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Article

Mice Exposed to Combined Chronic Low-Dose Irradiation and Modeled Microgravity Develop Long-Term Neurological Sequelae

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Presenters

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Background

The central nervous system (CNS) is vulnerable to irradiation and fluid shifts experienced during short- and long-term spaceflight (Kovalchuk, A wt al., 2017 and Lawley, J.S. et al., 2017)

Astronauts in spaceflight experience impairments in neurocognition and neurobehavior (De la Torre, G.G., 2014).

NASA Twins study post-flight cognitive decline persisted up to 6 months post-flight (Garrett-Bakelman, F.E. et al., 2019)

Rodent Research (RR)-1 mission ISS (33-days) monitored behavior → 16-week old at launch female mice unique hyperactivity, implying neurological alterations in flight (Ronca, A.E. et al., 2019)

Readaptation to chronic LDR and hindlimb unloading (HLU) resulted in increased aquaporin-4 (AQP4) protein expression, oxidative stress damage, apoptosis, blood–brain barrier (BBB) compromise and an increase in neurobehavioral risk-taking behavior (Bellone, J.A. et al., 2016; Mao, X.W. et al., 2016; and Mao, X.W. et al., 2017)

Aims and Hypothesis

NASA goal is to identify the effects of spaceflight-like conditions on the CNS to better prepare astronauts during long-duration missions to the Moon and Mars.

Aims

1. **Determine the pan-transcriptome responses to chronic LDR exposure, as encountered on space missions (<0.04 Gy/y) on neurohealth singly or in combination with HLU**

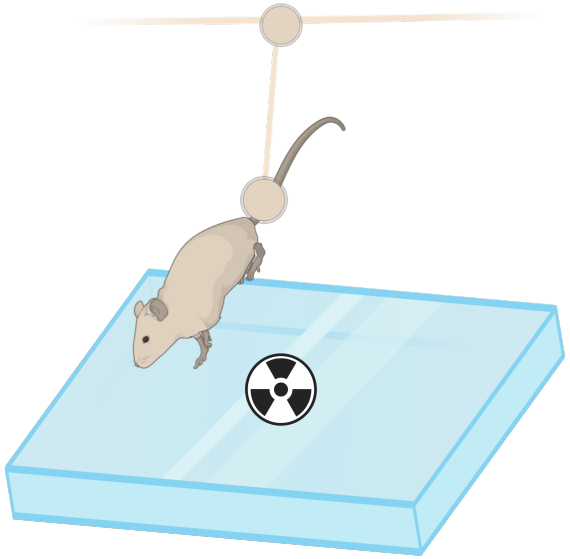
2. Determine the effects at a long-term readaptive timepoint

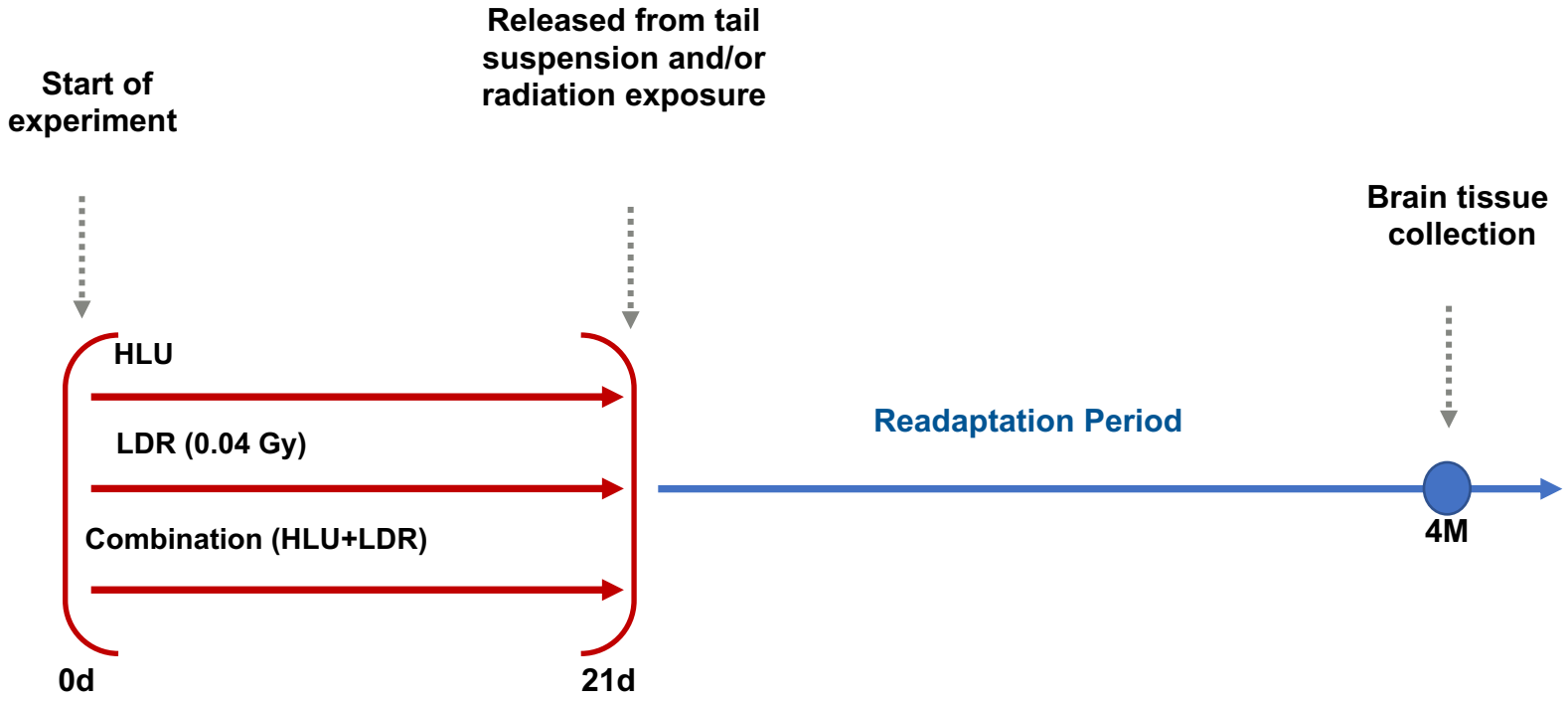
To address these aims → mice were exposed to chronic LDR γ-ray irradiation (0.04 Gy), HLU, or a combination of HLU+LDR for 21 days, followed by a 4-month readaptation period.

Hypothesis

Combined spaceflight factors (simulated microgravity+ irradiation) would result in an elevated expression level of neuroinflammation and neurological damage during re-adaptation.

In collaboration with Dr. Vivien Mao' group, mice were exposed to modeled microgravity (hindlimb unloading) and low-dose radiation (cobalt plates) for 21 days.





Experimental Design

Methods

Simulated microgravity
Age at onset: 6-month old Female *C57BL6/J* mice, Sample size: Control (n=3); HU (n=6); LDR (n=6); Combination HU / LDR (n=4)
Single-housed, hindlimb unloaded for 21 days followed by group housing (n=3/cage) during readaptation

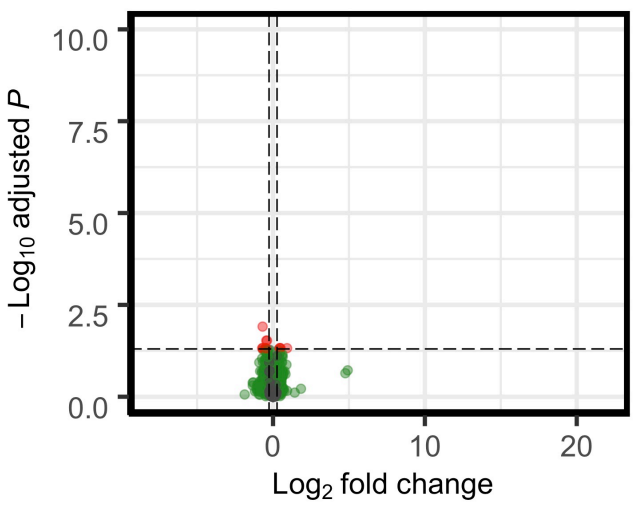
Low-Dose Radiation
Cobalt plates that release low-dose/low-dose rate gamma irradiation (total dose 0.04Gy, rate 0.01cGy/h for 21 days).

RNA-sequencing and GeneLab computational pipeline
Gene expression (transcriptome) of right caudal half hemisphere (housed the hippocampus, basal ganglia, substantia nigra, thalamus and major signaling hub for neuroendocrine system (hypothalamus/pituitary) brain tissue → area used to assess neurogenesis and hypothalamic-pituitary-axis responses, without contamination from cerebral cortex of cerebellum.

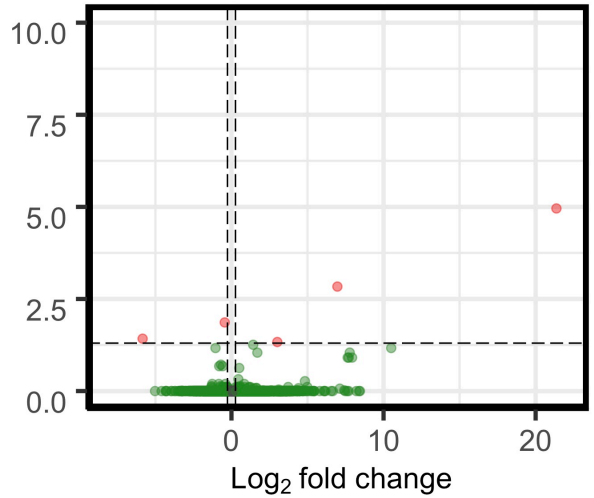
Higher-Order Analyses
Computational tools WebGestalt for gene ontology analysis and Reactome for pathway analysis

Differential Gene Expression Occurs Primarily with Combined Spaceflight Factors

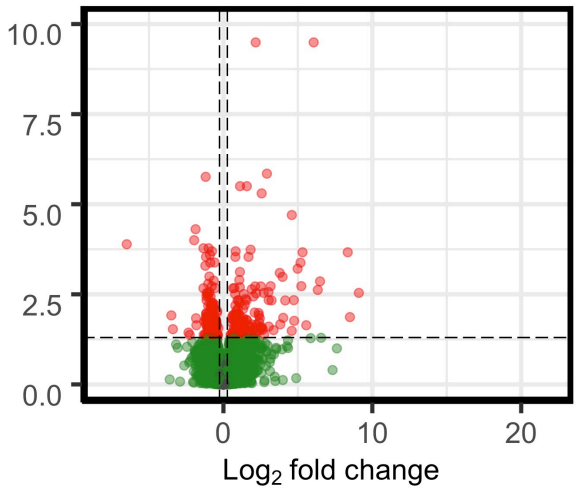
Hindlimb Unloading-Only



Radiation-Only

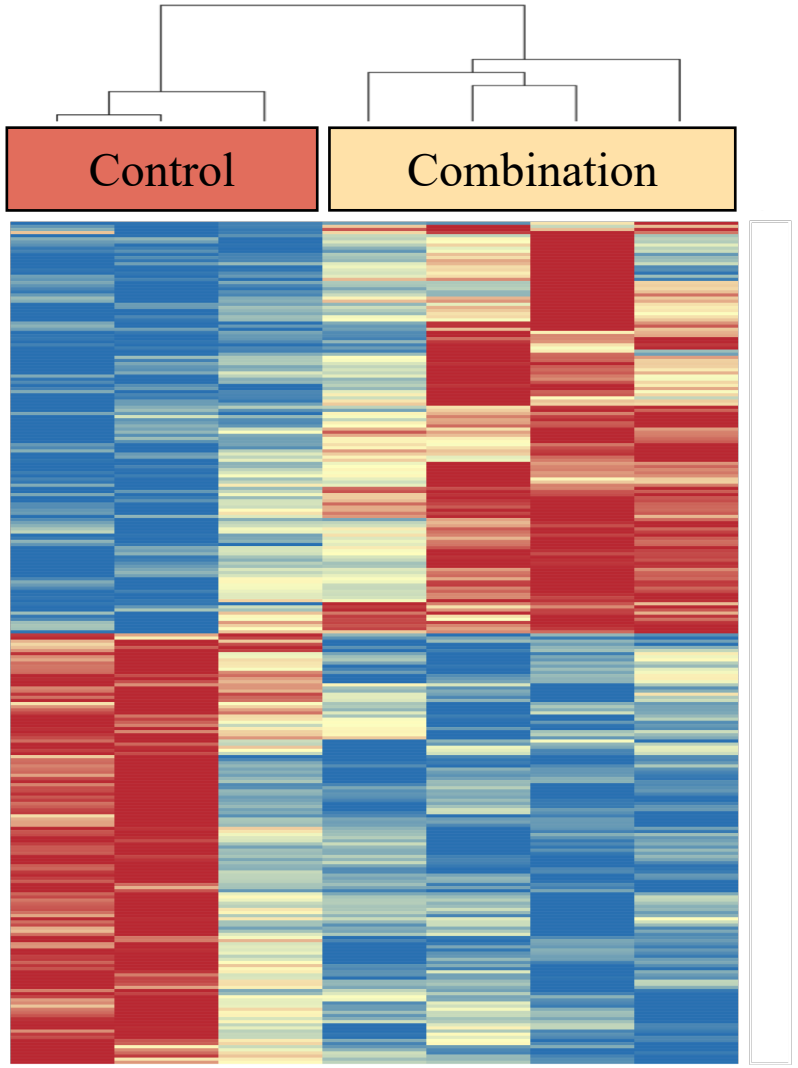


Combination



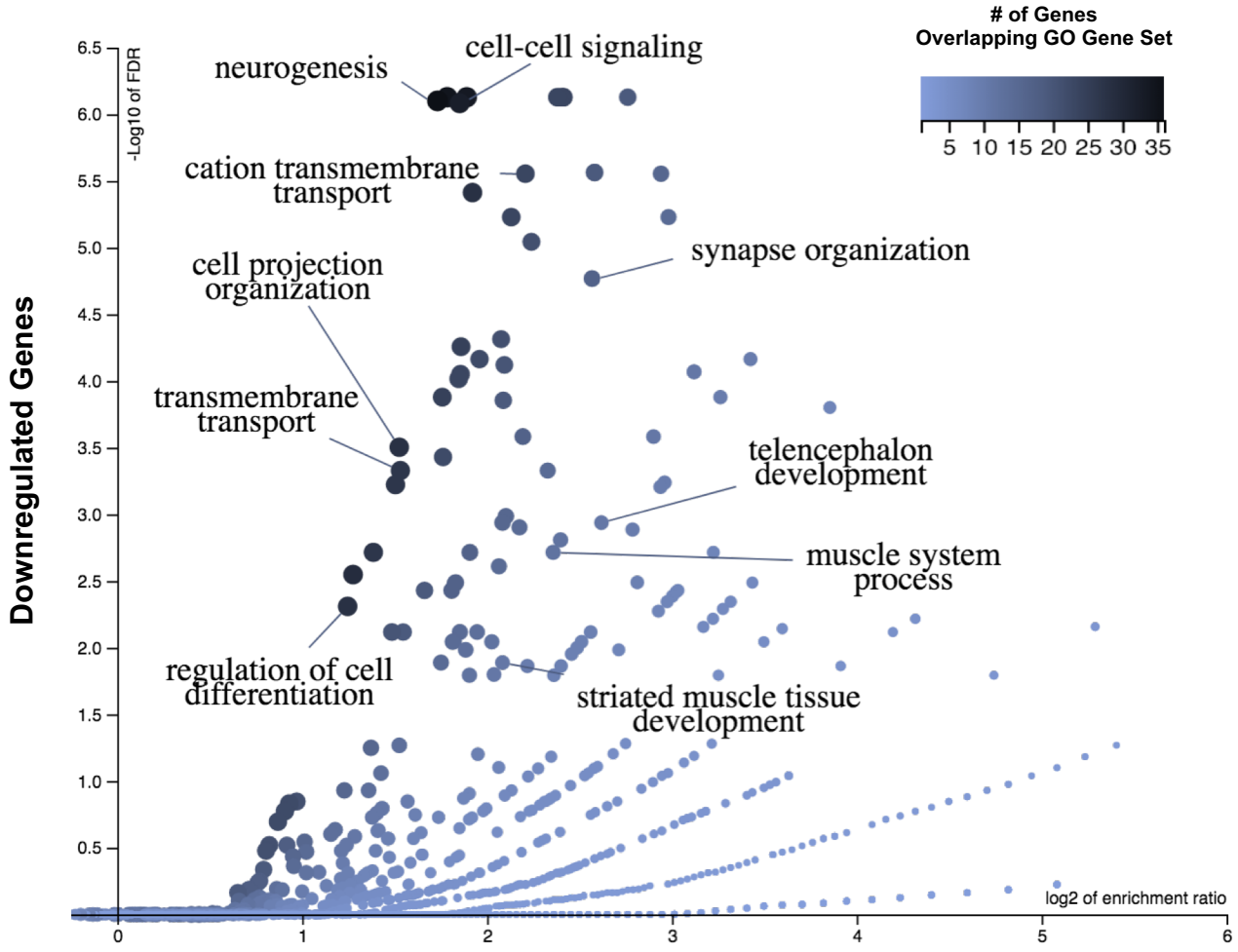
Each plotted point is a gene. Differentially expressed genes are in **red**, which means it has met the adjusted p-value and Log2 fold-change threshold for significance ($p<=0.05$, $|log2FC| > 0.263$). If it has only met the adjusted p-value threshold, it is in **green**.

Differentially Expressed Genes Profile for Combination Group

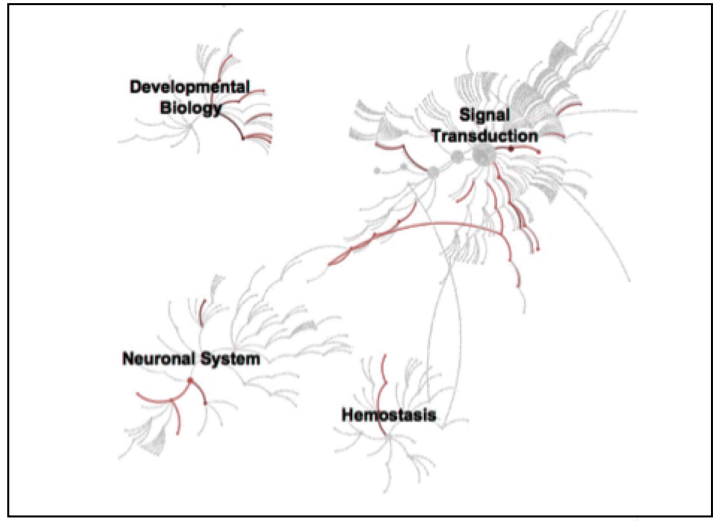


Gene Ontology (GO) and Pathway Analyses

Overrepresented GO categories



Mouse Pathways



Human Orthologs

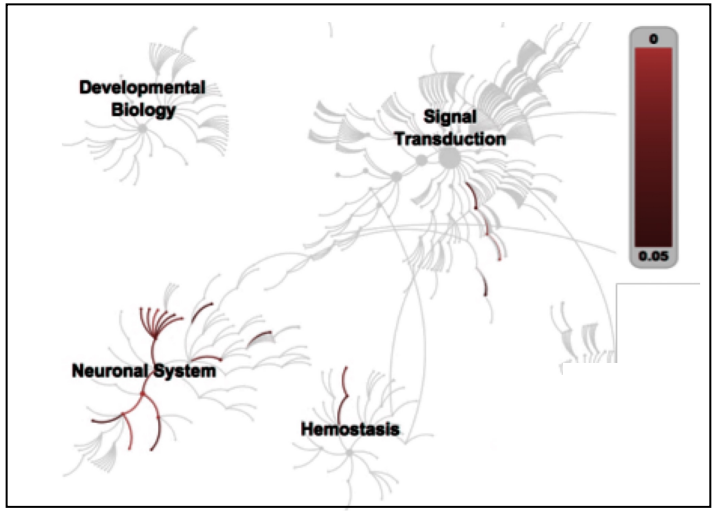


Table 3. Combination of LDR and HLU versus controls DEG and Reactome pathway IDs in mice that translated to human orthologs.

Pathway Name	Associated Mouse Genes	Mouse Pathway ID	Human Pathway ID
Neuronal System	*Gria3, Cacnb3, Hcn1, Camkk2, Kcnh1, Kcnma1, Kcnn1, Stx1a, Kcnv1, Kcnh5, Grin2a, Slc17a7, Dlgap1, Homer1, Snap25, Nrgn, Kcnh7, Slitrk1*	R-MMU-112316	R-HSA-112316
Potassium Channels	*Kcnn1, Kcnh5, Kcnma1, Hcn1, Kcnh1, Kcnv1, Kcnh7*	R-MMU-1296071	R-HSA-1296071
Voltage Gated Potassium Channels	*Kcnv1, Kcnh1, Kcnh5, Kcnh7*	R-MMU-1296072	R-HSA-1296072
cGMP effects	*Pde1a*	R-MMU-418457	R-HSA-418457
p75 NTR receptor-mediated signaling	*Rtn4r, Obscn, Lingo1, Kalrn*	R-MMU-193704	R-HSA-193704
Nitric oxide stimulates guanylate cyclase	*Pde1a*	R-MMU-392154	R-HSA-392154

Summary

- A **combination** of spaceflight-relevant factors **(fluid-shift** and **radiation**) created a different **gene expression profile** than either factor individually.
  - Some gene pathways including →reduced transcriptional machinery, increased neurogenesis and neuropeptide production, and dysregulated cell structure and cell signaling

- **Gene expression** differences can persist for at least **4 months** after a **21-day exposure** to a combination of fluid-shift and radiation in the **brain tissue** of mice.

- Brain-related transcriptional changes are **dynamic** during readaptation phase from exposure to spaceflight-like conditions, which may lead to **long-term neurological consequences**.

Acknowledgments

GeneLab Animal Working Group

GLDS-211 Members: Eliah G. Overbey, Amber M. Paul, Willian A. da Silveira, Candice G.T. Tahimic, Sigrid S. Reinsch, Nathaniel Szewczyk, Seta Stanbouly, Charles Wang, Jonathan M. Galazka, and Xiao Wen Mao

Technical contribution: Nina Nishiyama, Mary Campbell-Beachler and Peter Gifford