Region- and Cell Type–Specific Gene Expression Changes in the Mouse Brain Following Spaceflight: A Bulk and Single-Cell RNA-Seq Analysis

Michael A. Moshi [1,2], Edwin C. Oh[1,2,3,4]

[1]Laboratory of Neurogenetics and Precision Medicine, College of Sciences; [2]Neuroscience Interdisciplinary Ph.D. program; [3]Department of Brain Health; [4]Department of Internal Medicine, Kirk Kerkorian School of Medicine at UNLV, University of Nevada Las Vegas, Las Vegas, NV 89154, USA

OVERVIEW

This research performs secondary data analysis to explore how spaceflight alters brain gene expression. Publicly available RNA-seq datasets from three distinct brain regions were processed, applying a robust bioinformatics pipeline for quality control, alignment, and quantification. Additionally, single-cell data from OSD 612 (left cerebral hemisphere) was analyzed using CellRanger ARC and Seurat to identify specific cell populations and their transcriptional changes. Differential gene expression (DE) analyses and functional enrichment assessments were performed on both bulk and single-cell data, focusing on pathways such as *protein processing in the endoplasmic reticulum*, *RNA splicing*, and *phosphorylation regulation*. By comparing bulk datasets and examining single-cell clusters, we identified common molecular adaptions alongside cell type–specific responses to spaceflight.

INTRODUCTION

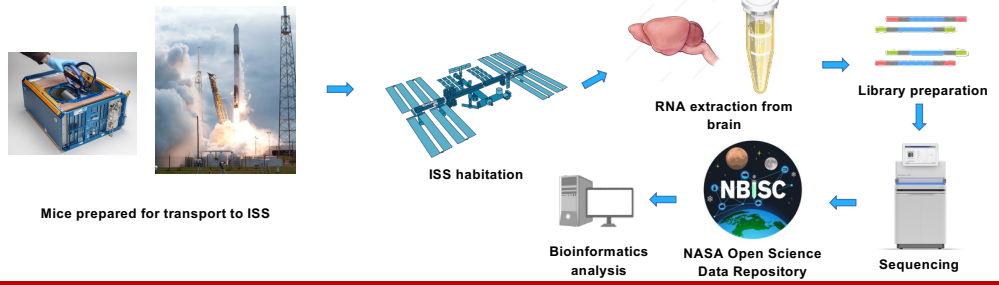
Spaceflight exposes organisms to unique stressors such as microgravity and cosmic radiation, which can have profound effects on brain physiology. In this study, secondary data analysis was performed on bulk RNA sequencing data from NASA's OSD projects (OSD 525, 564, and 612) to investigate the molecular impact of spaceflight on the mouse brain. To gain further resolution into cell type–specific responses, single-cell RNA-seq analysis was performed on data derived from OSD 612. The goal was to examine shared and region-specific transcriptional responses in the brain in response to the space environment and how certain cell populations may drive these changes.

METHODS

Raw bulk RNA sequencing data from NASA's OSD projects were obtained for the cerebellum (OSD 525), hippocampus (OSD 564), and left cerebral hemisphere (OSD 612). Quality control was performed using fastp (v0.24.0), followed by alignment to the Mus musculus reference genome with STAR (v2.7.11b), and transcript quantification with Salmon (v1.10.3). Differential expression analysis was carried out using DESeq2, with ground controls set as the reference group. Gene ontology (GO) and KEGG pathway enrichment analyses were conducted using the clusterProfiler package, and pairwise comparisons were made to assess molecular responses across the datasets.

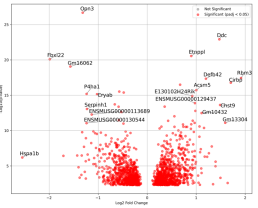
For single-cell analysis, the reads were aligned and quantified with CellRanger ARC (v.2.0.2), then created Seurat (v4.4.0) objects for the samples. The count matrix was filtered for genes with ≥100 total counts in ≥10 cells. After merging, data was normalized, scaled, and subjected to PCA (dims=1–20). A neighbor graph was built for clustered cells (resolution=0.5) and visualized via UMAP. Clusters were annotated using module scores for known marker genes (neurons, glia, etc.). DEGs were identified between flight and ground-control groups in each cell-type cluster, and enriched pathways were identified using gProfiler python package.

METHODS

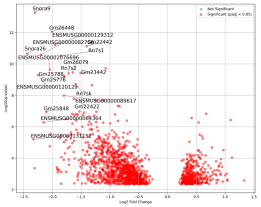


RESULTS

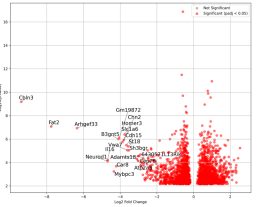
Cerebellum (Ground Control vs Flight)



Hippocampus (Ground Control vs Flight)



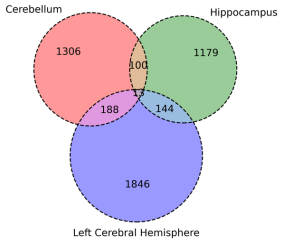
Left Cerebral Hemisphere (Ground Control vs Flight)



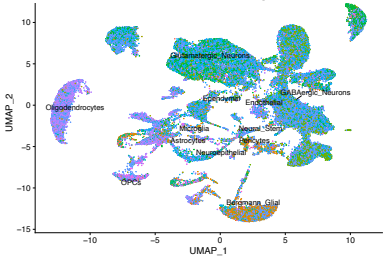
OSD Project Sample Size

	Ground Control	Flight
OSD 525	9	9
OSD 564	5	5
OSD 612 (bulk)	5	4
OSD 612 (single cell)	5	5

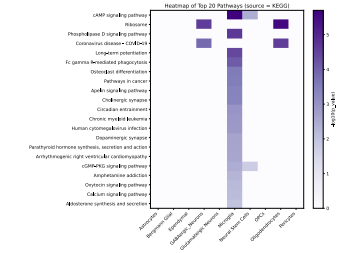
Overlap of Differentially Expressed Genes in Various Regions of the Brain



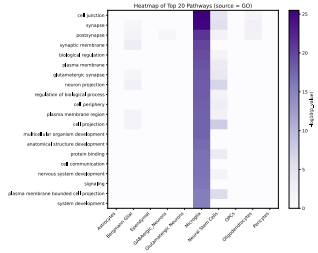
UMAP of Cell-Type Clusters Identified in the Left Cerebral Hemisphere



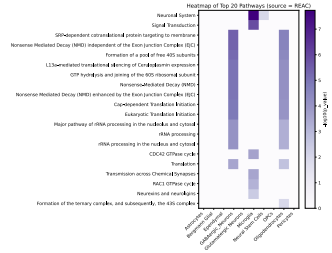
KEGG Cell-Specific Pathway Analysis



GO Cell-Specific Pathway Analysis



Reactome Cell-Specific Pathway Analysis



RESULTS

The analysis revealed that the KEGG pathway *Protein processing in the endoplasmic reticulum* was consistently enriched across all three brain regions, suggesting a common cellular stress response to spaceflight. Additionally, pairwise comparisons indicated that *RNA-related processes* and *negative regulation of phosphorylation* were prominent between the cerebellum and left cerebral hemisphere, while the hippocampus and left cerebral hemisphere shared enrichment in *ribosome biogenesis* and *RNA processing* pathways.

Single-cell analysis of the left cerebral hemisphere (OSD 612) identified distinct DE patterns between flight and ground control groups in multiple cell types, such as GABAergic neurons, microglia, and oligodendrocytes. Pathways involved in *cellular signaling*, *ribosome function*, *structural processes*, and *synaptic plasticity* showed cluster-specific enrichment, suggesting that spaceflight alters both glial- and neuron-specific mechanisms.

CONCLUSION

In summary, this study demonstrates that spaceflight induces significant alterations in brain gene expression at both the bulk tissue and single-cell levels, with a notable universal impact on *protein processing in the endoplasmic reticulum*. The identification of additional region-specific pathways, such as those involved in *RNA processing* and *phosphorylation regulation*, underlines how the brain adapts to spaceflight, while cell type specific pathways were highlighted by single-cell analysis. Microglia exhibited the most significant transcriptional changes, followed by GABAergic neurons and oligodendrocytes, highlighting distinct cellular responses to spaceflight. These findings contribute valuable insights into the molecular mechanisms underlying neural adaptation to space and may help guide future research aimed at mitigating adverse effects on astronaut health.

REFERENCES

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2. Chauhan, A., Martinez, P., Smith, L., & Rodriguez, R. (2023). Spatially Resolved Multiomics on the Neuronal Effects Induced by Spaceflight in Mice. Nature Communications, 15, Article 4778. https://doi.org/10.1038/s41467-024-48916-8

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