



# ***Invertebrate Model Organisms for Spaceflight***

**STAR Course**  
October 1st 2024

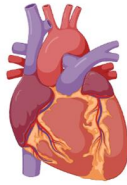
**Janani Iyer, *Ph.D***

**Siddhita Mhatre, *Ph.D***

***NASA Ames Research Center***



# *Physiological Response to Spaceflight*



**Cardiovascular  
deconditioning**



**Muscle atrophy**



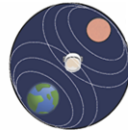
**Bone loss**



**Fluidic shift**



**Radiation**



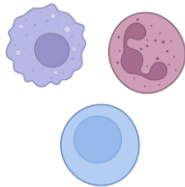
**Gravity**



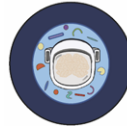
**Social isolation**



**Central Nervous system**



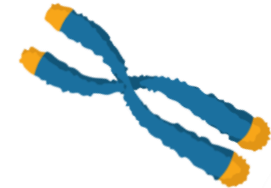
**Maladaptive immune system**



**Closed and hostile  
environment**



**Distance from Earth**



**Genetic changes - Telomeres**

# Spaceflight model systems



Skin



Brain



Cells

## *In vitro* systems



**Astronaut**



*C.elegans*



*D.melanogaster*

## **Invertebrates**



Rat



Mouse



Zebrafish

## **Vertebrates**

# *Invertebrate models in spaceflight research*

- **Genetic Conservation and Relevance**
  - Highly conserved genes and pathways
  - Relevance to human health
- **Well-annotated genome**
- **Short Lifespan - multiple generations**
- **Higher statistical power**
- **Cost-Effective**
- **Low resource requirement**
- **Ease of genetic manipulation**
- **Fewer ethical considerations**
- **Repositories - Wormbase, Flybase**





Volume 42, Issue 6, 15 September 2008, Pages 1072-1079



N.J. Szewczyk<sup>a,b</sup>  , J.                                                                                                                                        

S. Honda <sup>9</sup>, Y. Honda <sup>9</sup>, T.

N. Ishioka<sup>i</sup>, K. Fukui<sup>j</sup>, D.N. Ishioka<sup>i</sup>, K. Fukui<sup>j</sup>, D.

ARTICLE OPEN

Microgravity elicits reproducible alteration  
and metabolic gene and protein expression  
*Caenorhabditis elegans*

Published: 21 January 2016



iScience

## Article

# Loss of physical contact in space alters the dopamine system in *C. elegans*

Surabhi Sudevan,<sup>1,2,3,11</sup> Kasumi Muto,<sup>1,11</sup> Nahoko Higashitani,<sup>1,11</sup> Toko Hashizume,<sup>4,11</sup> Akira Higashibata,<sup>5</sup> Rebecca A. Ellwood,<sup>2,3</sup> Colleen S. Deane,<sup>6,7</sup> Mizanur Rahman,<sup>8</sup> Siva A. Vanapalli,<sup>8</sup> Timothy Etheridge,<sup>6,\*</sup> Nathaniel J. Szewczyk,<sup>2,3,9,10,\*</sup> and Atsushi Higashitani<sup>1,11,12,\*</sup>



*cells*

Cells 2023, 12, 2470. <https://doi.org/10.3390/cells12202470>



## Article

# Spaceflight Induces Strength Decline in *Caenorhabditis elegans*

Purushottam Soni <sup>1,†</sup>, Hunter Edwards <sup>2,‡</sup>, Taslim Anupom <sup>3</sup>, Mizanur Rahman <sup>1</sup>, Leila Lesanpezeshki <sup>1</sup>, Jerzy Bławdziewicz <sup>4,5</sup>, Henry Cope <sup>6,†</sup>, Nima Gharahdaghi <sup>6</sup>, Daniel Scott <sup>7</sup>, Li Shean Toh <sup>8</sup>, Philip M. Williams <sup>8</sup>, Timothy Etheridge <sup>9</sup>, Nathaniel Szewczyk <sup>6,10,\*</sup>, Craig R. G. Willis <sup>11,‡</sup> and Siva A. Vanapalli <sup>1,\*</sup>

Article

# Comparative Analysis of Muscle Atrophy During Spaceflight, Nutritional Deficiency and Disuse in the Nematode *Caenorhabditis elegans*

Ban-seok Kim <sup>1,†</sup>, Alfredo V. Alcantara, Jr. <sup>1,†</sup>, Je-Hyun Moon <sup>1</sup>, Atsushi Higashitani <sup>2,†</sup>, Nahoko Higashitani <sup>2</sup>, Timothy Etheridge <sup>3</sup>, Nathaniel J. Szewczyk <sup>4,†</sup>, Colleen S. Deane <sup>3,5,†</sup>, Christopher J. Gaffney <sup>6,†</sup>, Akira Higashibata <sup>7</sup>, Toko Hashizume <sup>8</sup>, Kyoung-hye Yoon <sup>9,†</sup> and Jin I. Lee <sup>1,\*</sup>

iScience

## Article

# Spaceflight affects neuronal morphology and alters transcellular degradation of neuronal debris in adult *Caenorhabditis elegans*

Ricardo Laranjeiro,<sup>1,7,\*</sup> Girish Harinath,<sup>1</sup> Amelia K. Pollard,<sup>2</sup> Christopher J. Gaffney,<sup>3,4</sup> Colleen S. Deane,<sup>3</sup> Siva A. Vanapalli,<sup>5</sup> Timothy Etheridge,<sup>3</sup> Nathaniel J. Szewczyk,<sup>2,6</sup> and Monica Driscoll<sup>1</sup>



# *Drosophila melanogaster* – the model organism

- 6 Nobel prizes in physiology or medicine for fruit fly research
- ~75% of human disease-causing genes have a functional homolog in the fruit fly
  - Model for human innate immunity
  - Model for Parkinson's, Alzheimer's, Fragile X syndrome, Rett syndrome, cancers, autism, diabetes
  - Cardiovascular disorders
- >75 years of spaceflight research in *Drosophila*
- Small, convenient for ISS experimentation
- Fast generation times, inexpensive to maintain

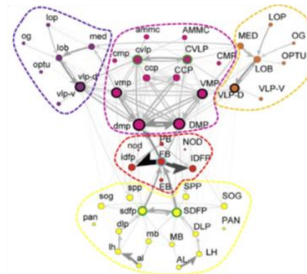
**Neural connectivity is strikingly similar across species**



*Drosophila melanogaster*



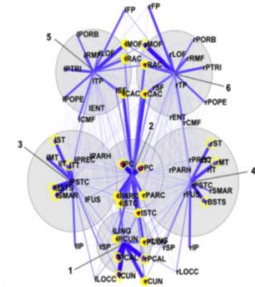
135000 neurons



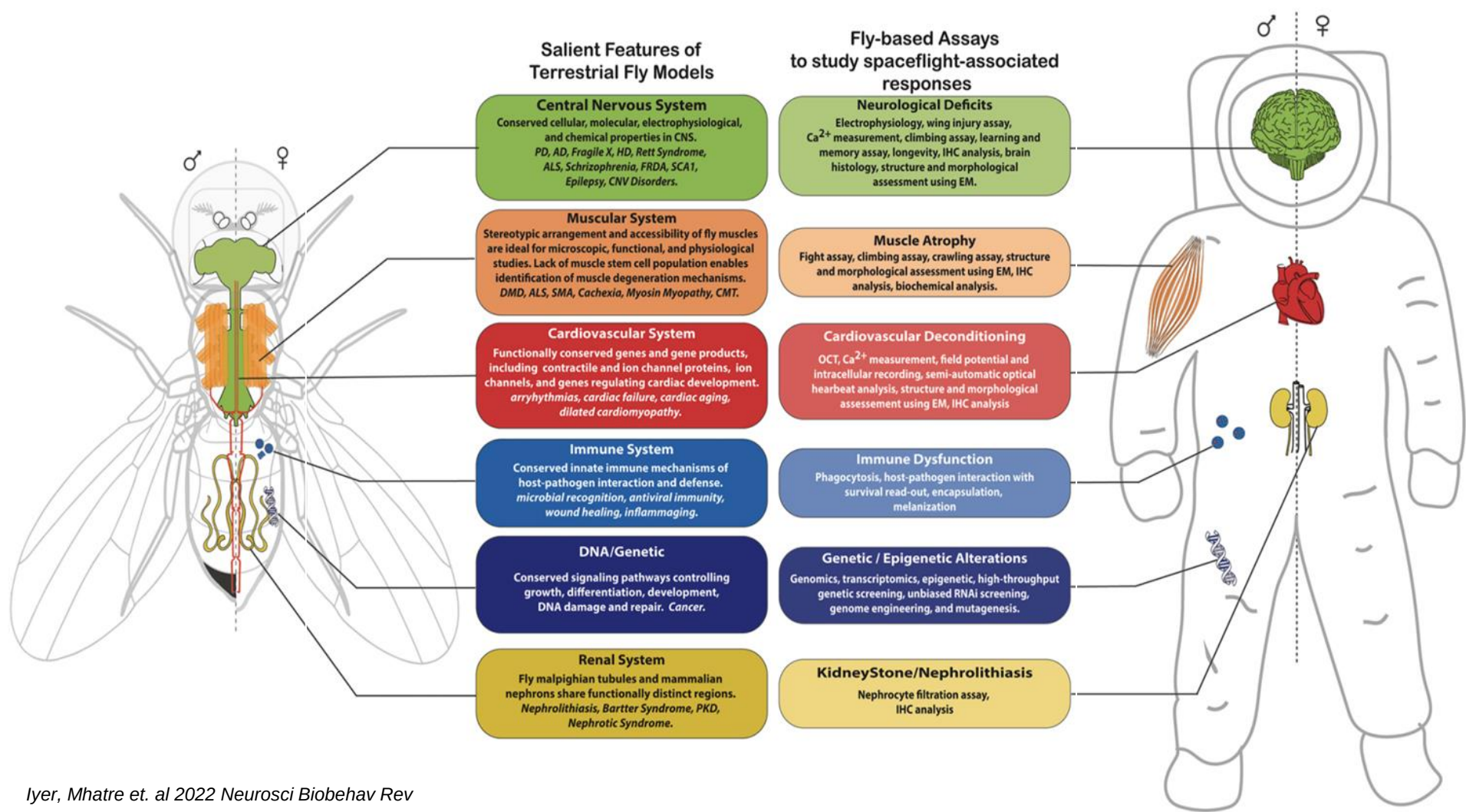
*Homo sapiens*



100 billion neurons



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

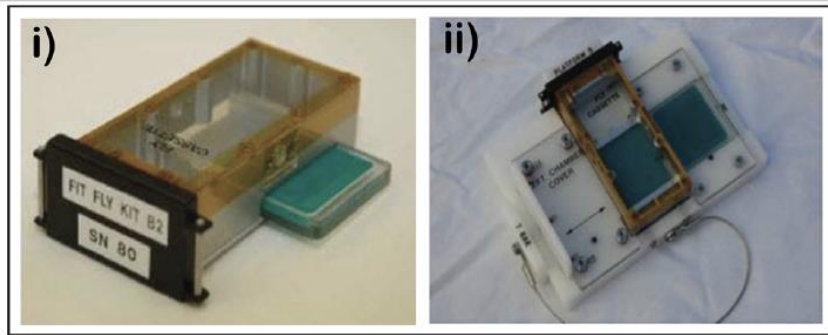


# Drosophila missions

| Year launched | Mission                       | Mission duration | Topic of study                                                                                                                 |
|---------------|-------------------------------|------------------|--------------------------------------------------------------------------------------------------------------------------------|
| 1947          | V-2 rocket                    | 3 mins           | Explore the effects of radiation exposure at high altitudes                                                                    |
| 1966          | Biosatellite I                | 0 days           | Effects of Weightlessness on Radiation Induced Somatic Damage in <i>Drosophila</i> ; experiment was reflown on Biosatellite II |
| 1967          | Biosatellite II               | 2 days           | Effects of Space on Radiation-Induced Damage to Reproductive Cells of <i>Drosophila</i> Adults and Pupae                       |
| 1975          | Bion 3<br>Cosmos (COS 782-10) | 19.5 days        | Effects of spaceflight on ageing                                                                                               |
| 1977          | Bion 4<br>Cosmos (COS 936-5)  | 18.5 days        | Genetic effects, development and ageing in space                                                                               |
| 1978          | Salyut-6                      | 49 days          | Effects of weightlessness on breeding and heredity                                                                             |
| 1985          | D-1                           | 7 days           | Effects of microgravity and space environment on the embryogenesis and lifespan                                                |
| 1989          | BION 9<br>(Cosmos-2044)       | 14 days          | Microgravity and space radiation effects: development, ageing, mitotic recombination rate and adaptation                       |
| 1992          | IML-1 (STS-42)                | 8 days           | Effect of spaceflight on the development and lifespan                                                                          |
| 1994          | IML-2 (STS-65)                | 14.5 days        | Effects of microgravity and cosmic radiation on <i>Drosophila melanogaster</i> development, behavior and aging                 |

| Year launched | Mission                    | Mission duration | Topic of study                                                                                                                                                 |
|---------------|----------------------------|------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1996          | STS-72                     | 9 days           | Effects of microgravity and space radiation                                                                                                                    |
| 1999          | STS-93                     | 5 days           | Spaceflights effects on neuronal development associated with muscle fibers in <i>Drosophila melanogaster</i> embryo and larvae; experiment re-flown on STS-106 |
| 2000          | STS-106                    | 12 days          | Spaceflights effects on neuronal development associated with muscle fibers in <i>Drosophila melanogaster</i> embryo and larvae                                 |
| 2003          | Spanish Soyuz (Cervantes)  | 11 days          | Effect of microgravity on motility behavior and ageing in <i>Drosophila melanogaster</i>                                                                       |
| 2006          | STS-121 (FIT)              | 13 days          | Study the cellular and molecular biological responses of <i>Drosophila</i> immune system to post-flight microbial and fungal infections.                       |
| 2014          | HEART FLIES (SpaceX-CRS-3) | 30 days          | Spaceflight effects on cardiovascular system                                                                                                                   |
| 2014          | Foton-M No.4               | 44.5 days        | Gene expression profiling of multiple generations of larvae during spaceflight and subsequent adaptation to Earth                                              |
| 2015          | FFL-1 (SpaceX-CRS-5)       | 30 days          | Effects of spaceflight on the immune response through host-pathogen interaction along with new hardware testing                                                |
| 2017          | FFL-2 (SpaceX-CRS-11)      | 30 days          | Effects of spaceflight on the cardiovascular system and development of countermeasures                                                                         |
| 2018          | MVP-Fly01 (SpaceX-CRS-14)  | 33 days          | MVP hardware validation mission                                                                                                                                |
| 2018          | FFL-3 (SpaceX-CRS-14)      | 33 days          | Effect of spaceflight on innate immune system using host-pathogen interaction paradigm                                                                         |

# FIT hardware



OPEN ACCESS Freely available online

January 2011 | Volume 6 | Issue 1 | e15361

PLOS one

## Innate Immune Responses of *Drosophila melanogaster* Are Altered by Spaceflight

Oana Marcu<sup>1,2</sup>, Matthew P. Lera<sup>1,3</sup>, Max E. Sanchez<sup>3a</sup>, Edina Levic<sup>1a</sup>, Laura A. Higgins<sup>1b</sup>, Alena Shmygelska<sup>1,4</sup>, Thomas F. Fahlen<sup>3ac</sup>, Helen Nichol<sup>5</sup>, Sharmila Bhattacharya<sup>1\*</sup>

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January 2014 | Volume 9 | Issue 1 | e86485

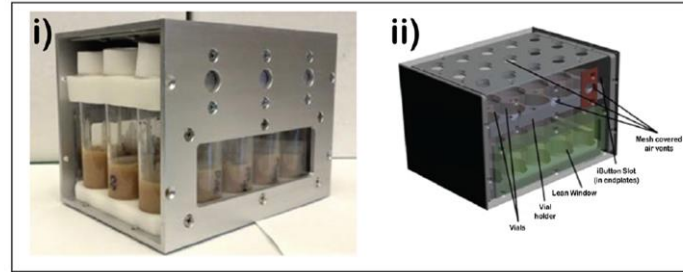
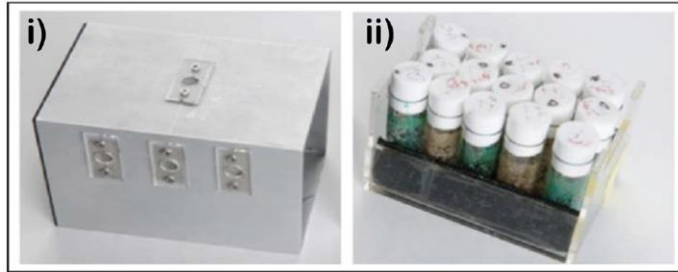
PLOS ONE

## Toll Mediated Infection Response Is Altered by Gravity and Spaceflight in *Drosophila*

Katherine Taylor<sup>1a</sup>, Kurt Kleinhesselink<sup>1b</sup>, Michael D. George<sup>2</sup>, Rachel Morgan<sup>3</sup>, Tangi Smallwood<sup>3</sup>, Ann S. Hammonds<sup>4</sup>, Patrick M. Fuller<sup>5ac</sup>, Perot Saelao<sup>1</sup>, Jeff Alley<sup>6</sup>, Allen G. Gibbs<sup>7</sup>, Deborah K. Hoshizaki<sup>7</sup>, Laurence von Kalm<sup>3</sup>, Charles A. Fuller<sup>5</sup>, Kathleen M. Beckingham<sup>8</sup>, Deborah A. Kimbrell<sup>1\*</sup>



# Vented Fly Box (VFB)



Cell Reports

Cell Reports 33, 108445, December 8, 2020 © 2020 The Authors.

CellPress  
OPEN ACCESS

ARTICLE

## Prolonged Exposure to Microgravity Reduces Cardiac Contractility and Initiates Remodeling in *Drosophila*

Stanley Walls,<sup>1</sup> Soda Diop,<sup>1</sup> Ryan Birse,<sup>1,5</sup> Lisa Elmen,<sup>1,5</sup> Zhuohui Gan,<sup>3</sup> Sreehari Kalvakuri,<sup>1,5</sup> Santiago Pineda,<sup>1,5</sup> Curran Reddy,<sup>2</sup> Erika Taylor,<sup>1,5</sup> Bosco Trinh,<sup>1,5</sup> Georg Vogler,<sup>1,5</sup> Rachel Zarndt,<sup>1,5</sup> Andrew McCulloch,<sup>3</sup> Peter Lee,<sup>4</sup> Sharmila Bhattacharya,<sup>2</sup> Rolf Bodmer,<sup>1,\*</sup> and Karen Ocorr<sup>1,6,\*</sup>

## HEART FLIES (SpaceX-3)

Heart Effect Analysis Research Team conducting FLY Investigations and Experiments in Spaceflight



iScience

iScience 27, 108759, January 19, 2024 © 2023 The Author(s)

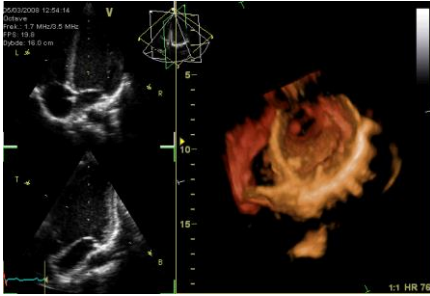
Article

## *Drosophila* parasitoids go to space: Unexpected effects of spaceflight on hosts and their parasitoids

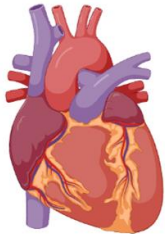
Jennifer Chou,<sup>1</sup> Johnny R. Ramroop,<sup>1,9</sup> Amanda M. Saravia-Butler,<sup>2,4,9</sup> Brian Wey,<sup>1,3,9</sup> Matthew P. Lera,<sup>4</sup> Medaya L. Torres,<sup>4,5</sup> Mary Ellen Heavner,<sup>1,6</sup> Janani Iyer,<sup>2,4,7</sup> Siddhita D. Mhatre,<sup>2,4</sup> Sharmila Bhattacharya,<sup>4,8</sup> and Shubha Govind<sup>1,3,4,10,\*</sup>

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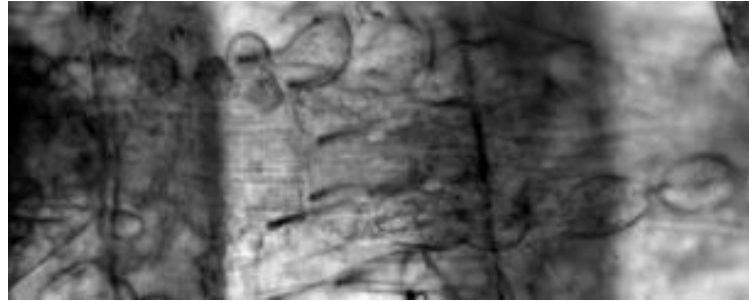
# *Drosophila heart*



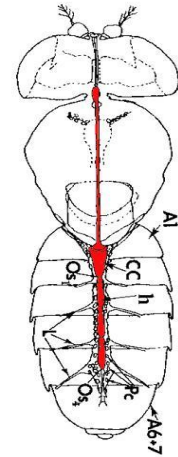
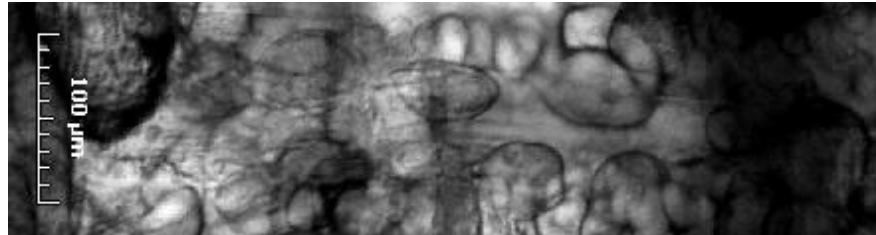
# Human heart



**1 week fly (~10 years old in human years)**



**7 week old fly (~70 years old in human years)**



# Fly hearts show age-related arrhythmias

# Mitochondrial Investigation of Oxidative Stress in Fruit Flies for CNS, Muscle, and Cardiac Health (*MitoFlyght* mission)

Spaceflight Hazards

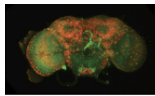


Antioxidants

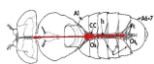
ROS

**Oxidative stress**

Tissue specific  
**Overexpression**  
of mitochondrial  
superoxide  
dismutase  
(SOD2)



CNS



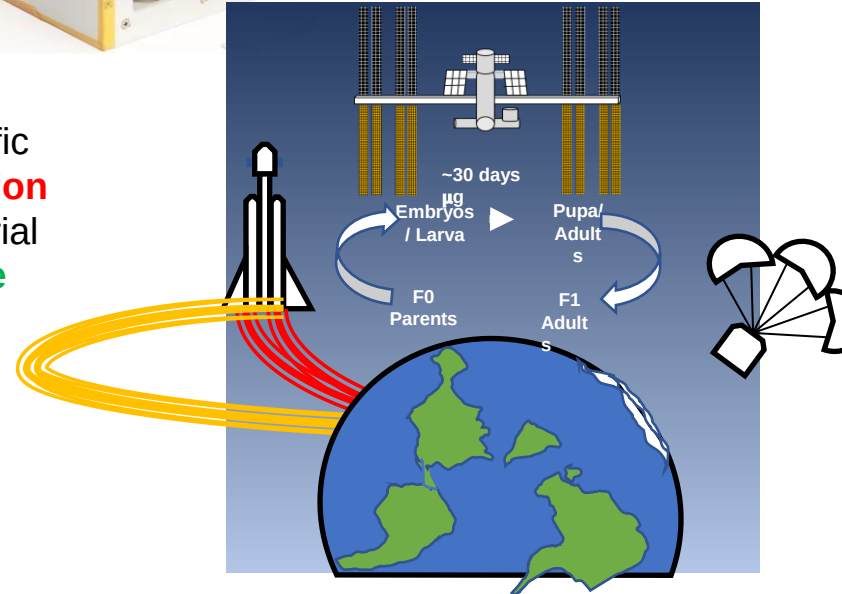
Cardiac



Muscle



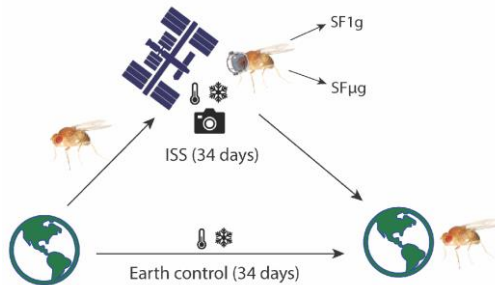
Vented Fly Box (VFB)



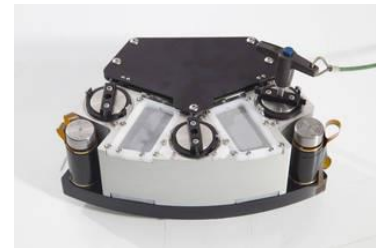
Space Biology funded

# Multi-use Variable-g Platform (MVP)

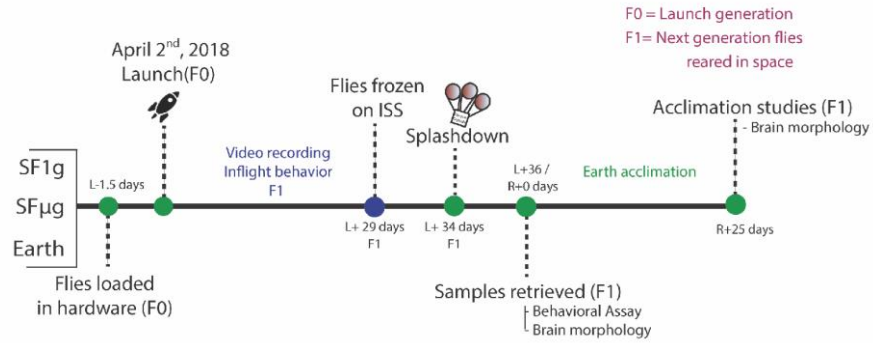
- Optimized for life/physical science research in microgravity
- **2 independent centrifuges (each can spin up to 2g)**
- **Controls temperature, relative humidity**
- **Cycles fresh cabin air into habitat**
- Telemetry/real-time video & other data; ground commanding
- Reusable/reconfigurable MVP facility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



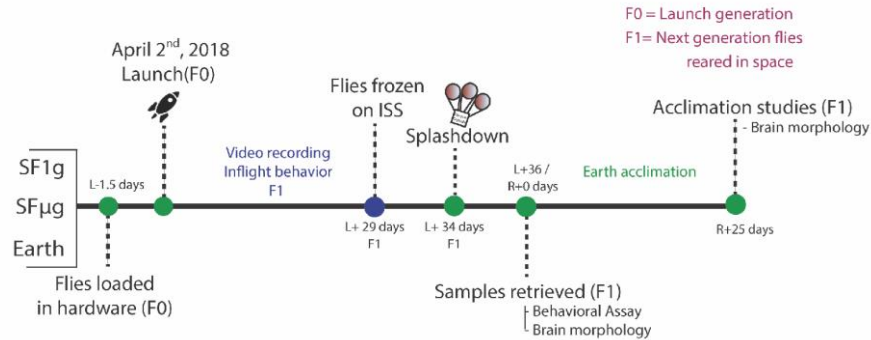
- Earth – Ground control
- SFug – Space microgravity
- SF1g – Earth gravity simulated on ISS (Artificial gravity)



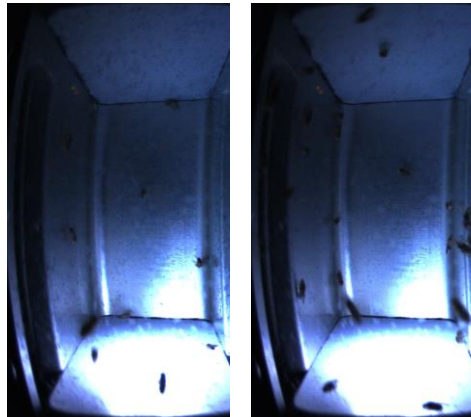
# Artificial gravity partially protects space-induced neurological deficits in *Drosophila melanogaster*



# Artificial gravity partially protects space-induced neurological deficits in *Drosophila melanogaster*

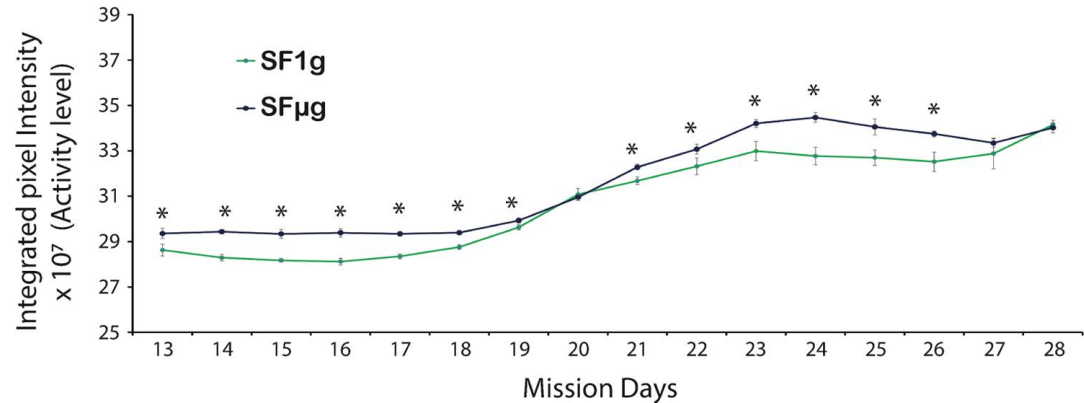


## In-flight activity assay



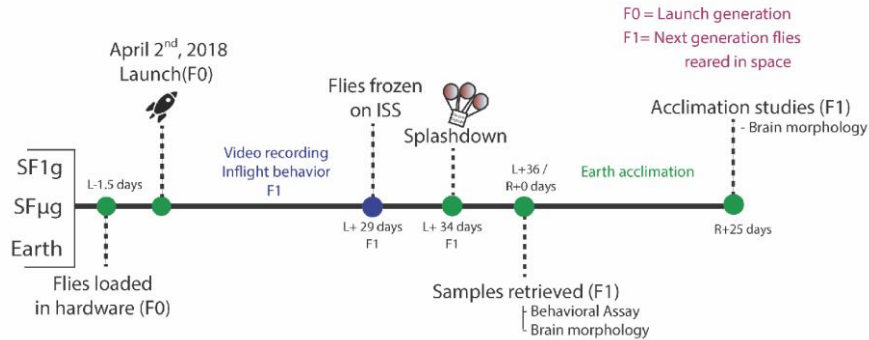
Space 1g

Space ug



***SFμg flies exhibit increased activity on ISS***

# Artificial gravity partially protects space-induced neurological deficits in *Drosophila melanogaster*

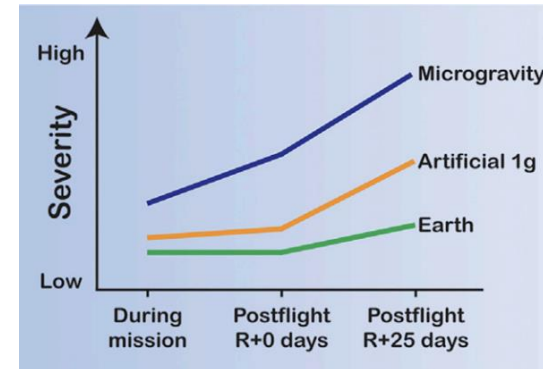


## Immediately post flight

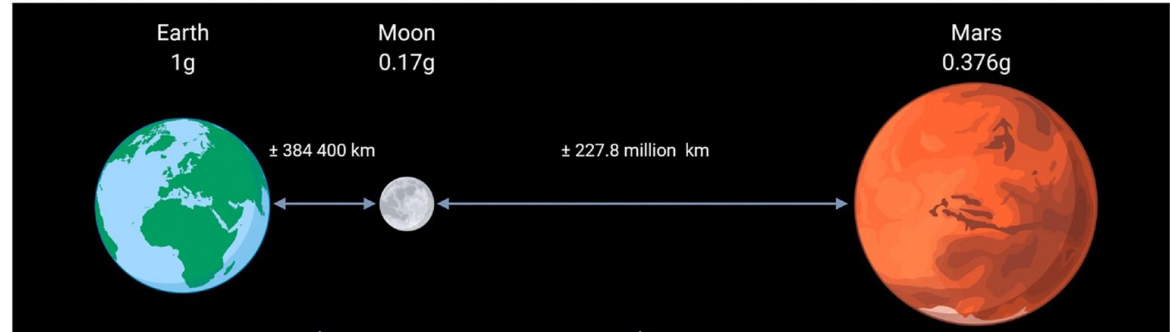
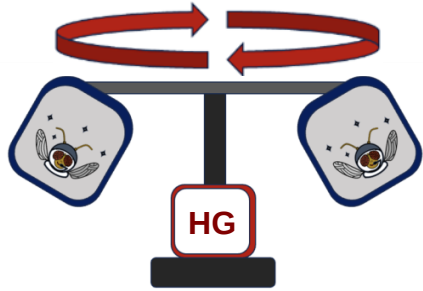
- Behavioral impairments - climbing deficits - **SFμg**
- Neurological changes – neuronal loss, changes in glia, increased apoptosis, oxidative damage - **SFμg**
- Metabolic pathways affected: oxidative phosphorylation, glycolysis, fatty acid metabolism, amino acid metabolism - **SFμg, SF1g**

## Post-acclimation to Earth conditions – changes observed in SFμg and SF1g

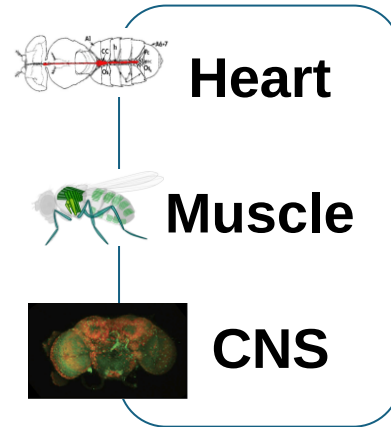
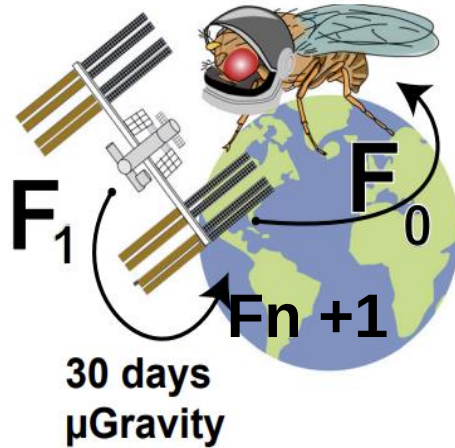
**Artificial gravity may provide partial protection to the brain, but long-term solutions need to be explored, especially for long-duration missions**



# Integrated physiological responses of CNS and muscle in *Drosophila* and *C. elegans* along a gravity continuum (iGCE mission)



Moroni et al., 2022 Trends in Biotechnology



Structure  
Function  
Omics



# *Spaceflight versus Ground-Based Analogs*

## Spaceflight

- Ideal model for space environment
- Expensive, few, and rare
- Limited n (sample size)
- Small margin of error

## Ground-Based Studies

- Spaceflight Analog
- Can look at individual + combined spaceflight stressors
- Larger sample sizes with ability to run longitudinal assays
- Ability to redo experiments

# Ground based *Drosophila* Hypergravity studies

> [Genomics](#). 2016 Oct;108(3-4):158-167. doi: 10.1016/j.ygeno.2016.09.002. Epub 2016 Sep 10.

## Transcriptomic response of *Drosophila melanogaster* pupae developed in hypergravity

Shannon Hateley <sup>1</sup>, Ravikumar Hosamani <sup>2</sup>, Shilpa R Bhardwaj <sup>3</sup>, Lior Pachter <sup>4</sup>,  
Sharmila Bhattacharya <sup>5</sup>

MAG: 3049770188

### Hypergravity-induced altered behavior in *Drosophila*

Ravikumar Hosamani, Judy Wan, Oana Marcu, Sharmila Bhattacharya

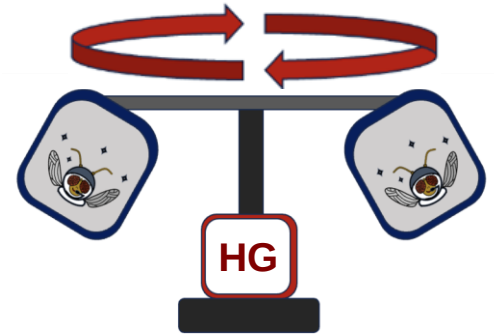
[Astrobiology](#) [Gene](#) [Hypergravity](#) 2012 39th COSPAR Scientific Assembly →

[Review](#) > [Comp Biochem Physiol Comp Physiol](#). 1993 Jul;105(3):389-96.

doi: 10.1016/0300-9629(93)90408-v.

## Hypergravity, aging and longevity in *Drosophila melanogaster*

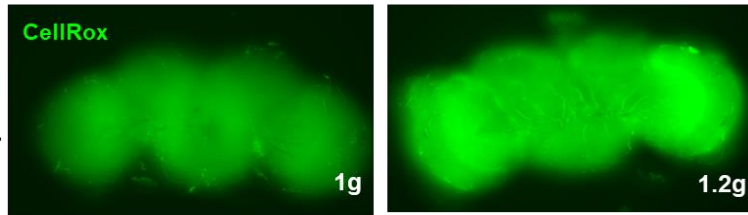
E Le Bourg <sup>1</sup>, F A Lints, N Fresquet, P Bullens



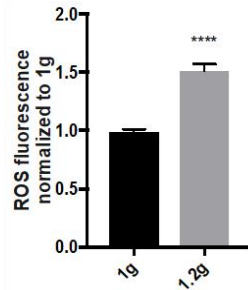
# Hypergravity induces oxidative stress resulting in dopaminergic neuronal loss and apoptosis

A

Reactive oxygen species

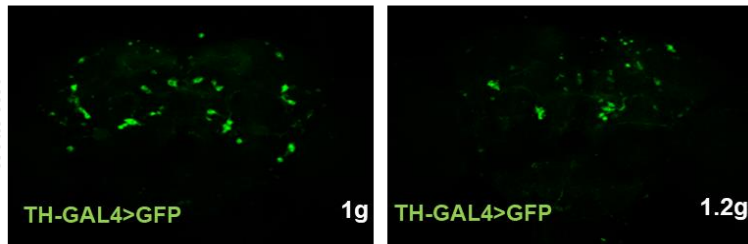


B

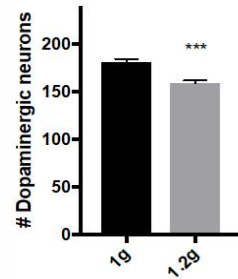


C

Dopaminergic neurons

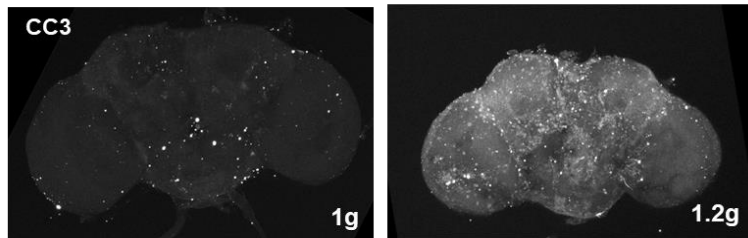


D

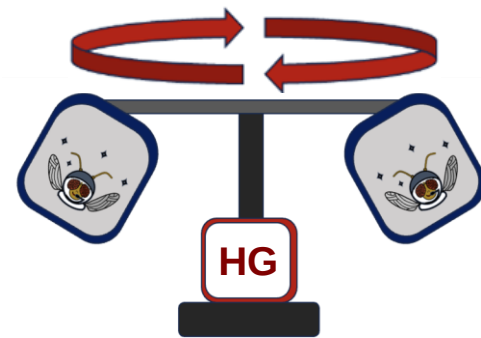
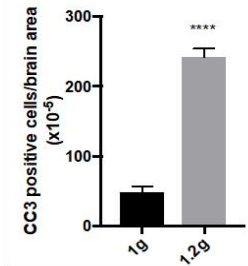


E

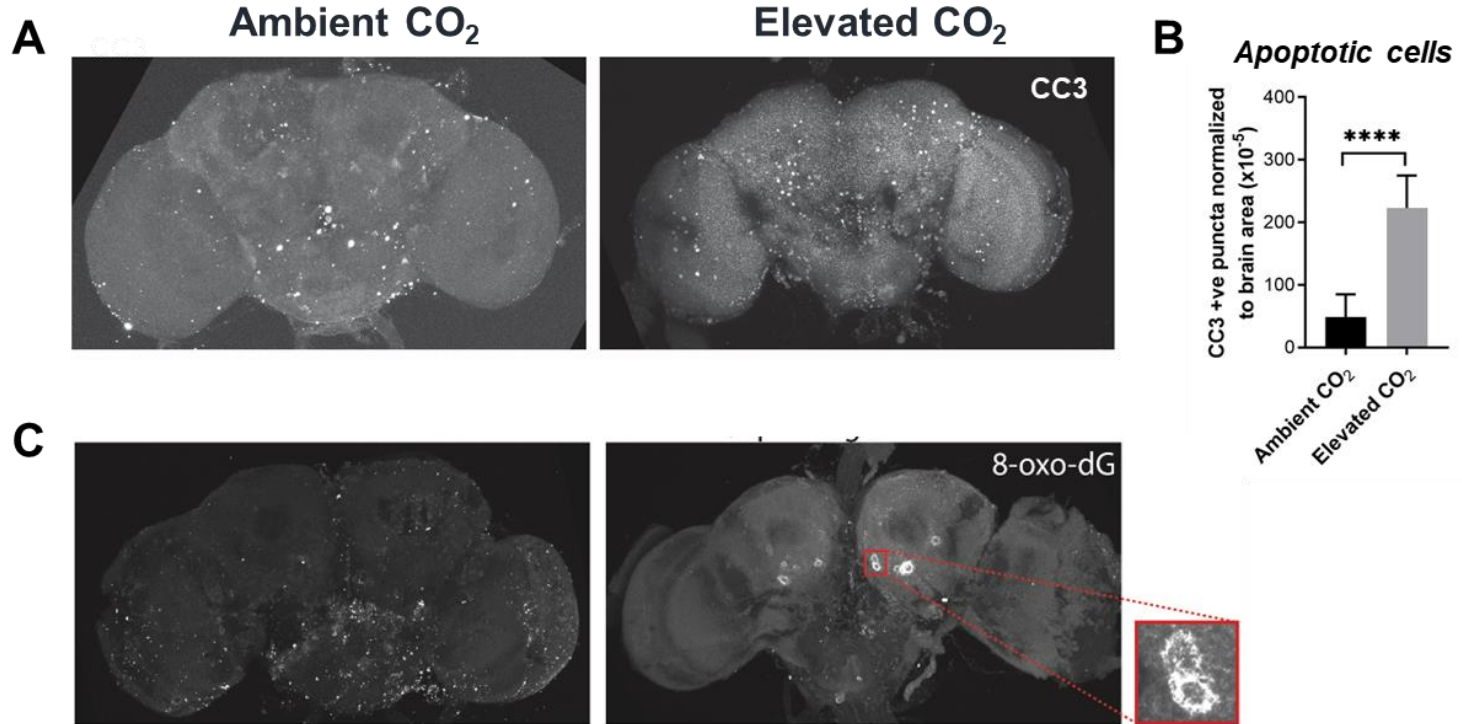
Apoptotic cells



F

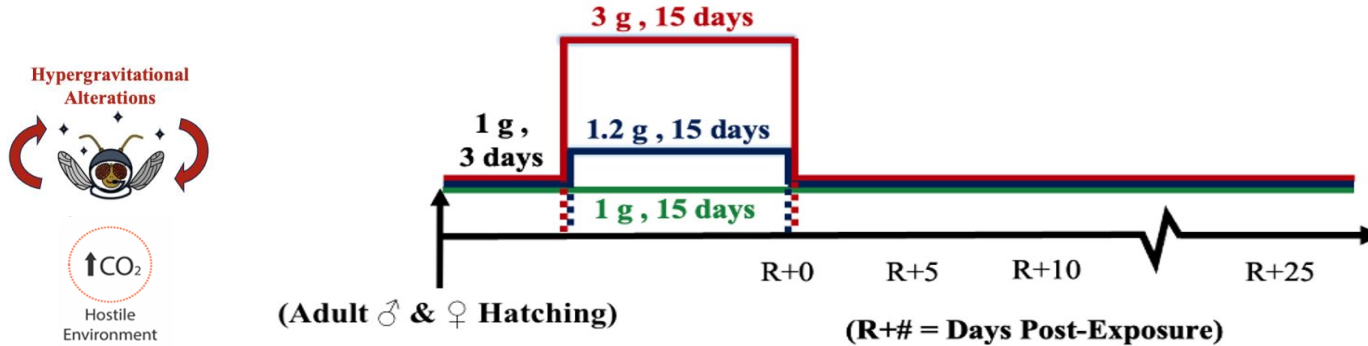


# Elevated $\text{CO}_2$ leads to apoptosis and oxidative damage

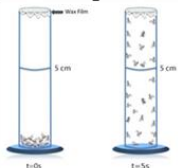




# Sex-specific physiological and transcriptomic responses of CNS to combined effects of spaceflight stressors in *Drosophila melanogaster*



## Neuro-behavioral assays



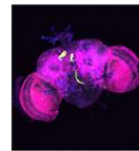
Climbing ability

## Biochemical assays

- Mitochondrial membrane potential
- ATP assay
- NADH/NAD assay
- ROS measurements
- Western Blot- OXPHOS

Adult heads

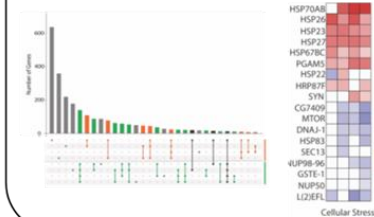
## Neural morphology



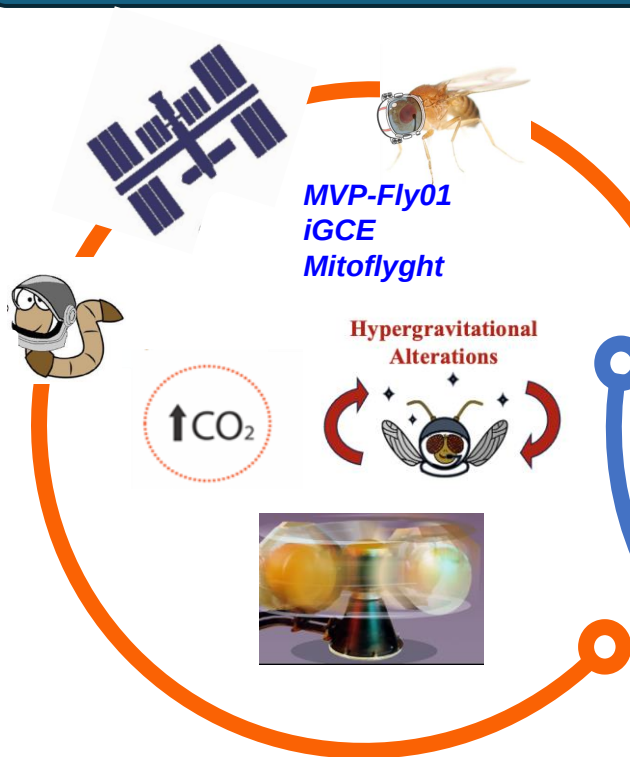
Adult Brain

- Neuron
- Mushroom body
- Glia
- DA neurons
- Apoptosis
- Oxidative damage
- Mitochondria

## Gene expression changes



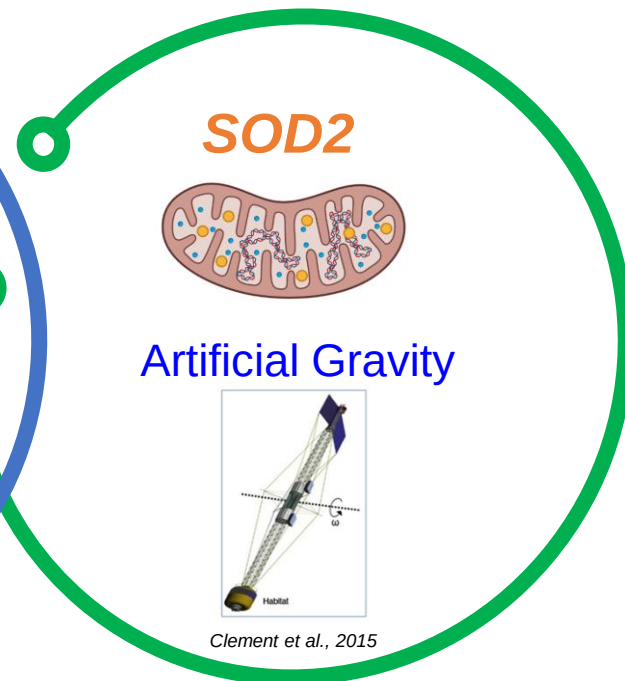
## Summary



## Modeling Spaceflight Stressors



## Identifying mechanisms and pathways



## Testing countermeasures

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