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Spaceflight Modulates Neurodegeneration-Associated Transcriptomic Pathways Across Brain Cell Types

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Abstract

Spaceflight induces stress that damages tissues and promotes disease. The brain may be particularly vulnerable due to its high bioenergetic demand and to microgravity-induced headward fluid shifts that impose mechanical and vascular stress. Prior work has shown that spaceflight can alter the structure and function of the brain and reshape its transcriptome in ways that implicate disease-relevant gene pathways. Here, we used single-nucleus transcriptomic analyses to test whether spaceflight engages neurodegeneration-associated transcriptional programs and activates disease-associated microglia. Young (3 months) or older (7 months) female mice were flown to the International Space Station or retained as terrestrial controls for 55–58 days before euthanasia. Brain nuclei were processed for single-nucleus RNA sequencing, annotated, and grouped into seven primary cell types. All cell types exhibited large numbers of differentially expressed genes as a function of spaceflight, whereas few genes were affected by age. Pathway analysis showed that these genes were enriched for processes upstream of neurodegenerative pathology, including mitochondrial and oxidative stress, proteostasis, and cell death, rather than for brain-specific outcomes such as synaptic and neuroplasticity pathways. Spaceflight further drove an increase in microglial gene signatures associated with activated states, including disease-associated, MHC-II, interferon-responsive, and inflammatory microglia. Together, these findings indicate that spaceflight induces a transcriptional landscape consistent with elevated risk for neurodegenerative disease, likely reflecting cellular sensitization rather than established pathology at this exposure duration. These results underscore the need for further studies to define relative risk and to identify countermeasures as human missions venture farther and longer into space.

1. Introduction

As humans increase frequency and duration of spaceflight, it is critical to understand how the biological stressors encountered in this environment affect physiology and long-term risk for disease. Spaceflight exposes crews to a unique combination of physiological and psychological challenges, the most distinctive of which are microgravity and ionizing radiation in the form of galactic cosmic radiation (GCR)¹⁻³. Unlike terrestrial radiation, GCR comprises high-energy, high-charge particles that deposit dense ionization tracks through tissue and are only partially attenuated by spacecraft shielding, making the central nervous system a tissue of particular concern^{2,3}.

A substantial body of work using simulated microgravity and ground-based GCR analogs points to meaningful neurological consequences of these exposures. Radiation induces cognitive deficits⁴⁻⁹ that have been attributed to several convergent mechanisms, including microglial dysfunction and neuroinflammation^{5,6,10}, metabolic disruption⁴, and physiological changes to neurons^{7,9}. Terrestrial radiation exposure of varying types also promotes hippocampal apoptosis, neuroinflammation, and oxidative stress¹⁰⁻¹⁴. Simulated microgravity is independently associated with cognitive deficits¹⁵ and hippocampal dysfunction in rodents^{16,17} and can induce neuroinflammation and disrupt synaptic plasticity *in vitro*¹⁸. Together, these findings indicate that the individual components of the spaceflight environment are each capable of perturbing brain function, raising the possibility that their combined, prolonged exposure poses a cumulative risk to the brain¹³.

Observations from space flown astronauts and rodents corroborate these results. Microgravity drives a headward fluid shift that alters the brain's position within the cranium and redistributes free water¹⁹, increasing hydrostatic pressure and altering ventricular volume, periventricular white matter hyperintensities, gray matter volume, and functional connectivity²⁰⁻²². At the molecular level, gene and protein expression studies in space flown animals reveal differentially expressed genes tied to apoptosis, immune function, neuroplasticity, proteostasis, and core aspects of cellular metabolism²³⁻²⁷, alongside ultrastructural changes in the cerebral

cortex and cerebellum^{28,29}. Behavioral effects in astronauts are detectable but generally subtle, affecting tasks involving motor function and motion perception³⁰ and spatial cognition³¹, without large or consistent global decrements³².

Many of these reported effects converge on mechanisms that are also central to neurodegenerative disease. Although individual neurodegenerative disorders have distinct primary etiologies, they share a set of tissue responses, including mitochondrial and bioenergetic dysfunction, excess oxidative stress, glial-mediated neuroinflammation, and failures of protein folding and clearance, that translate diverse insults into progressive pathology³³. The brain may be especially susceptible to these stressors given its high energetic demand and dependence on tightly regulated mitochondrial function, which together render it sensitive to even modest bioenergetic perturbation. Accordingly, the cumulative burden of spaceflight stressors, integrated over longer missions, has been proposed to increase risk for, or directly drive, neurodegeneration processes^{24,25}. This risk is expected to scale with mission duration and with travel beyond low Earth orbit, where GCR dose rises sharply due to the loss of shielding from Earth's magnetosphere^{2,34}.

Despite this convergence, whether spaceflight engages neurodegeneration-associated transcriptional programs across the diverse cell types of the brain remains unresolved, as does the extent to which different cell populations share or diverge in their responses. Here, we directly address these questions using a single-nucleus RNA-sequencing (snRNA-seq) dataset from female mice housed on the International Space Station for just under two months. We find that spaceflight induces large numbers of differentially expressed genes across all major brain cell types, that these genes enrich for pathways underlying neurodegenerative disease, and that disease-associated microglial phenotypes are upregulated with spaceflight. These results provide further evidence that spaceflight perturbs the brain in ways consistent with elevated neurodegenerative risk and underscore the potential importance of countermeasures for long-duration missions beyond low Earth orbit.

2. Results

2.1 Spaceflight induces widespread gene expression changes across brain cell types

To interrogate the effects of spaceflight on distinct brain cell populations, we analyzed single-nucleus RNA-sequencing (snRNA-seq) data from the NASA Open Science Data Repository (OSDR) study OSD-613. Female C57BL/6Ntac mice, aged 12 (young) or 29 (old) weeks at experimental onset, were assigned to either ground control (GC) or spaceflight conditions (FLT; 55–58 days aboard the International Space Station), yielding four groups: young-GC (n=5), young-FLT (n=4), old-GC (n=4), and old-FLT (n=5). Mice were euthanized aboard the International Space Station where they were immediately frozen until they were returned to Earth. Tissue was dissected from a single hemisphere (Allen Brain Atlas slices 60–74), spanning cortex, hippocampus, thalamus, hypothalamus, amygdala, caudate-putamen, and major white matter tracts including corpus callosum, fimbria, and internal capsule (Fig. 1A). After quality control, 88,338 nuclei were retained, clustered, and annotated with MapMyCells³⁵ into seven major cell classes: astrocytes, oligodendrocytes (OLGs), oligodendrocyte precursor cells (OPCs), microglia, vascular/epithelial cells (vascular), glutamatergic neurons (Glut), and GABAergic neurons (GABA) (Fig. 1B). Annotations were validated by module scoring with canonical marker genes (Fig. 1C; Supplementary Table 1) and manually curated where necessary.

cell type composition differed between conditions ($p < 0.05$): GC mice showed higher proportions of GABAergic neurons in both cohorts, whereas FLT mice showed increased relative abundance of OLGs, OPCs, and microglia (Fig. 1D).

We next quantified differential expression using a pseudobulk approach with DESeq2, treating each mouse as a biological replicate. A full model (gene ~ age + condition + age:condition) was fit, with genes considered differentially expressed (DEGs) at a Benjamini-Hochberg-adjusted $p < 0.05$ and an absolute shrunken $\log_2$ fold change ($\log_2 \text{FC}_{\text{shr}} > 0.6$).

Shrinkage was applied to reduce variance from lowly expressed genes and rare populations, yielding a more comparable measure of effect size across genes and cell types. No gene in any cell type showed a significant age $\times$ condition interaction, so main effects were estimated using a reduced model (gene $\sim$ age + condition; Supplementary Tables 2–4).

Spaceflight altered expression of a large number of genes across all cell types, whereas few genes were affected by age (Fig. 2A). This limited age effect is consistent with the old cohort being substantially younger than the onset of most age-related transcriptomic changes in the mouse³⁶, as well as principal component analysis, in which samples segregated primarily by condition rather than age (Supplementary Fig. 1). To evaluate whether heterogeneity of variance might confound differential expression, we examined DEG variability at the pseudobulk level; within-group variance/dispersion was comparable between flight and ground across cell types (Levene's test, $p > 0.05$; Supplementary Fig. 2), arguing against variance-driven artifacts. Subsequent analyses therefore focused on the main effect of flight condition.

The majority of DEGs were upregulated (Fig. 2A). Neurons (GABA and Glut) and OLGs yielded the highest DEG counts, but this may in part reflect their greater abundance, which confers higher statistical power and limits direct cross-cell type comparison of DEG number. To compare response magnitude independent of power, we examined the $|\log_2 \text{FC}_{\text{shr}}|$ of the top 50 DEGs per cell type (Fig. 2B). Pairwise differences were widespread (Cohen's $d = 0.05$ – 6.12 ; Fig. 2C): astrocytes and GABA neurons showed the smallest fold changes among their most significant genes, while OLGs showed the largest. Pairwise correlations of $\log_2 \text{FC}_{\text{shr}}$ across all genes revealed strong joint regulation between GABA and Glut neurons ($r = 0.86$), with vascular cells least similar to other types (Fig. 2D). No cell type pair was negatively correlated, indicating broadly concordant directionality across the brain. DEG identity, however, overlapped only partially; GABA and Glut populations shared the most DEGs, glial cells overlapped moderately, and vascular cells were again most distinct (Jaccard index; Fig. 2E).

Together, these analyses indicate that all cell types respond to ~8 weeks of spaceflight with a generally concordant direction of regulation, apart from a subset of downregulated genes in OLGs and vascular cells. The partial overlap in DEG identity nonetheless suggests cell type specific components of the response that may underlie differential vulnerability.

2.2 Spaceflight activates neurodegeneration-associated transcriptional programs linked to oxidative and proteostatic stress

To assess whether spaceflight engages transcriptional programs relevant to neurodegenerative disease, we performed gene set enrichment analysis (GSEA) on KEGG pathways for human neurodegenerative disorders: Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, prion disease, spinocerebellar ataxia, Huntington's disease, and pathways of neurodegeneration—multiple diseases (Fig. 3A). All pathways were significantly upregulated across every cell type (adjusted $p < 0.05$), with the sole exception of spinocerebellar ataxia in microglia. Leading-edge analysis showed these enrichments were predominantly driven by oxidative phosphorylation (OXPHOS) genes shared across multiple pathways. To test whether enrichment depended on this overlap, we removed OXPHOS genes and repeated the analysis; the overall pattern was preserved (Supplementary Fig. 3).

Because neurodegenerative disease arises through both convergent and disease-specific mechanisms, we next examined a curated panel of Gene Ontology (GO) terms covering neurodegenerative outcomes (neuroinflammation, neuroplasticity, synaptic vesicle function/transport) and upstream cellular processes implicated in degeneration (reactive oxygen stress, antioxidant response, proteostasis, autophagy, cell death, endoplasmic reticulum stress) (Fig. 3). We observed significant enrichment across these categories, corroborating the KEGG results. Enrichment was particularly pronounced for upstream processes—antioxidant and reactive oxygen stress pathways, proteostasis, subsets of cell death, and endoplasmic reticulum stress—whereas neuroinflammation, neuroplasticity, and synaptic vesicle pathways were only

sparsely enriched. A notable exception was significant upregulation of microglial cell activation within microglia, an increasingly recognized transdiagnostic feature of neurodegenerative disease³⁷. Full GO and KEGG results per cell type are provided in Supplementary Tables 5 and 6.

These findings indicate that spaceflight engages broad cellular stress responses while largely sparing brain-specific outcome pathways such as neurotransmission and synaptic vesicle effects. This pattern is consistent with the induction of neurodegeneration precursor pathways, although the duration of the present mission may be insufficient to produce overt, transcriptome visible, neurodegenerative pathology.

2.3 Spaceflight reprograms microglia toward inflammatory and antigen-presenting states

Microglia are central contributors to neurodegenerative disease phenotypes³⁷. To determine whether spaceflight drives disease-associated microglial states, we assembled curated gene sets for five microglial phenotypes, including disease-associated microglia (DAM), MHC-II, interferon-response (IFN), inflammatory, and homeostatic, from a consensus annotation of mouse single-cell transcriptomic studies³⁷ (Methods; Supplementary Table 7). Two-way ANOVA (age $\times$ condition) on per-mouse module scores revealed significant condition effects for the DAM, inflammatory, IFN, and MHC-II signatures, all elevated in FLT relative to GC mice, whereas the homeostatic signature did not reach significance (Fig. 4A; Supplementary Table 8; Supplementary Fig. 4). Effect sizes were large ($\eta^2 = 0.27\text{--}0.43$), supporting an increase in activated microglial signatures with spaceflight.

To localize this response, we subclustered microglia into seven clusters (Fig. 4B) and tested condition composition by cluster. Clusters 1, 2, 5, and 6 differed significantly in FLT/GC membership (chi-square test with Benjamini-Hochberg correction); cluster 1 was FLT-dominated (72% FLT-derived), while cluster 2 was GC-dominated (77% GC-derived) (Fig. 4C; Supplementary Table 9). Per-cluster module scoring (Fig. 4D) showed that cluster 0 (balanced

across conditions) and cluster 2 (GC-dominated) were enriched for homeostatic markers, whereas cluster 1 (FLT-dominated) was enriched for DAM, inflammatory, and MHC-II signatures. This suggests that clusters 0 and 2 represent surveillant, baseline microglia, while spaceflight promotes a shift toward activated, disease-associated states.

GO enrichment analysis of the three largest clusters supported this functional differentiation. Cluster 0, the largest, with balanced FLT and GC representation, was enriched for cell motility and morphology, immune surveillance, phagocytosis, and tissue maintenance (Fig. 4E), consistent with a homeostatic state primed for response. Cluster 1 was enriched for activation-associated processes, including migration, cell death, regulation of inflammation, synapse pruning, and antigen processing (Fig. 4F), whereas cluster 2 was enriched for terms reflecting reduced motility, maintenance of synaptic connectivity, and cellular organization (Fig. 4G). Collectively, these data indicate that spaceflight promotes inflammatory and disease/injury-related transcriptomic phenotypes in microglia, which are processes strongly linked to neurodegenerative disease.

3. Discussion

Here we show that 55–58 days of spaceflight in low Earth orbit is sufficient to reprogram the transcriptome across all major brain cell types in a manner consistent with elevated risk for neurodegeneration. This suggests that spaceflight induces a transcriptional landscape that may sensitize neural tissue to neurodegenerative processes rather than directly recapitulating the disease itself.

Individual neurodegenerative diseases have distinct primary etiologies, but a growing body of work suggests that these etiologies converge on overlapping downstream tissue responses that drive the gradual accumulation of pathology, including cell death, chronic neuroinflammation, and loss of synaptic connectivity³⁸. Core responses that translate etiology into degeneration include mitochondrial and bioenergetic dysfunction, oxidative stress, and

failure of proteostasis³⁹. In the present study, spaceflight altered each of these processes, with the strongest and most global effects in pathways tied to mitochondrial dysfunction, oxidative stress, and proteostasis, whereas fewer neurodegeneration outcome pathways, including synaptic and neuroplasticity-related terms, reached significance. This pattern is consistent with spaceflight setting the stage for degeneration, while the present exposure (55–58 days) appears insufficient to produce transcriptomically overt neurodegenerative outcomes.

The brain may be especially vulnerable to spaceflight stressors because of its high energetic demand and reliance on tightly regulated mitochondrial function. Oxidative phosphorylation (OXPHOS) accounts for the large majority of cellular ATP production, and the brain consumes roughly 20% of resting systemic energy despite representing only ~2% of body mass, rendering it acutely sensitive to bioenergetic deficits^{40,41}. Consistent with this, a central hypothesis in the etiology of Alzheimer's disease, and of neurodegeneration more broadly, holds that declining mitochondrial function compromises cellular homeostasis and drives pathology⁴². Disruption of normal neuronal metabolism is thought to be a primary route by which both acute and chronic spaceflight produces neurological effects^{43,44}. Mitochondrial dysfunction during spaceflight has been documented across in vitro models, rodent systems, and human astronaut biospecimens. Low-dose radiation elicits excess mitochondrial reactive oxygen species (mROS) in human and mouse cells, producing mitochondrial damage and reduced OXPHOS activity^{45,46}, while tissues from mice aboard the ISS show broad OXPHOS inhibition and upregulation of mitochondrial stress markers⁴⁷. In parallel, blood profiling from the NASA Twins Study, JAXA, and Inspiration4 missions revealed that prolonged spaceflight impaired mitochondrial function, depleted antioxidant defenses, and elevated circulating mitochondrial damage-associated molecular patterns (DAMPs), including mtDNA and mtRNA^{43,48}. These perturbations were accompanied by upregulation of the pro-inflammatory cytokines IFN- γ , IL-1, and TNF- α , linking spaceflight-induced mitochondrial stress to systemic inflammatory signaling^{47,48}.

Against this backdrop, mitochondrial pathways were among the most consistently altered across cell types in our data. We observed upregulation of OXPHOS transcripts, which may reflect a compensatory response to bioenergetic insufficiency or increased mitochondrial biogenesis and turnover, consistent with coordinated efforts to restore mitochondrial homeostasis. This was accompanied by enrichment of oxidative stress, antioxidant defense, proteostasis, and autophagy/mitophagy pathways. These changes plausibly represent downstream effects of mitochondrial and endoplasmic reticulum stress responses, including the unfolded protein response and integrated stress response, potentially driven by elevated mROS. Such responses are likely adaptive in the short term, but sustained activation under chronic stress can become maladaptive, leading to accumulating damage, proteotoxic stress, and eventual cellular dysfunction. Indeed, enrichment of cell death pathways was modest but detectable across multiple cell types. Together with the observation that several neurodegeneration-outcome pathways were enriched but not fully engaged across cell types, these findings are consistent with a model of cellular sensitization rather than established pathology. Transcriptomic profiles alone are insufficient to conclude that pathology was not yet present in this tissue, however, previous studies directly analyzing tissue from space flown mice corroborate our analyses. Various durations of spaceflight have been shown to alter histologically verified brain apoptosis, lipid peroxidation from reactive oxygen stress, and cerebrovascular remodeling^{11,49,50}, with terrestrial models showing similar patterns of changes^{9,13}. As such, tissue neurodegeneration may be within the scope of this duration of spaceflight, though it may not be adequately reflected in transcriptomic data.

By using snRNAseq data, we established that spaceflight-induced alterations in molecular pathways are broadly similar across brain cell types. Correlations in gene expression changes across the transcriptome revealed qualitatively similar patterns between cell types (Pearson's $r = 0.36\text{--}0.86$), although the extent of overlap in the specific genes identified as DEGs varied considerably (12–57%). Together, these findings suggest that spaceflight elicits

coordinated shifts in gene expression across multiple cell types, however, differences in the specific genes involved and the magnitude of changes indicate that the relative vulnerability or resilience of each cell type may differ. This differing vulnerability may confer distinct consequences for overall brain function depending on which cells are most affected. Vulnerability may be driven by specialized functions and energetic requirements of the various cell types. Neurons expend substantial energy to maintain ion gradients essential for action potential generation and propagation, with energy consumption rising in tandem with firing frequency. This high metabolic demand may render neurons particularly susceptible to oxidative damage from GCR-induced ROS^{51,52}. Myelinating OLGs also must sustain significant metabolic and lipid synthesis activity to produce expansive myelin membranes⁵³, while cells that are actively dividing or state-switching (OLG precursor cells, microglia, and astrocytes during state transitions) may be especially sensitive to oxidative and metabolic disturbances. As such, specific subpopulations of each cell type may be particularly vulnerable to spaceflight. The cell types characterized in the present study are aggregates, each comprising numerous subpopulations with potentially divergent metabolic profiles and proliferative behaviors. Therefore, within each cell type, especially susceptible subpopulations may be obscured, limiting the resolution of our current findings. Future studies specifically focused on cell type and subtype vulnerability will be essential to fully clarify cell type vulnerability.

While it is difficult to parse cell vulnerability from the present data, OLGs demonstrated significantly larger fold changes in their strongest DEGs compared to all other cell types (Cohen's $d = 2.35\text{--}6.12$). As the central nervous system's myelinating cells, OLGs undergo large-scale membrane outgrowth to generate lipid-based myelin sheaths that insulate axons to maintain coordinated information processing across the brain. This membrane expansion places a substantial metabolic and lipid synthesis burden on OLGs, potentially increasing sensitivity to mitochondrial dysfunction and redox imbalance⁵⁴. This membrane outgrowth results in a large membrane surface area that may interact with GCR and associated ROS. This is consistent

with observations from brain radiation studies, which have identified OLGs as especially radiosensitive among central nervous system cell types⁵⁵. Impaired OLG health could compromise myelin maintenance and axonal support, which, in turn, could erode functional network synchrony by altering the speed of information flow within distinct, but converging neural networks.

In an *a priori* planned analysis we examined neuroimmune involvement more directly by profiling microglia in depth. Microglia adopt distinct transcriptomic states according to their functional activation, and microglia from spaceflight mice showed clear upregulation of non-homeostatic signatures, indicating early neuroimmune engagement. Particularly notable was enrichment of the disease-associated microglia (DAM) signature. This microglial phenotype was first described in Alzheimer's-model mice⁵⁶, with cells accumulating near amyloid plaques⁵⁷ and activating immune-response and phagocytic gene programs, which appear early in disease and strengthen with disease progression³⁷. Similar states have been described across a number of neurodegenerative diseases^{58–61} suggesting a conserved injury response. Beyond DAM, the inflammatory, MHC-II, and interferon-response (IFN) signatures were all elevated with spaceflight. Excessive or prolonged IFN signaling can promote neurodegeneration through aberrant synaptic phagocytosis and has been implicated in Alzheimer's and Parkinson's diseases^{62,63}, while the MHC-II phenotype reflects antigen presentation during disease states³⁷, including neurodegenerative disease^{64,65}. Given the established role of chronic microglial activation in neurodegeneration, these findings suggest that sustained spaceflight could amplify neuroinflammatory processes in conjunction with mitochondrial dysfunction and may compound vulnerability to neurodegeneration.

These risks are likely driven primarily by a combined effect of microgravity and exposure to radiation and thus will scale with mission duration. Astronauts have remained in microgravity for up to a year aboard the ISS, but these missions occur in low Earth orbit, where Earth's magnetosphere attenuates radiation exposure by half compared to deep-space⁶⁶. Long-duration

missions beyond low Earth orbit, such as a mission to Mars, would impose prolonged microgravity together with markedly higher radiation exposure over periods approaching 500–600 days—far exceeding the ~13-day duration of the longest Apollo excursions beyond low Earth orbit. This combination of extended microgravity and elevated radiation raises particular concern for neurological risk during deep-space exploration⁴³. While there have been few studies directly addressing the effects of GCR at the doses that these mice experienced (~18.65 mGy across the duration of the experiment; <https://visualization.osdr.nasa.gov/eda/>), studies of simulated GCR at doses predicted to occur during these deep flight missions can induce cognitive and emotional deficits in mice and rats^{5,9,14,67}, activate microglia in the brain, induce peripheral inflammation and synaptotoxicity^{5,9}, and inhibit excitatory neuronal communication⁹, all hallmarks of neurodegenerative disease. While radiation exposure is surely a critical driver of these effects, headward fluid shifts caused by microgravity increase hydrostatic pressure and thus, exerts mechanical stress in the brain, resulting in spaceflight-associated neuro-ocular syndrome. Terrestrial -based models of these fluid shifts demonstrate that this can also cause oxidative stress, reduction in hippocampal neurons, and altered neuronal structure^{68,69}, among other effects²⁷. Given these overlapping results, a combination of both microgravity induced fluid shifts and radiation likely contribute to the effects observed in the present study.

Several limitations moderate the strength of the conclusions to be drawn from the present study. First, only female mice were studied. Biological sex contributes to substantial differences in the prevalence and progression of many neurodegenerative diseases⁷⁰, while sex chromosomes and gonadal hormones are strong regulators of microglial function in the aging brain. Consequently, whether males are similarly vulnerable to spaceflight-induced neurodegenerative risk cannot be determined here. Further, females appear to be more resilient to the effects of GCR⁷¹. Therefore, it's possible that the present results show a low threshold effect that may be stronger in males. Second, although the study included both 3- and 7-month-old mice and we detected no age x condition interactions, this should not be interpreted as

evidence that age does not modulate vulnerability. In terrestrially housed mice, transcriptomic aging effects in the brain are often not apparent before ~10 months of age and many are substantially delayed³⁶. Indeed, a laboratory-housed mouse's median lifespan is ~25 months⁷². As such, the "old" cohort here is comparatively young and likely pre-senescent. Third, because spaceflight mice were euthanized in orbit, these data capture the ongoing transcriptional state during flight rather than after return to Earth; whether these changes resolve, persist, or progress upon return to Earth cannot be assessed. Fourth, it is important to acknowledge the limited sample size in the present study because the main effect of spaceflight was determined from only nine mice per condition. The finding that spaceflight may predispose the brain to neurodegeneration is critically important to all future long duration missions should stimulate robust efforts to develop, brain-focused countermeasures. Given the gravity of this issue, it is essential to conduct studies that include more animals and a broader range of conditions to establish the robustness of these results. Fifth, it is important to note that these data combined a number of brain regions, each of which may be differentially vulnerable/resilient to the effects of spaceflight, and thus, future studies with greater spatial acuity are called for. Finally, it is important to note that mice experienced 55–58 days of spaceflight, corresponding to 7–8% of a typical mouse lifespan, which represents ~5.5 years in human terms. However, it is difficult to compare mouse days to human-equivalent days because cross-species time scales are non-linear and process-dependent. Given that adult brains in both species are overwhelmingly composed of cells that are either terminally differentiated or quiescent, and that each species is exposed to similar doses of radiation per unit time, it is unclear how much the difference in lifespan may affect results. These caveats make clear that future studies incorporating varied model organisms, flight durations and post-flight recovery intervals will be needed to fully understand these effects and their reversibility, and analyses that pair transcriptomic with proteomic or functional readouts would help establish how transcriptomic signatures translate into measurable cellular consequences.

4. Conclusion

Collectively, these data demonstrate that even relatively short-term spaceflight induces widespread transcriptional reprogramming in the brain, characterized by mitochondrial stress, activation of adaptive stress response pathways, and early neuroimmune engagement. These changes appear to sensitize neural tissues to neurodegenerative processes that could become pathogenic under prolonged exposure. This concern is particularly relevant for planned Mars missions, which combine long-duration with departure from Earth's protective magnetosphere and the associated rise in radiation dose. Studies integrating behavioral, biochemical, and longitudinal analyses will be required to determine the functional consequences of these transcriptional changes and to define the thresholds at which adaptive responses give way to pathology. Such work will be essential for developing targeted countermeasures to mitigate neurological risk on long-duration missions⁷³.

5. Methods

5.1 Dataset and experimental design

Single-nucleus RNA-sequencing data were obtained from the NASA Open Science Data Repository (OSDR) study OSD-613. Briefly, female C57BL/6Ntac mice aged 12 weeks ("young") or 29 weeks ("old") at experimental onset were assigned to either ground control (GC) or spaceflight (FLT; 55–58 days aboard the International Space Station) conditions, yielding four groups: young-GC (n = 5), young-FLT (n = 4), old-GC (n = 4), and old-FLT (n = 5). Mice were euthanized aboard the International Space Station, immediately frozen, and maintained in a frozen state until brain tissue was collected on Earth. Nuclei were isolated from a single brain hemisphere spanning Allen Brain Atlas slices 60–74, a region encompassing cortex, hippocampus, thalamus, hypothalamus, amygdala, caudate-putamen, and major white matter tracts (corpus callosum, fimbria, and internal capsule).

5.2 Analysis Pipeline

Per-sample expression matrices were aggregated and all analyses were performed in R (v4.4.2) using the Seurat pipeline (v5.3.0). For each sample, nuclei were filtered using adaptive thresholds (mean $\pm 3 \times$ median absolute deviation) applied to mitochondrial gene percentage, number of detected features, and total UMI counts; mitochondrial genes were removed from the expression matrix prior to downstream analysis. Putative doublets were identified using scDbtFinder (v1.20.2), and filtered matrices were inspected for outliers. Following quality control, 88,338 nuclei were retained.

Expression data were normalized and variance-stabilized with SCTransform, then merged and integrated across samples using Harmony (v1.2.4) to correct for batch effects. Dimensionality reduction and clustering were performed following the standard Seurat workflow, and clusters were visualized in two dimensions using Uniform Manifold Approximation and Projection (UMAP). Nuclei were annotated with MapMyCells³⁵ and assigned to seven broad cell classes: astrocytes, microglia, oligodendrocytes, oligodendrocyte precursor cells, vascular/endothelial cells, GABAergic neurons, and glutamatergic neurons. Annotations were validated by module scoring with canonical cell type marker genes (Supplementary Table 1) and manually curated where necessary before subsetting for cell type-specific analyses.

5.3 cell type composition analysis

Differences in cell type composition between FLT and GC conditions were assessed separately within young and old cohorts. The statistical significance of proportional shifts for each cell type was evaluated at a threshold of $p < 0.05$.

5.4 Differential Gene Expression

For each of the seven cell types, counts were aggregated into pseudobulk profiles with each mouse treated as an independent biological replicate, and differential expression was assessed using DESeq2 (v1.46.0). A full model (gene ~ age + condition + age:condition) was first fit to test the main effects of age and flight condition and their interaction (Supplementary Table 2). Because no gene in any cell type showed a significant age x condition interaction, a reduced model (gene ~ age + condition) was used to estimate the main effects of age (Supplementary Table 3) and condition (Supplementary Table 4). Genes were considered differentially expressed (DEGs) if they exhibited a Benjamini-Hochberg-adjusted $p < 0.05$ and an absolute shrunken $\log_2$ fold change ($\log_2 \text{FC}_{\text{shr}}$) > 0.6 . Shrinkage was applied using the *ashr* method⁷⁴ (v2.2.63), which stabilizes effect size estimates for lowly expressed genes and rare populations, providing a more robust and comparable measure of effect size across genes and cell types.

5.5 Cross cell type comparison of transcriptional responses

To compare the magnitude of the spaceflight response across cell types independent of statistical power, we extracted the 50 most significant condition DEGs within each cell type and compared their $|\log_2 \text{FC}_{\text{shr}}|$ values. Effect sizes of DEG expression are reported using Cohen's *d*. To quantify the directional similarity of responses, pairwise Pearson correlations were computed between cell types across $\log_2 \text{FC}_{\text{shr}}$ values for all genes. Overlap in DEG identity between cell types was quantified using the Jaccard index.

5.6 Pathway Analysis

Gene set enrichment analysis (GSEA) was performed against both the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) databases, with genes ranked by their DESeq2 test statistic for the main effect of condition. Normalized enrichment scores (NES) were reported as measures of effect size. A panel of KEGG pathways relevant to

human neurodegenerative disease was selected a priori for focused analysis: Alzheimer's disease (mmu05010), Parkinson's disease (mmu05012), amyotrophic lateral sclerosis (mmu05014), Huntington's disease (mmu05016), spinocerebellar ataxia (mmu05017), prion disease (mmu05020), and "pathways of neurodegeneration—multiple diseases" (mmu05022). A complementary curated set of neurodegeneration-related GO terms (spanning neurodegeneration, proteostasis, reactive oxygen and nitrosative stress, antioxidant pathways, oxidative phosphorylation (OXPHOS), cell death, endoplasmic reticulum stress, autophagy, neuroplasticity, synaptic vesicle function, and neuroinflammation) was likewise selected a priori. For these a priori collections, p-values were adjusted for multiple comparisons within the selected pathway set. Because leading-edge analysis revealed that the neurodegenerative KEGG enrichments were predominantly driven by shared OXPHOS genes, the KEGG GSEA was repeated after removing OXPHOS-annotated genes to confirm that enrichment patterns were not solely attributable to OXPHOS overlap (Supplementary Fig. 2). Additional post hoc GO enrichment analyses were performed across all GO terms, with p-value correction by the Benjamini-Hochberg method. Full KEGG and GO enrichment results for each cell type are provided in Supplementary Tables 5 and 6.

5.7 Microglial state analysis

To interrogate microglial functional states, we assembled curated gene sets representing established microglial subtypes: Homeostatic, Disease-associated, Inflammatory, MHC-II, and Interferon-response microglia. Marker genes were derived from a previous paper that compiled significant markers from three foundational studies³⁷. Only genes significant in at least two of the three publications were included in each marker set.

Each microglial subtype gene set was tested for enrichment in the pseudobulk differential expression results for microglia, with genes ranked by their differential expression

test statistic for the main effect of condition. GSEA was performed for each of the five gene sets across the entire population of microglia.

To further characterize activation states, mean scaled expression of module genes was calculated per sample, and comparisons of module scores across conditions were tested using two-way ANOVA (age x condition).

Microglia were then subclustered following the standard Seurat workflow, yielding seven clusters. Differences in the proportions of FLT- versus GC-derived nuclei within each cluster were tested with chi-square tests, with Benjamini-Hochberg correction across clusters (Supplementary Table 9). Subtype module scores were summarized per cluster (z-scored) to relate clusters to functional states. Finally, the three largest clusters were functionally annotated by GO enrichment analysis.

6. Disclaimer

The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs, the National Aeronautics and Space Administration, or the United States Government.

7. Data Availability

The snRNA-sequencing data are available in the NASA Open Science Data Repository under accession OSD-613 (<https://doi.org/10.26030/cevt-ja62>).

8. Acknowledgments

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9. Author Contributions

David Piekarski: Conceptualization, Methodology, Formal Analysis, Writing — original draft

Joseph Guarnieri: Writing – original draft, Methodology **Shehbeel Arif:** Writing – original draft

Elizabeth Aslinger: Methodology **M Windy McNerney:** Conceptualization, Methodology, Formal Analysis, Writing — original draft

10. Figure Legends

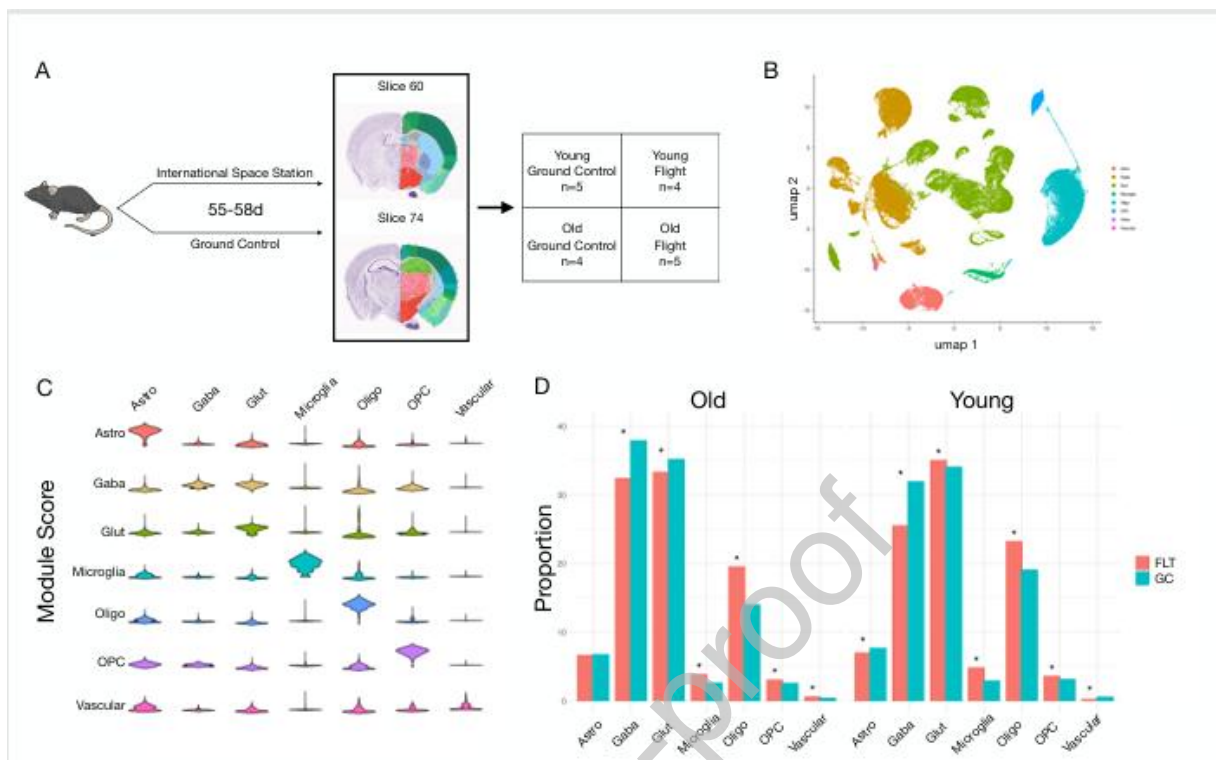


Fig. 1: Single-nucleus transcriptomic profiling of the mouse brain following spaceflight.

(A) Schematic of experimental design. Female C57BL/6Ntac mice, young (12 weeks) or old (29 weeks) at onset, were housed aboard the International Space Station (FLT; 55–58 days) or maintained as terrestrial ground controls (GC), yielding four groups; young-GC (n = 5), young-FLT (n = 4), old-GC (n = 4), and old-FLT (n = 5). Nuclei were isolated from a single brain hemisphere spanning Allen Brain Atlas slices 60–74. (B) UMAP projection of 88,338 nuclei, colored by seven annotated cell classes (Astro, astrocytes; Gaba, GABAergic neurons; Glut, glutamatergic neurons; Microglia; Olig, oligodendrocytes; OPC, oligodendrocyte precursor cells; Vascular, vascular/endothelial cells). (C) Module Scores for canonical marker gene sets (rows) across cell types (columns), confirming annotation specificity. (D) Relative proportion of each cell type by flight condition, shown separately for old and young cohorts. Asterisks denote significant compositional differences between FLT and GC (*p<0.05).

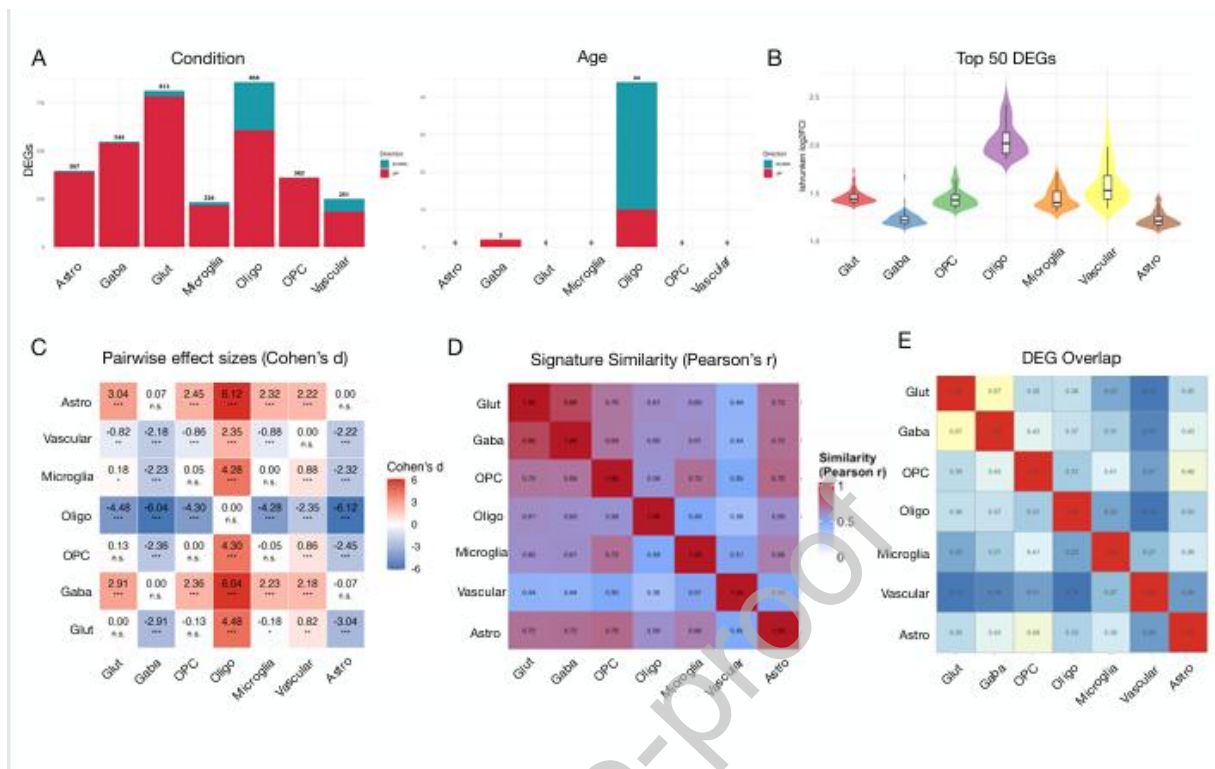


Fig. 2: The transcriptional response to spaceflight varies across brain cell types. (A) Number of differentially expressed genes (DEGs; Benjamini-Hochberg adjusted $p < 0.05$ and $|\log_2 \text{FC}_{\text{shr}}| > 0.6$) per cell type for the main effect of condition (left) and the main effect of age (right), colored by direction of regulation (up, red; down, teal). (B) Distribution of absolute shrunken $\log_2$ fold changes ($|\log_2 \text{FC}_{\text{shr}}|$) for the top 50 condition DEGs within each cell type; box plots show the median and interquartile range with whiskers extending to $1.5 \times \text{IQR}$, overlaid on the full density. (C) Pairwise effect sizes (Cohen's d) for differences in $|\log_2 \text{FC}_{\text{shr}}|$ of the top 50 DEGs between cell types; positive values (red) indicate larger fold changes in the row cell type. (D) Signature similarity between cell types, quantified as Pearson's r across $\log_2 \text{FC}_{\text{shr}}$ values for all genes. (E) Pairwise overlap in DEG identity between cell types, quantified by Jaccard index. * $p < 0.05$.

A

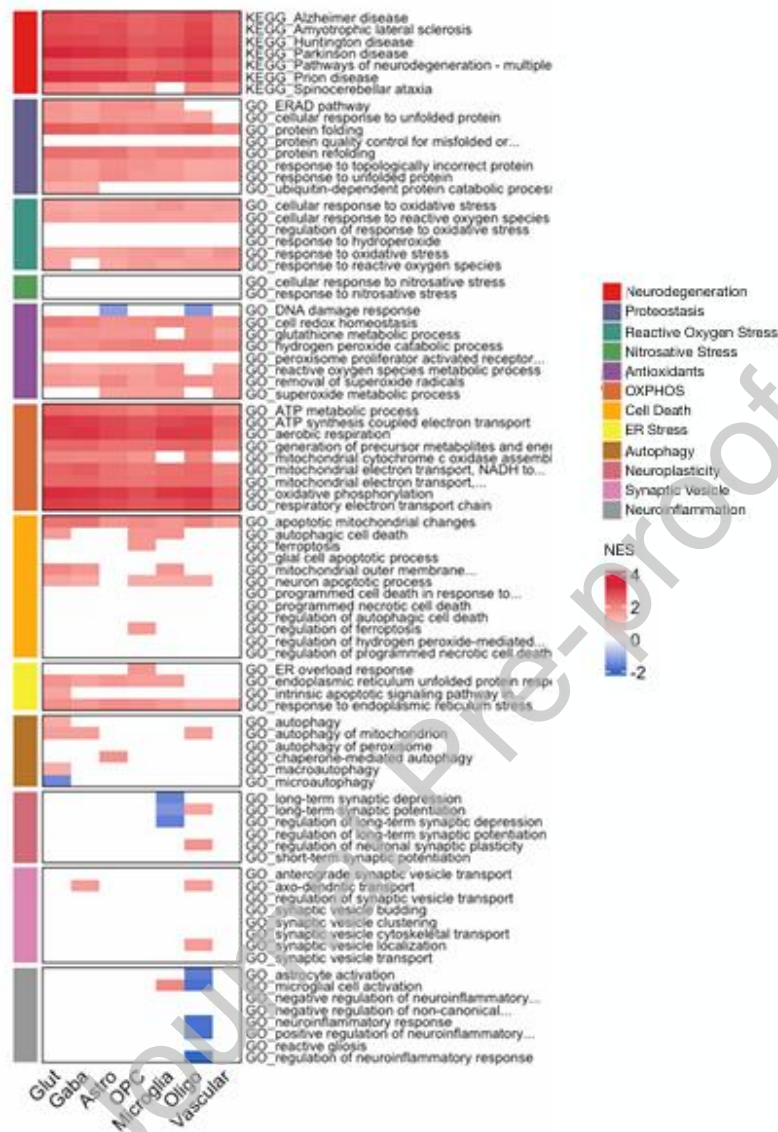


Fig. 3: Spaceflight upregulates neurodegeneration-associated transcriptional pathways across cell types. Gene set enrichment analysis (GSEA) for a priori selected KEGG and Gene Ontology (GO) pathways (rows), grouped by functional category (color bar, left), across the seven cell types (columns). Cell color denotes the normalized enrichment score (NES; red,

positive enrichment; blue, negative enrichment); only enrichments significant at adjusted $p < 0.05$ are shown.

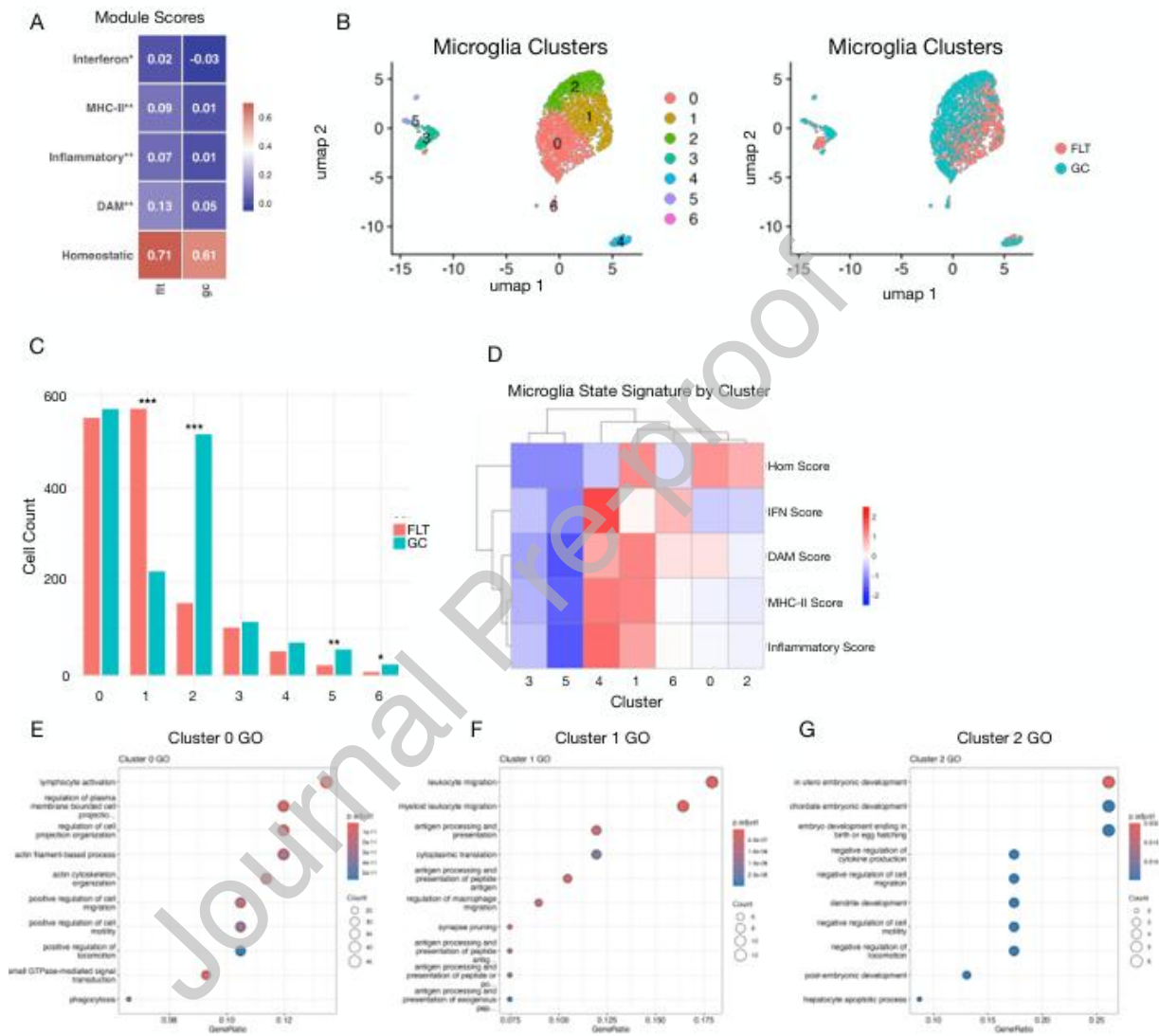


Fig. 4: Spaceflight reprograms microglia toward reactive, disease associated states. (A) Module scores for five microglial subtype signatures (DAM, disease-associated microglia; MHC-II; inflammatory; interferon-response; homeostatic) compared between FLT and GC microglia. Significance was assessed by two-way ANOVA (age $\times$ condition); asterisks denote a significant

main effect of condition (* $p < 0.05$, ** $p < 0.01$). (B) UMAP projections of subclustered microglia colored by cluster (left) and by flight condition (right). (C) Number of nuclei per cluster split by flight condition (FLT, red; GC, teal). Significance of compositional differences was assessed by chi-square test with Benjamini-Hochberg correction (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). (D) Mean subtype module scores (z-scored) per cluster, with hierarchical clustering of rows and columns. (E-G) GO enrichment results for the three largest clusters, cluster 0 (E), cluster 1 (F), and Cluster 2 (G) with dot size denoting gene count and color denoting adjusted p-value.

Supplementary figure legends

Supplementary Figure 1: Samples cluster by flight condition rather than age. Principal component analysis of pseudobulk expression profiles for each cell type. Individual samples are colored by flight condition (GC, FLT) and labeled by age (young, old); the proportion of variance explained is indicated on each axis. Samples segregate primarily along flight condition rather than age.

Supplementary figure 2: Pairwise within group variability does not differ across cell types. Normalized mean expression of significant genes from the main effect of condition show homogenous variability across pairwise groups, within all cell types (Levene test, $p > 0.05$).

Supplementary figure 3: Neurodegeneration pathway enrichment is preserved after removal of OXPHOS genes. GSEA of the a priori neurodegeneration-related KEGG pathways (rows) across cell types (columns), repeated after excluding oxidative phosphorylation (OXPHOS)-annotated genes. Dot size denotes $-\log_{10}$ (FDR) and color denotes NES; the overall pattern of enrichment is unchanged relative to the full analysis (Fig. 3).

Supplementary figure 4: Mean module scores per animal by condition. Boxplots of module scores across conditions, with age highlighted by point color, for each of the 5 microglia subtypes. Means and statistical significance is reported in Fig. 4A.

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Conflict of Interest

No Conflicts of Interest are acknowledged