokines)
- AKhR (Neural Signaling system)
- CG13575 (Neural peptides)
- Galphaf (Protein receptor binding)
- AdoR (Stem Cell proliferation/Differentiation)

All of which are **Orthologous to humans**

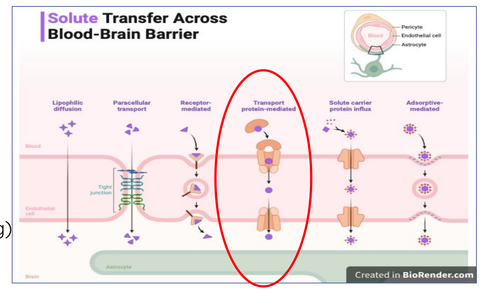


Figure 2: Image of Blood Brain Barrier solute transportation, illustrating its semi-permeable nature.

ORIGINAL DATASET METADATA

OSD-588: Drosophila parasitoids were launched into space during *Fruit Fly Lab-03* mission.

Figure 3: OSD-588 Icon Image

- **Vented Fly Box hardware system**: housed fruit fly adults, embryos/larvae, and parasitic wasps at International Space Station for 32 days
- **Samples**: Yellow White F1 generation larvae infected by Leptopilina boulardi (Lb), we compared four samples from space flight to four samples from ground conditions.

TRANSCRIPTOMICS ANALYSIS & RESULTS

Tools from the UseGalaxy.org open analysis platform were use to conduct the following transcriptomic analyses based on the spaceflight and ground control datasets from OSD-588:

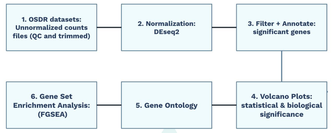


Figure 4: Transcriptomics analysis workflow derived from standardized OSDR pipelines

Methods:

- **UseGalaxy** facilitating following analyses**:**
- **GOSeq Tool:** RNA seq data from OSD-588 after trimmed & Normalization ensuring statistical significance
- **Volcano Plots:** RNA seq data from OSD-588
- **ShinyGO Gene Ontology Analysis**: Done by uploading Top volcano plot data to ShinyGo v0.741 (Figure 8)

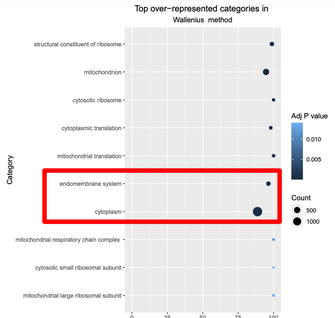


Figure 5: Top over-represented categories are shown, indicating a strong biological signal toward these pathways compared to the overall gene population

Using the GOseq tool, we identified pathways of interest, highlighting those that were significantly altered.

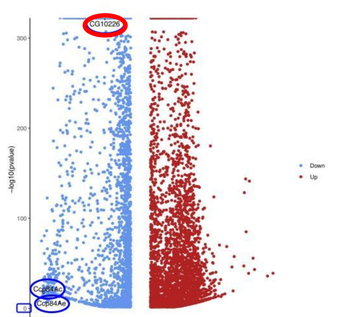
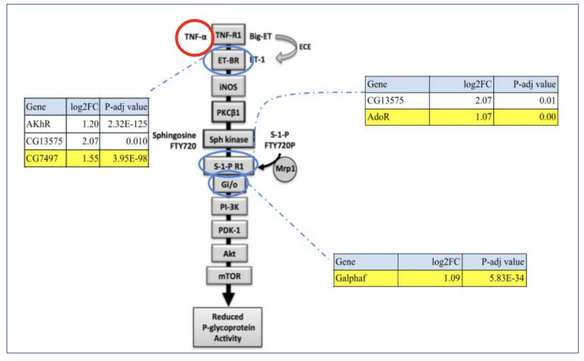


Figure 6: Volcano plots highlight differential gene expression, where upregulations are in red and downregulation are in blue.

The volcano plot highlights the significant **downregulation of CG10226, or P-glycoprotein (PGP),** a **gene orthologous** to humans both in genetic structure and functional pathways.

Upstream and Downstream Differential Gene Expression

In addition to PGP downregulation**,** we identified other **orthologous genes** that when **upregulated lead to reduction of PGP** expression—all of which have a Log2FC > +/-1 and P-adj value < 0.05.



- Overexpression of pro-inflammatory cytokines facilitates the upregulation of over 6 genes leading to PGP downregulation.

Figure 7: TNF-alpha (red circle) overexpression, genes found in analysis (Blue circles) to PGP reduction cascade.

Figure 8: Figure below, ShinyGO analysis from Volcano pot where pathways/ categories with Fold Enrichment> 100.00 are showcased. **Significance**: Illustrates overrepresentation of ABC proteins→ATP transporter activity (Red represented ABC protein pathways)

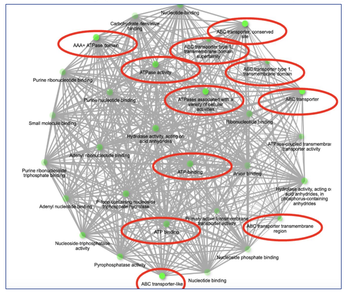
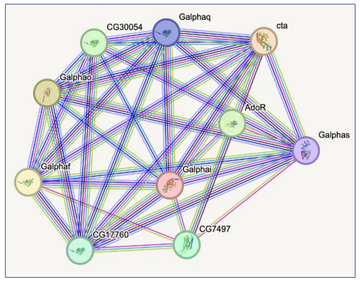


Figure 9: StringDB protein-protein & drosophila gene interactions.

Significance: Shows interplay of TNF-alpha to PGP cascade genes previously mentioned.



PROPOSED EXPERIMENT

HYPOTHESIS: **We hypothesize that Valproic Acid, a PGP Upregulator, will stabilize the Blood brain barrier, preventing BBB leakage, despite the presence of microgravity.**

This study evaluates potential therapeutic uses for PGP (MDR1) upregulation in spaceflight through Valproic acid (VPA: decreases BBB permeability) treatment on rodent models.

Methods:

Eighty drosophila will be divided into four groups: 40 on Earth and 40 in space, with each group further split between VPA-dosed (20) and control (20) subgroups. VPA will be dissolved in ethanol, with 1 µl dispensed per 1 ml of food for the VPA groups, while controls receive plain ethanol. Separate habitats will prevent cross-contamination, and the study will last 34 days.

Dosing Procedure and Preparation:

A 50 mM VPA solution in ethanol will be prepared, with 1 µl dispensed daily into the food of the VPA groups on both Earth and in microgravity simulations. Controls will receive pure ethanol to control for any effects from ethanol exposure.

Measuring BBB Permeability Post-Spaceflight:

After 34 days, BBB permeability will be assessed using Evan's blue dye. All *Drosophila* will be immersed in 2.5% dye for 45 minutes, rinsed with PBS, and prepared for tissue collection. Brains will be dissected, sectioned, and observed under a fluorescence microscope to analyze dye penetration. Tissues will be fixed in PFA for additional Pgp expression analysis using Western blotting, further investigating how VPA dosing impacts BBB integrity and Pgp expression.

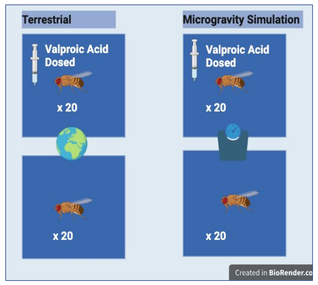


Figure 10: Graphical representation of experimental design

Figure 11: Vented Fly box and VPA food pellets

Anticipated Results:

- **We expect** the VPA-dosed groups to display reduced BBB permeability in comparison to control groups in the spaceflight study, as shown by lower Evan's blue dye penetration in brain tissue.

SIGNIFICANCE & CONCLUSION

Therapeutic methods to prevent neurological degeneration and accelerated aging will be necessary as NASA transitions to long-term space flight travel. Previous studies have been conducted regarding the decreased function of the BBB in space. However, there **has yet** to be research on molecular specifics, such as Pgp function.

Our experimental proposal evaluates the impact of spaceflight on Pgp and evaluates a potential therapeutic option, all in one continued and comprehensive study. This is an angle that is **underrepresented in literature**.

- By studying the intricacies of BBB function and their impacts on neurological function, we work towards a better understanding of spaceflight impact on the brain, and subsequently, the development of novel treatments and preventative measures to ensure astronaut wellbeing.
- **Reduction of PGP** has been **already confirmed** in multiple studies to increase **drug delivery** effort by increasing blood brain barrier permeability.
- **p53**, a tumor suppressor gene **already confirmed** to be differentially expressed in space, is **co-expressed with Pgp,** so further studies into this relationship could open up new avenues for spaceflight-induced cancer risk research.

We conclude changes in ABC transport activity, particularly reduced PGP production by CG10226, will be connected to Blood Brain Barrier dysfunction and leakage.

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