Investigating neuro-consequences of spaceflight using *Drosophila melanogaster*

Janani Iyer

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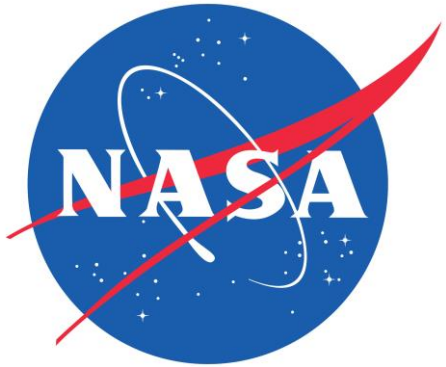
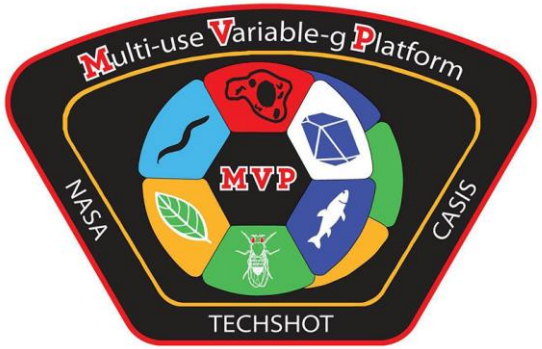
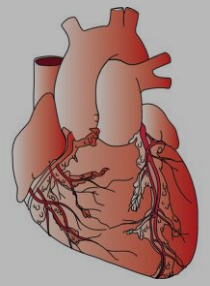


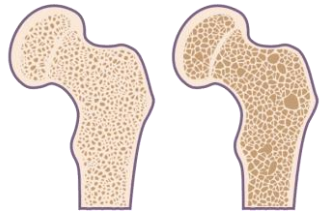


Physiological response to spaceflight

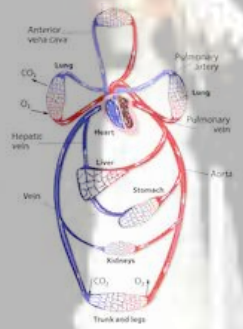


Cardiovascular deconditioning

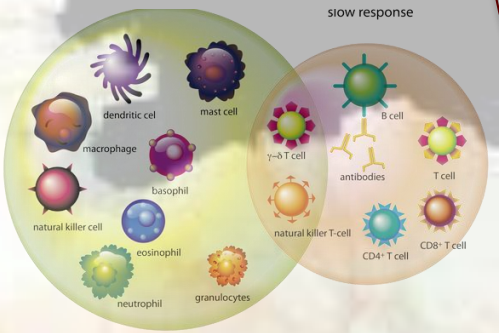
Muscle atrophy



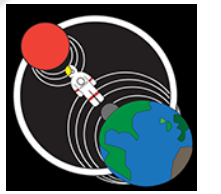
Bone loss



Fluidic shift



Maladaptive immune system



*Micrograv*ity
(Lack of gravity)

Radiation

Social Isolation

Central Nervous system

Genetic changes - Telomeres

Drosophila melanogaster (fruit fly) – the model organism

- ~75% of human disease-causing genes have a functional homolog in the fruit fly
  - Model for human innate immunity, circadian rhythms, development, neurobehavior etc.

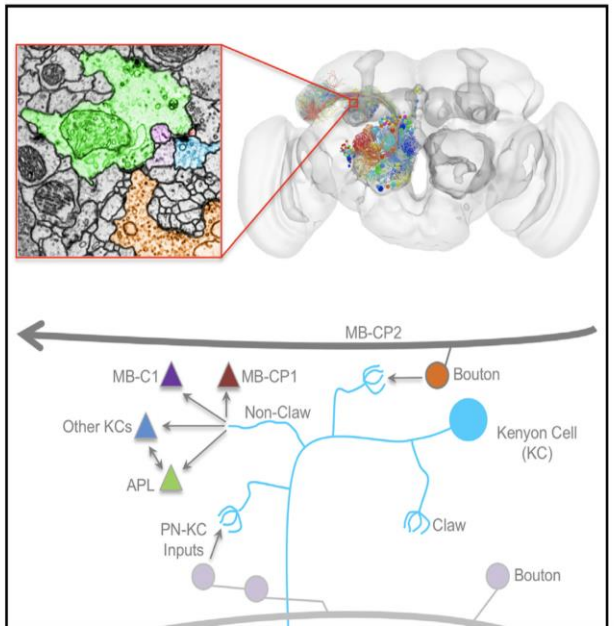
- Amenable to experimental manipulation
  - Readily available genetic tools and fly lines
  - Well-annotated genome
  - Fast generation time
  - Low gene redundancy

- Small, convenient for ISS experimentation (requires little power, mass, volume) to get statistically significant sample sizes

Cell 2018

A Complete Electron Microscopy Volume of the Brain of Adult *Drosophila melanogaster*

Graphical Abstract



Authors

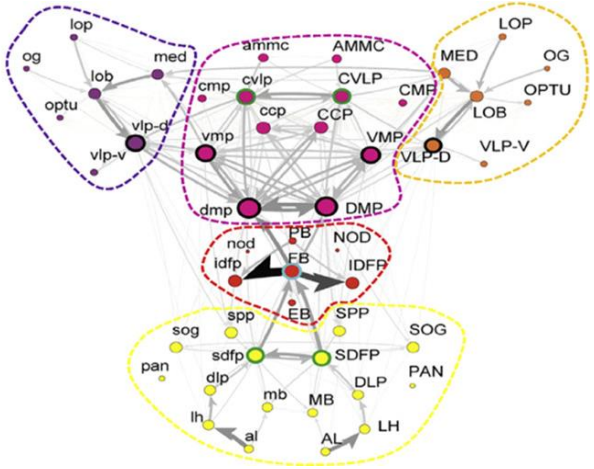
Zhihao Zheng, J. Scott Lauritzen, Eric Perlman, ..., Stephan Saalfeld, Richard D. Fetter, Davi D. Bock

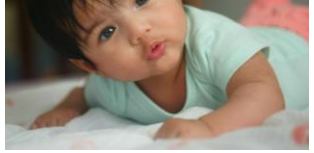
In Brief:
Electron microscopy imaging of the entire adult fruit fly brain at synapse resolution reveals circuitry spanning multiple regions and connectivity between known and previously unknown cell types.

Neural connectivity is strikingly similar across species

Drosophila melanogaster

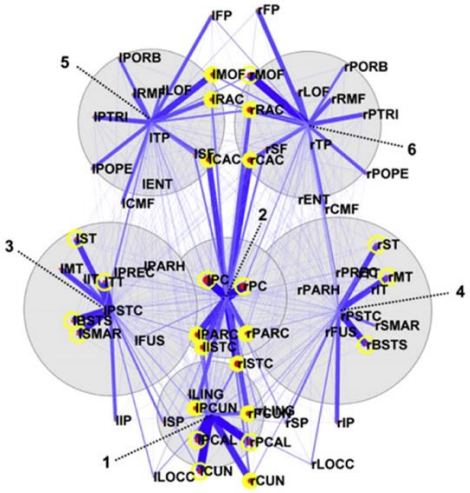
135000 neurons





Homo sapiens

100 billion neurons



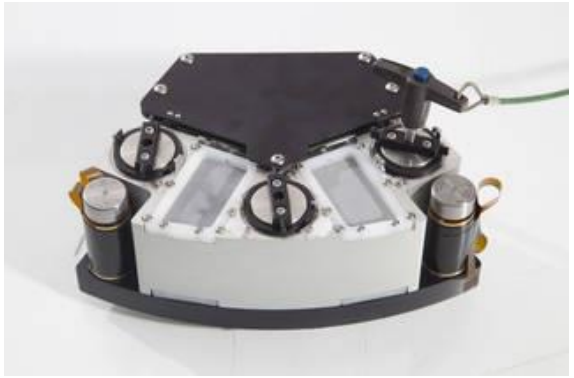
Shi et al., 2017; Kaiser M 2015; Heuvel et al., 2016

Multi-use Variable-g Platform (MVP) Validation

techshot

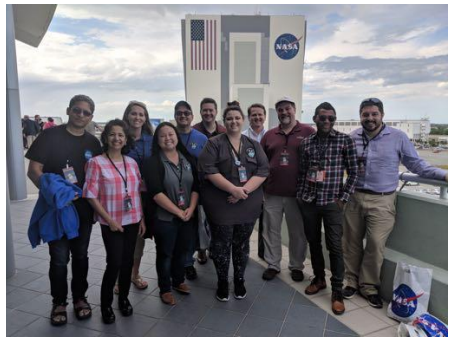
MVP-Fly-01 experiment on SpaceX CRS-14:
Launched on: 4/2/18
Returned on: 5/5/18 (33 days)
Ground control dates: 5/27/18 – 6/29/18

- Optimized for life/physical science research in microgravity
- 2 independent centrifuges (each can spin up to 2g)
- Controls temperature, relative humidity
- Cycles fresh cabin air into habitat
- Telemetry/real-time video & other data; ground commanding
- Reusable/reconfigurable MVP facility
- Up to 12 simultaneous experiment modules

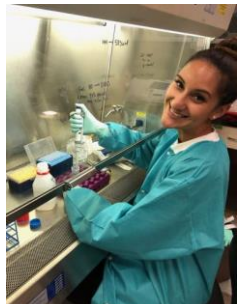


Credits: Techshot Inc.







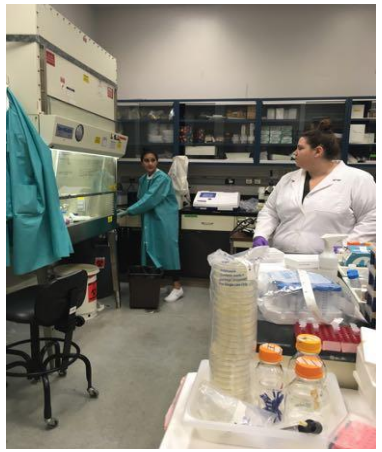
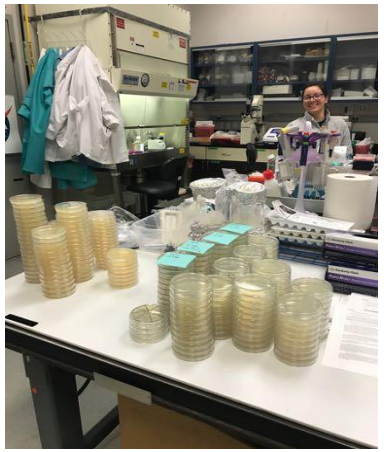


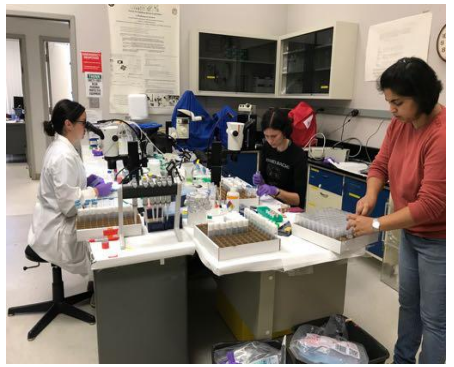

LARGE SAMPLE NUMBERS RETRIEVED POST-FLIGHT

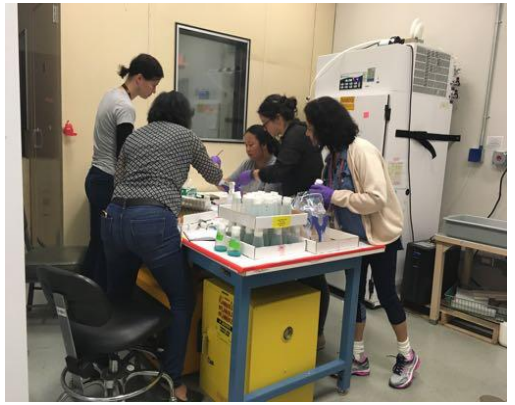
- 1,160 total Micro-g adult flies: 506 males, 654 females
- 1,076 total Space 1g adult flies: 517 males, 559 females

(+ thousands more larvae and eggs in each case)

- 883 total asynchronous ground flies: 362 males, 521 females

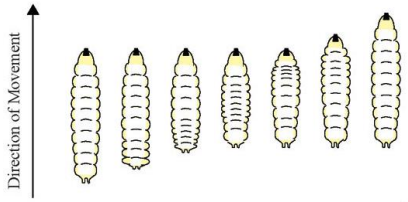





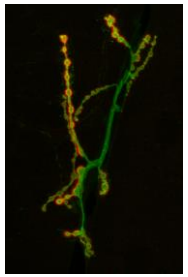



Measuring neurobehavioral health of the flies

A) Behavior: Contraction and crawling

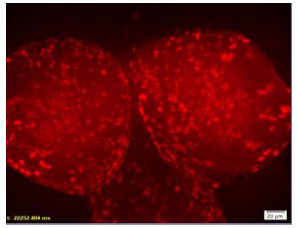


B) Synaptic architecture using NMJ



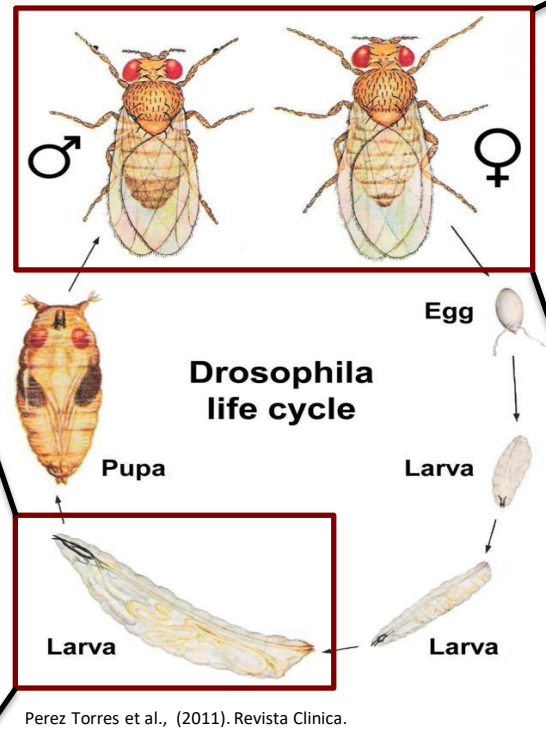
Glutamatergic synapses

C) Brain morphology



- Neuron
- Glia
- DA neurons
- Apoptosis
- Proliferation

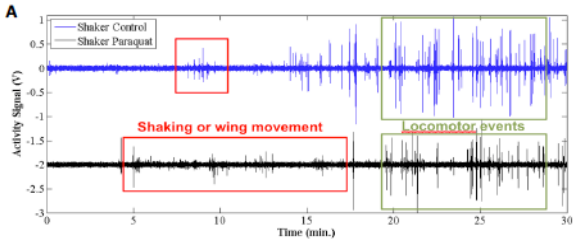
D) Genomic and proteomic analysis



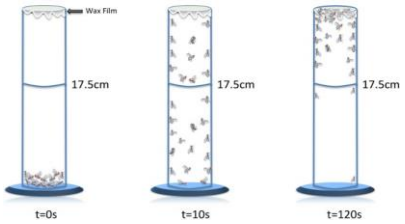
Perez Torres et al., (2011). Revista Clinica.

- Multi-generation studies

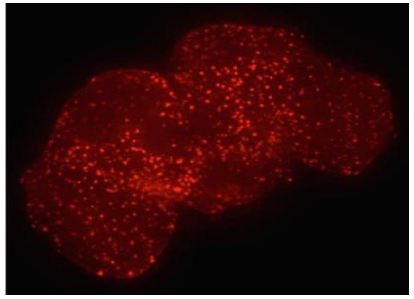
A) Behavior: In-flight locomotion assay



B) Behavior: Climbing ability



C) Brain morphology

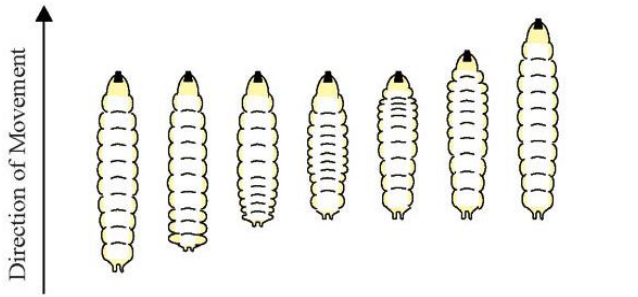


- Neuron
- Glia
- DA neurons
- Apoptosis
- Oxidative stress markers

D) Genomics and Proteomics analysis

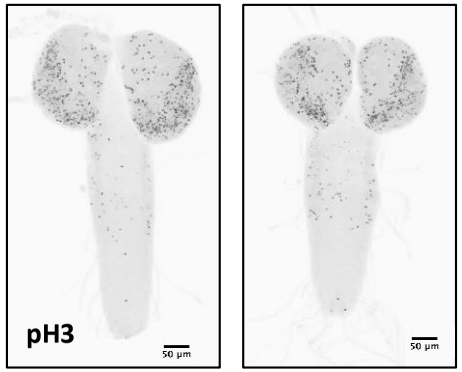
Janani Iyer,Siddhita Mhatre, Amber Paul and Jhony Zavaleta

Behavioral and brain morphology defects in spaceflight larvae suggest neurological impairments



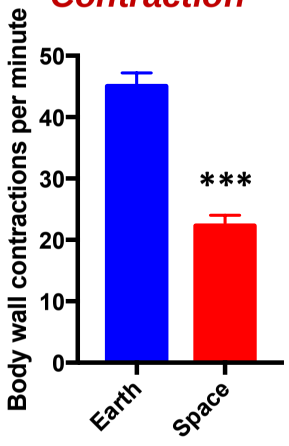
Song et al., 2007

Larval brain

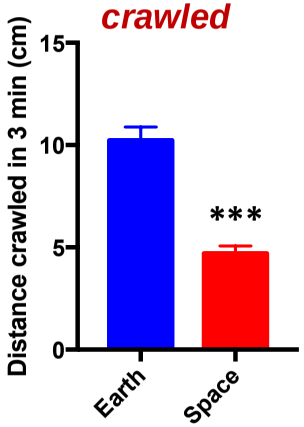


Ground 1g Space µg

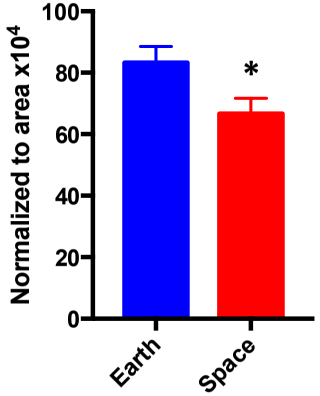
A ***Contraction***



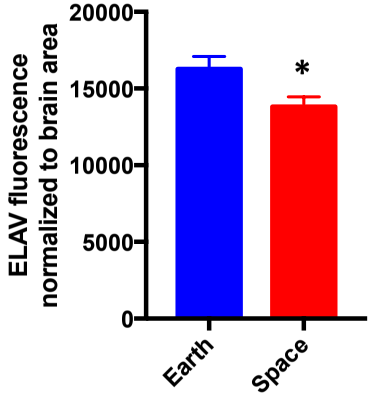
B ***Distance crawled***



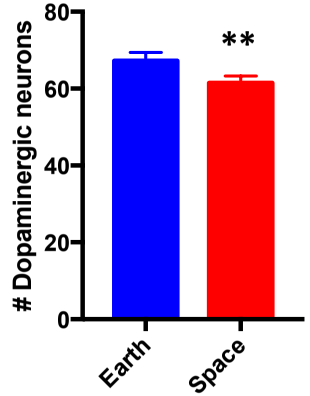
C ***Proliferation marker***



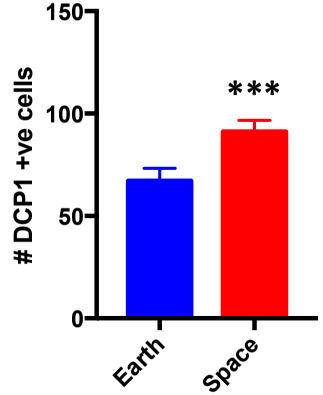
D ***Neuronal marker***



E ***DA neuron marker***



F ***Apoptotic marker***



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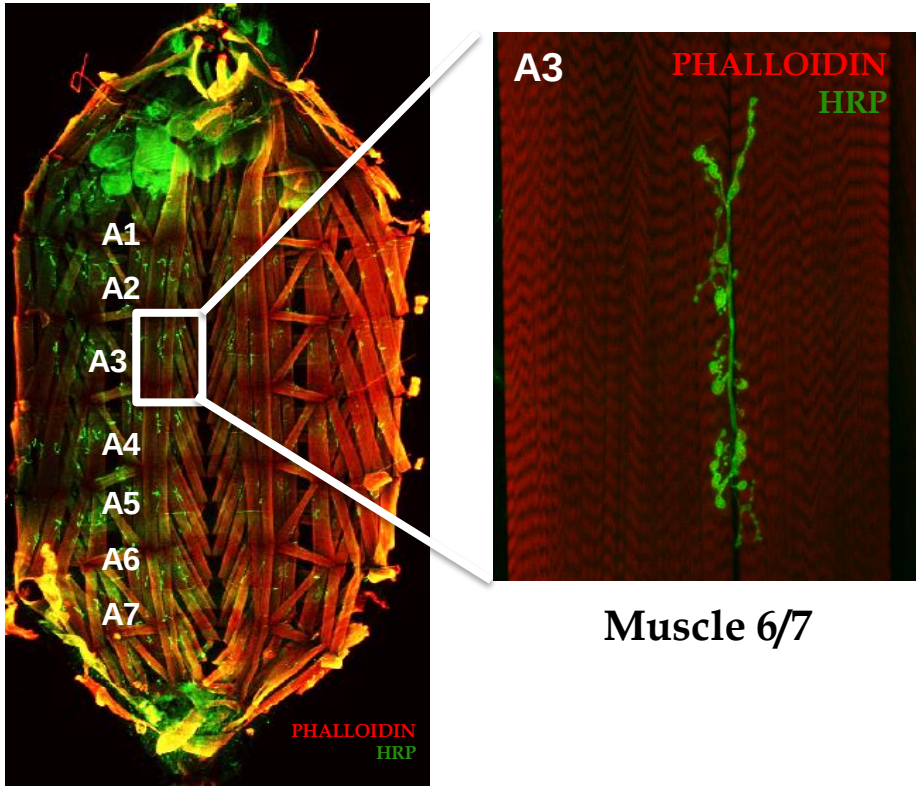
Synaptic abnormalities in spaceflight larvae



Muscle 6/7

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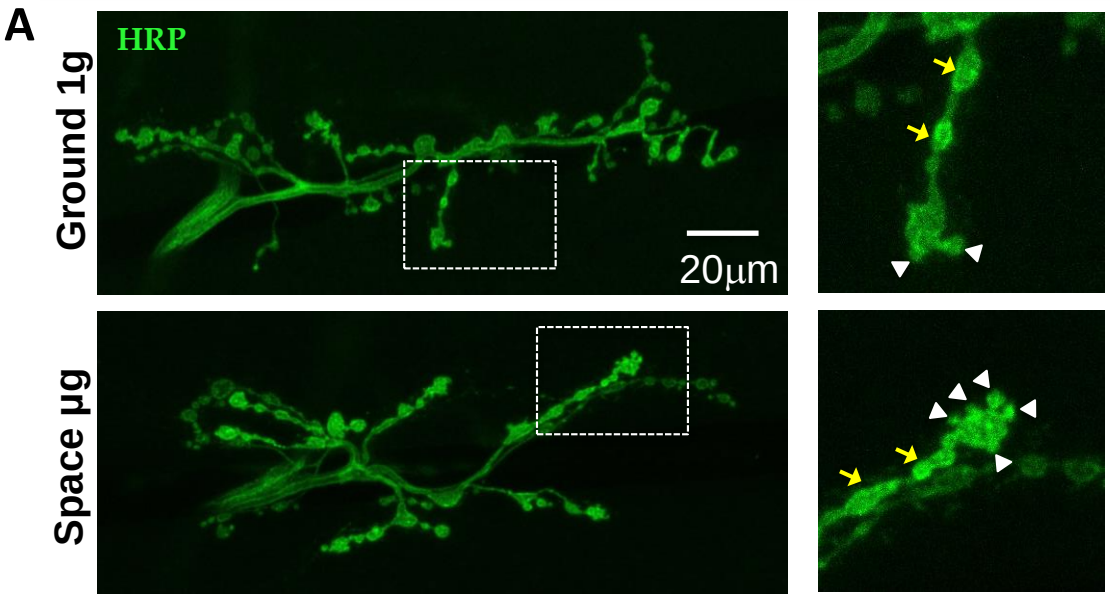
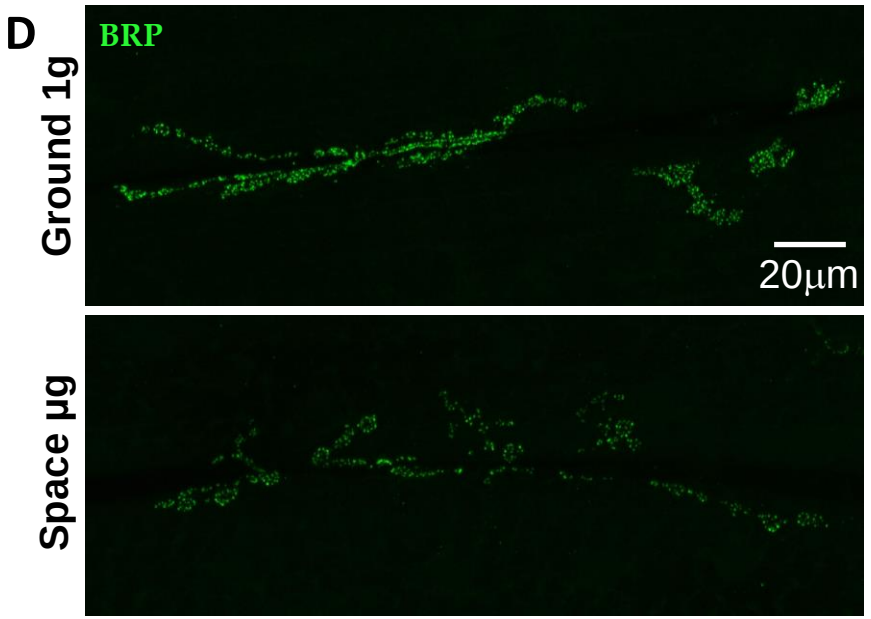
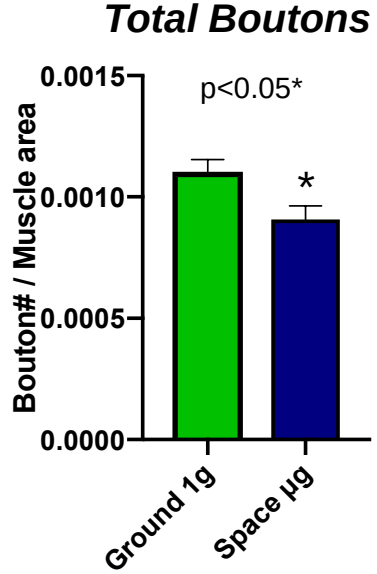
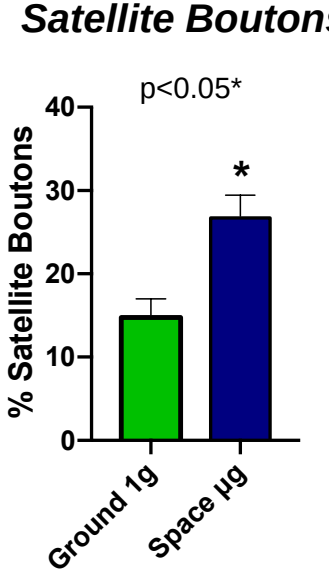
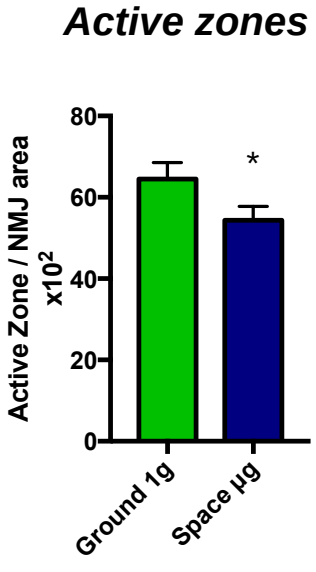
Synaptic abnormalities in spaceflight larvae

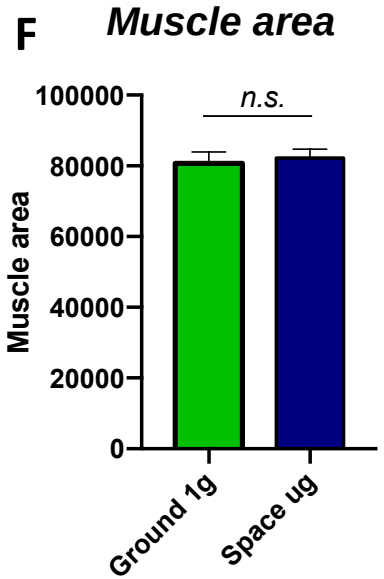










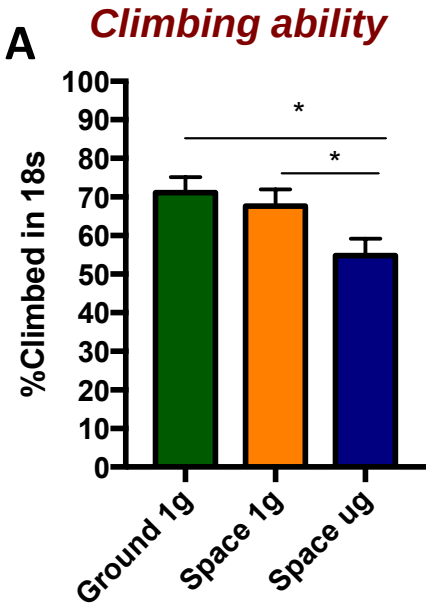


Decreased total number of boutons and active zones while increased number of satellite boutons in spaceflight larvae suggests decreased synaptic transmission

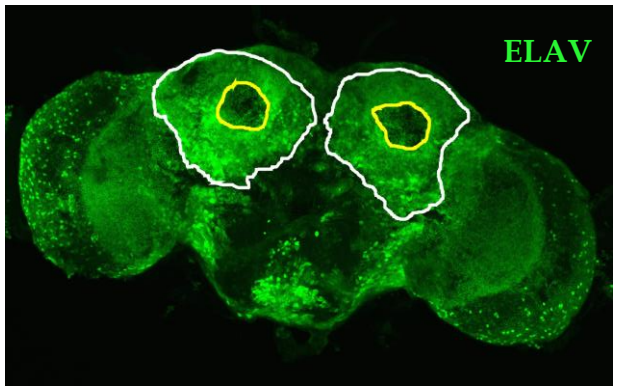
Janani Iyer, Siddhita Mhatre

Neuronal loss in spaceflight condition: Adult flies

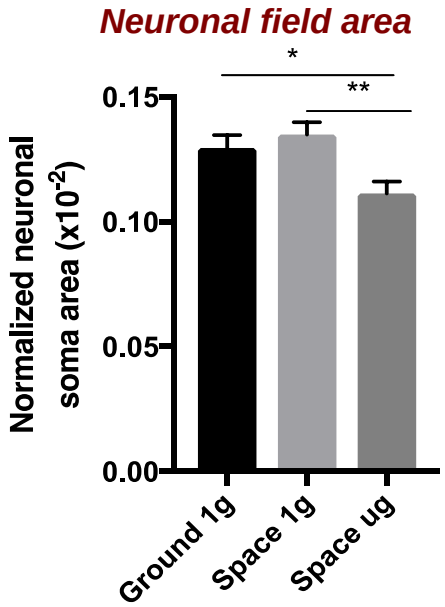
A



B



C



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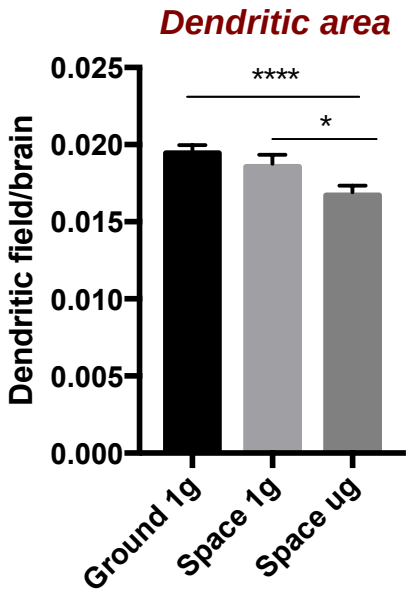
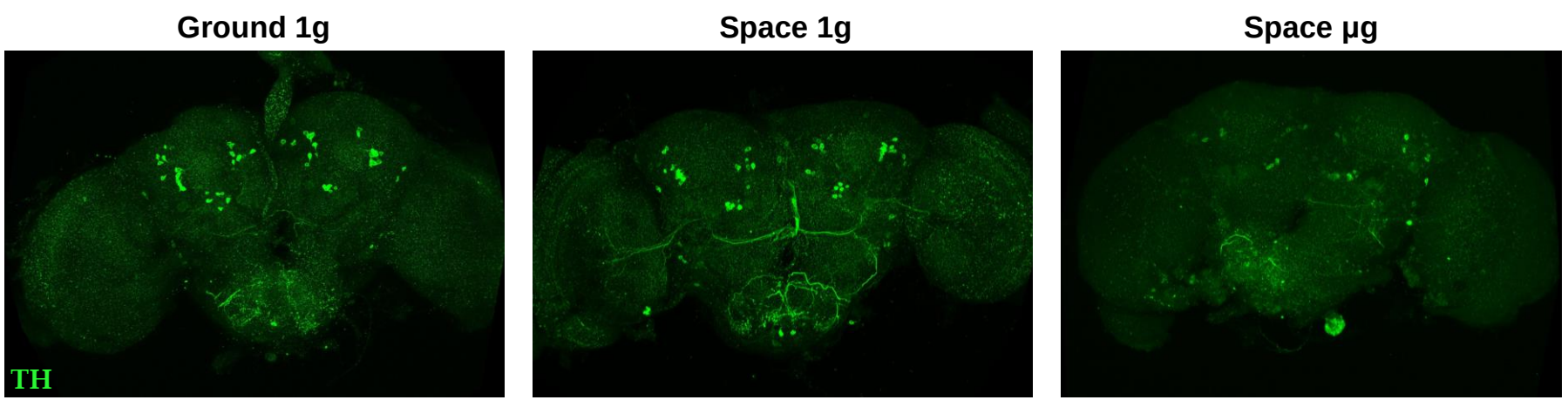


E



F

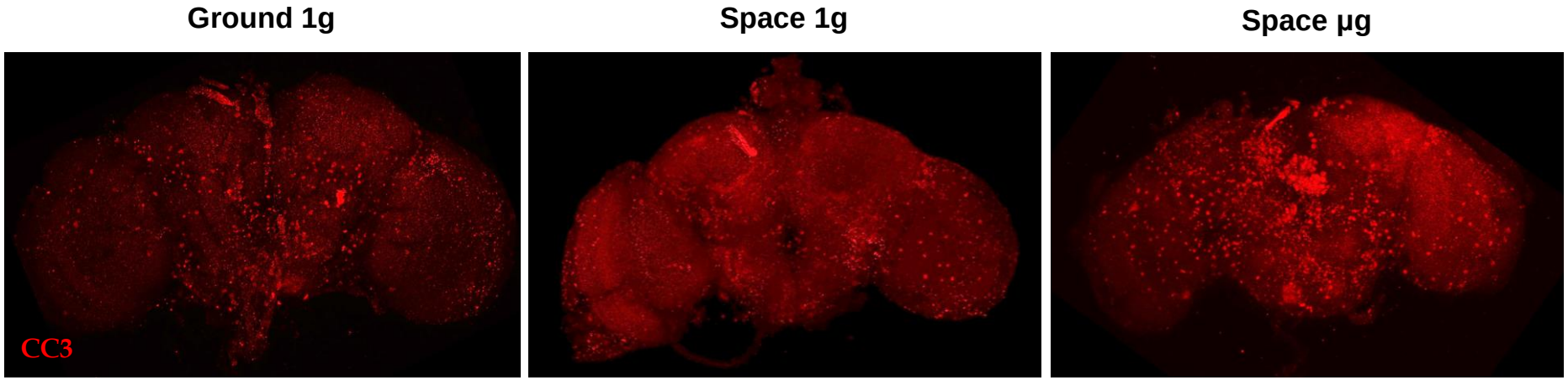
Dopaminergic neurons

Decreased neuronal area and dendritic field, and reduced dopaminergic neurons in space reared flies suggests neuronal loss

Janani Iyer, Siddhita Mhatre

Neuronal loss in spaceflight condition: Adult flies

A



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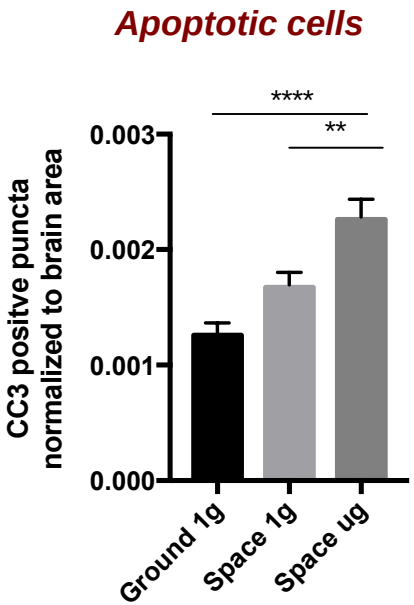


Increased apoptotic cells and decreased neuronal area and dendritic field suggests neuronal loss

Janani Iyer, Siddhita Mhatre

Summary and Future Directions

- Validation spaceflight mission for *Drosophila* MVP hardware → Successful
- Novel study assessing spaceflight effect on nervous system
- Behavioral deficits in larvae and adults correlates with observed morphological defects
- Adult fly brain results suggest **gravity as a major factor** in neuronal deficits in spaceflight
- Results from this mission would help in shaping hypothesis for future missions and ground based studies
- *On going:* Ground acclimatization and multi-generational studies along with genomic and proteomic analysis on adult flies

This study will help elucidate the underlying anatomical, functional and molecular changes in the nervous system resulting from spaceflight, which in future will help identify putative gene pathways for countermeasure studies

Principal Investigator:

Sharmila Bhattacharya

Scientists:

Janani Iyer

Rachel R. Gilbert

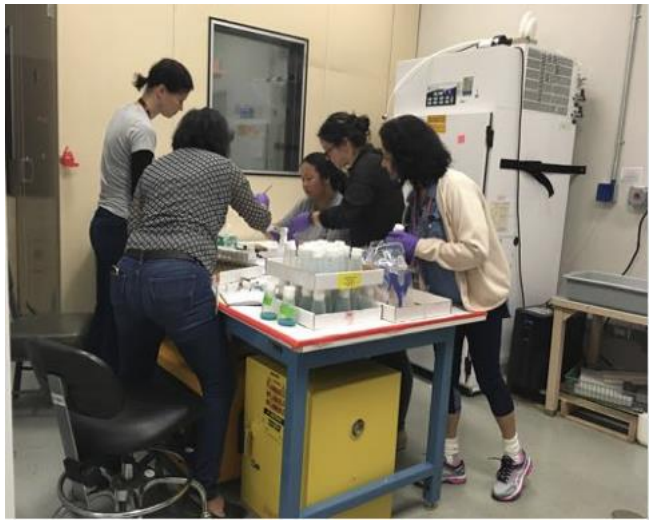
Amber M. Paul

Egle Cekanaviciute

Christina Cheung

Medaya Torres

THANK YOU!!



Students :

Ivy Fernandes

Jhony Zavaleta

COMMERCIAL PARTNER: TECHSHOT, INC.

ISS Research Integration Office for support and funding

KSC/ARC project management and staff for flight support