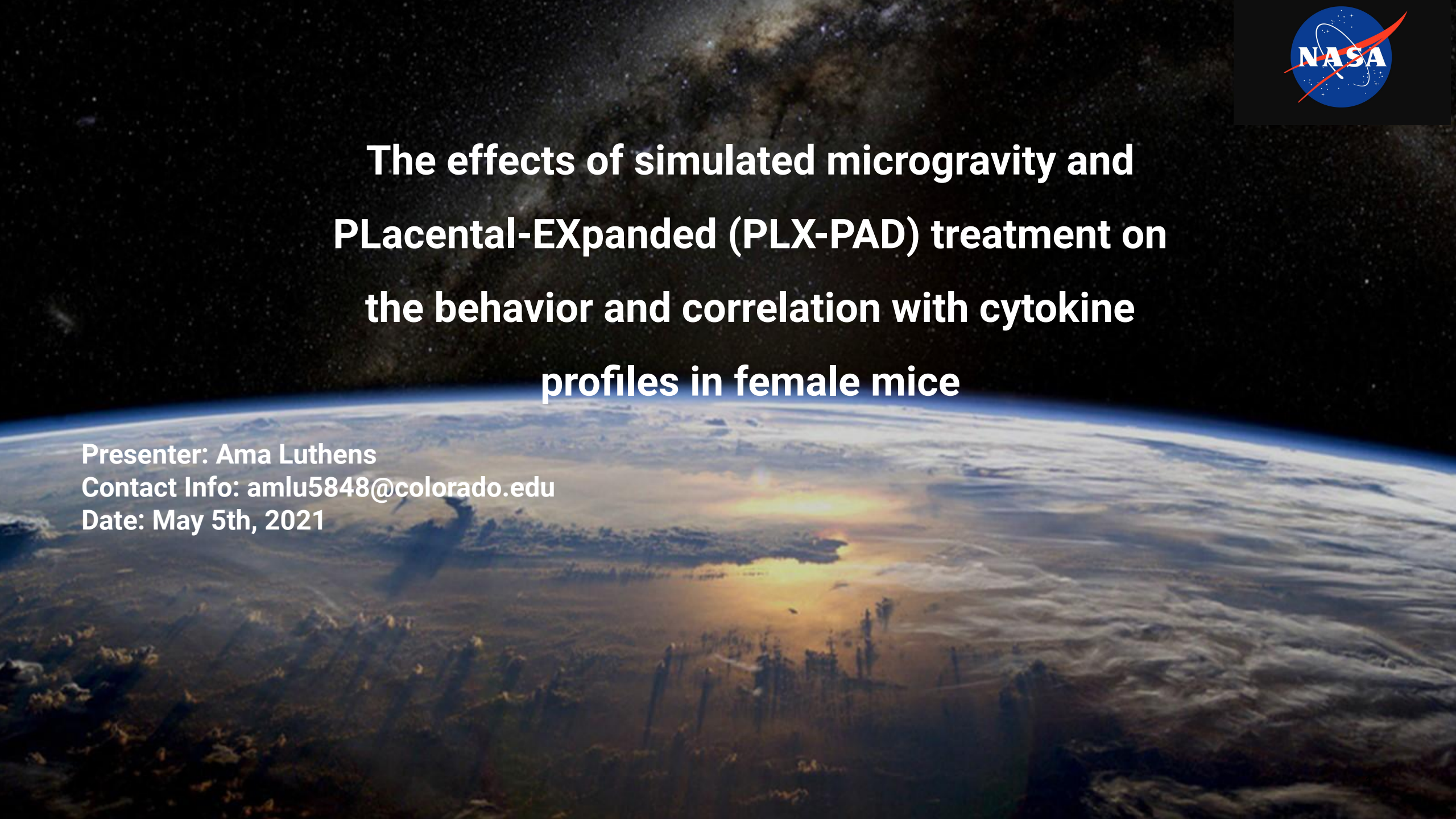
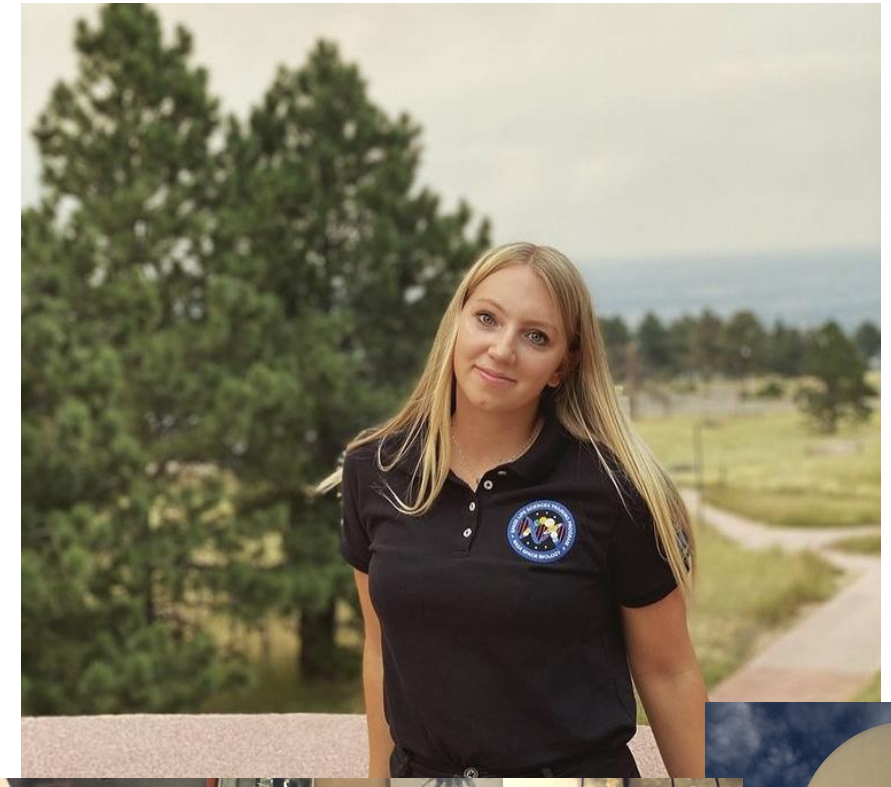


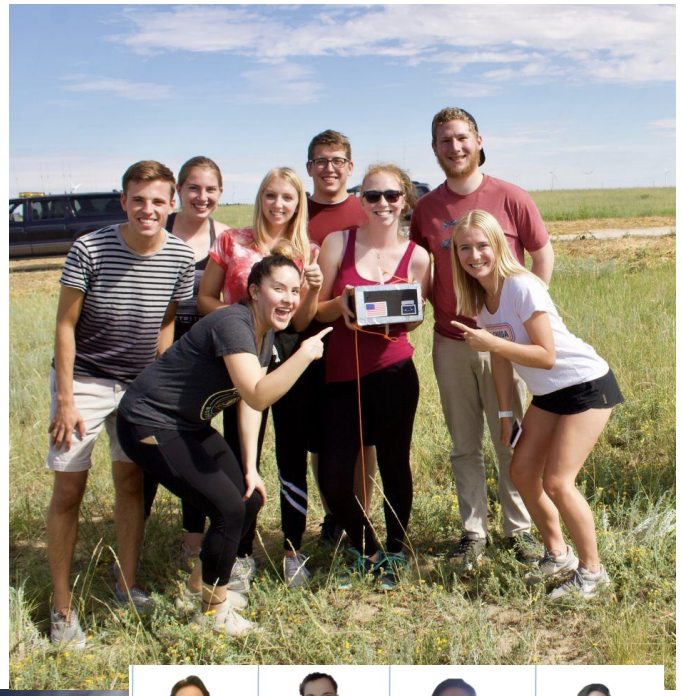
The effects of simulated microgravity and PLacental-EXpanded (PLX-PAD) treatment on the behavior and correlation with cytokine profiles in female mice

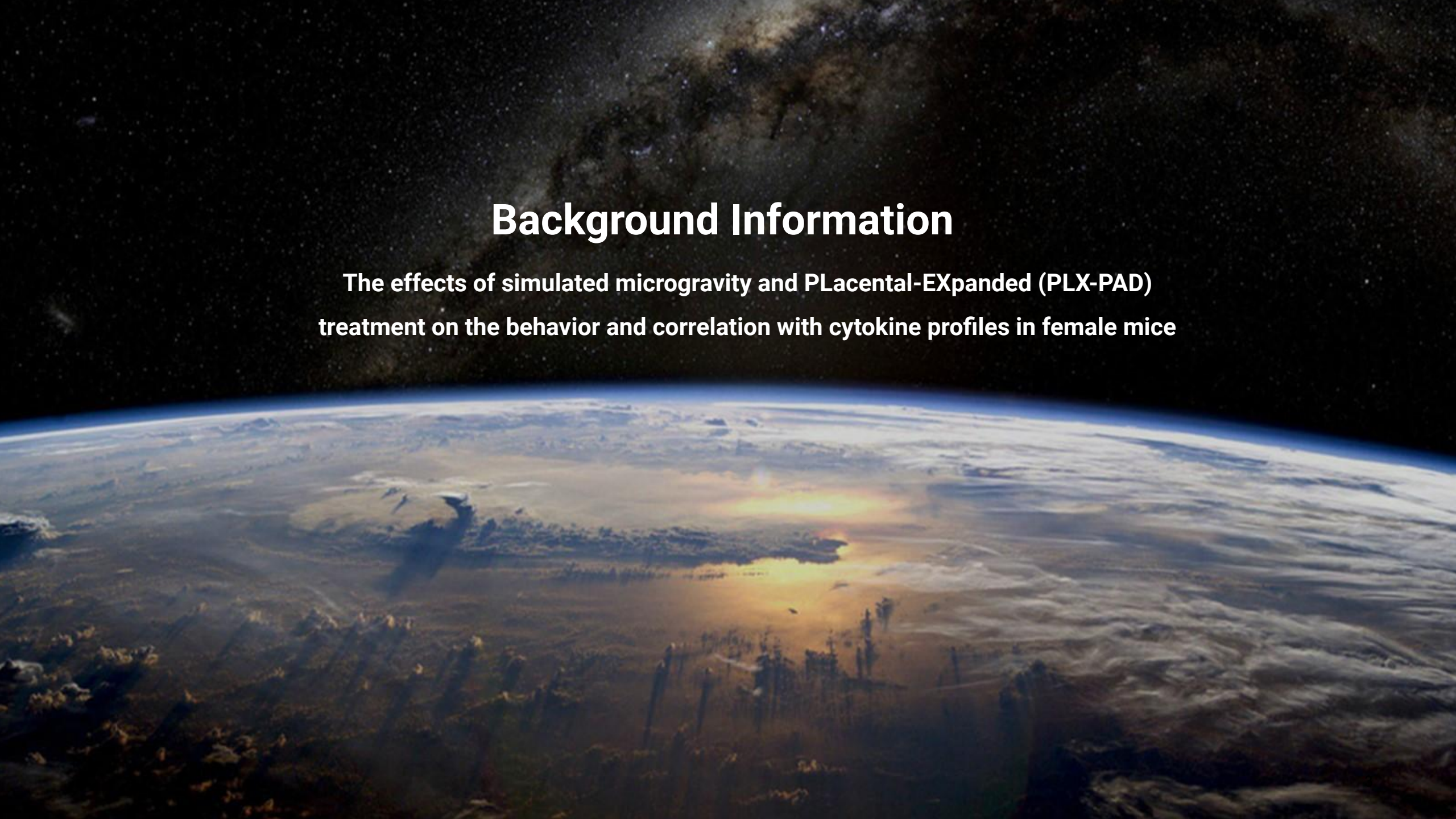
Presenter: Ama Luthens
Contact Info: amlu5848@colorado.edu
Date: May 5th, 2021

About me

- **21 years old**
- **Incoming senior at the University of Colorado Boulder**
  - Major: Neuroscience
  - Minors: Business and Molecular Biology
- **Started space research in 2019 with the Colorado Space Grant Consortium (COSGC)**
- **Interned for NASA Ames Space Life Sciences Training Program (SLSTP) in summer 2020**
- **This summer, working as an SLSTP mentor**



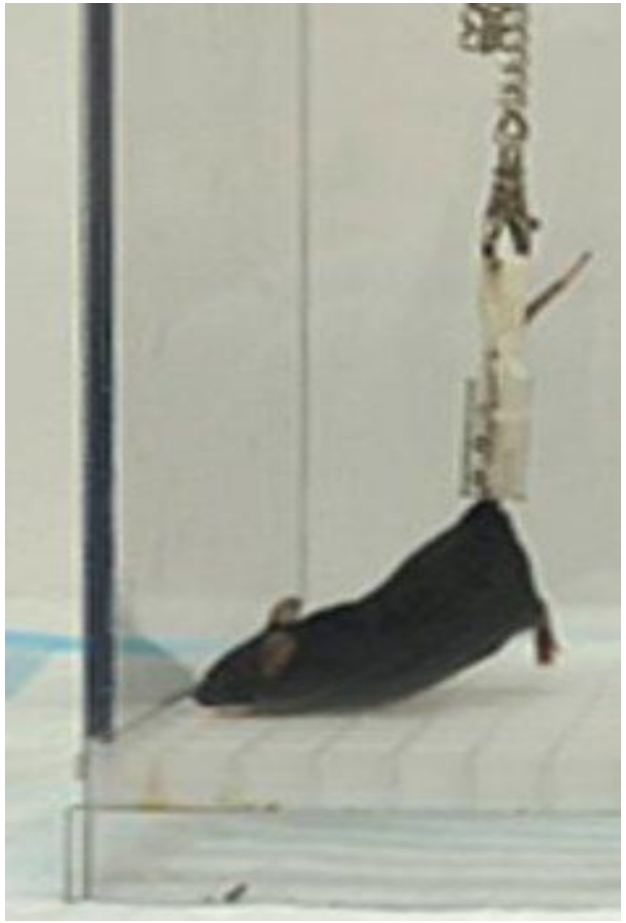




Background Information

The effects of simulated microgravity and PLacental-EXpanded (PLX-PAD) treatment on the behavior and correlation with cytokine profiles in female mice

Hindlimb Unloading (HU): A Microgravity Analog

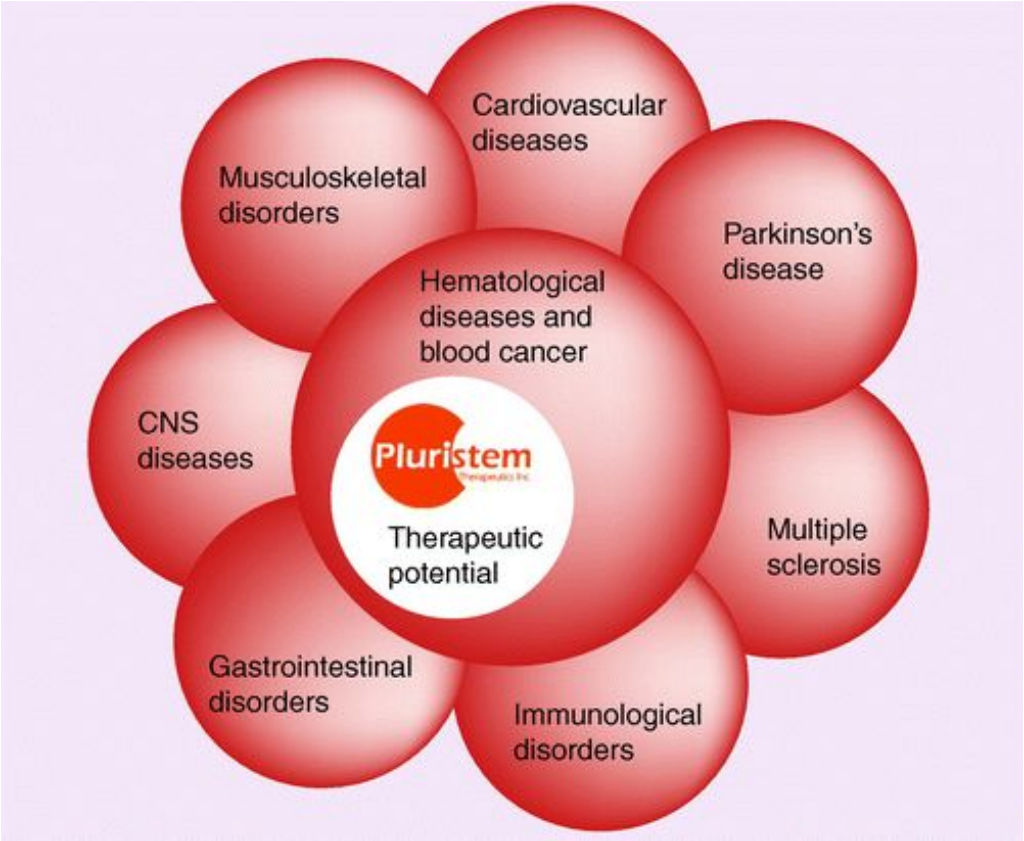


Tahimic et al. 2019

- **Hindlimb Unloading (HU)**
  - Simulated microgravity
  - Space flight and HU result in negative physiological consequences
  - Currently, a lack of behavioral HU studies

PLX-PAD stromal cells

- **PLacental eXpanded cells (PLX-PAD)**
  - Stromal cell type derived from the maternal placenta (PLX-PAD)
  - Therapeutic effects
  - Promising treatment used in many clinical trials[1] (image on right) and previous physiology studies with HU[2]
- **Currently, no studies on how PLX-PAD affects behavior**



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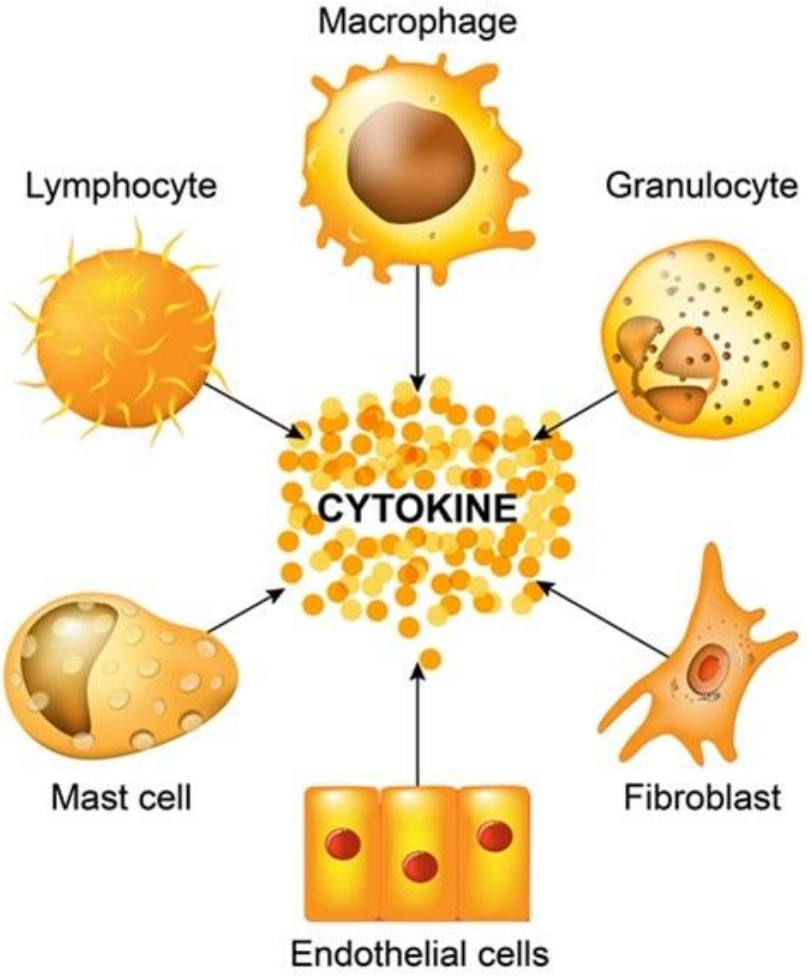
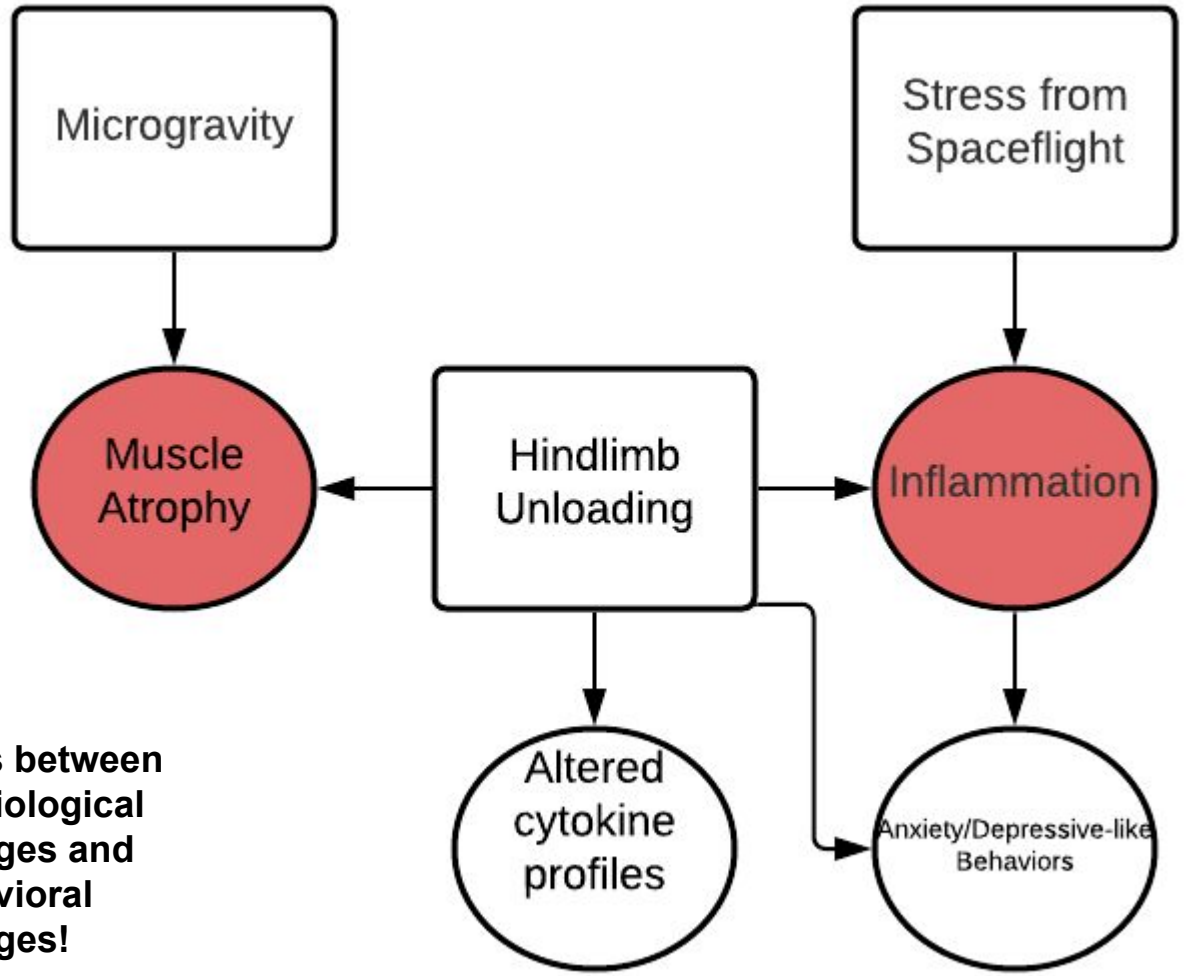
Cytokines

- **Small proteins released by cells that are important for cell signaling and immune system function**
  - Pro-inflammatory, anti-inflammatory, or participate in regulating activity of different immune cells



News Medical

What do we know from previous HU and spaceflight studies?



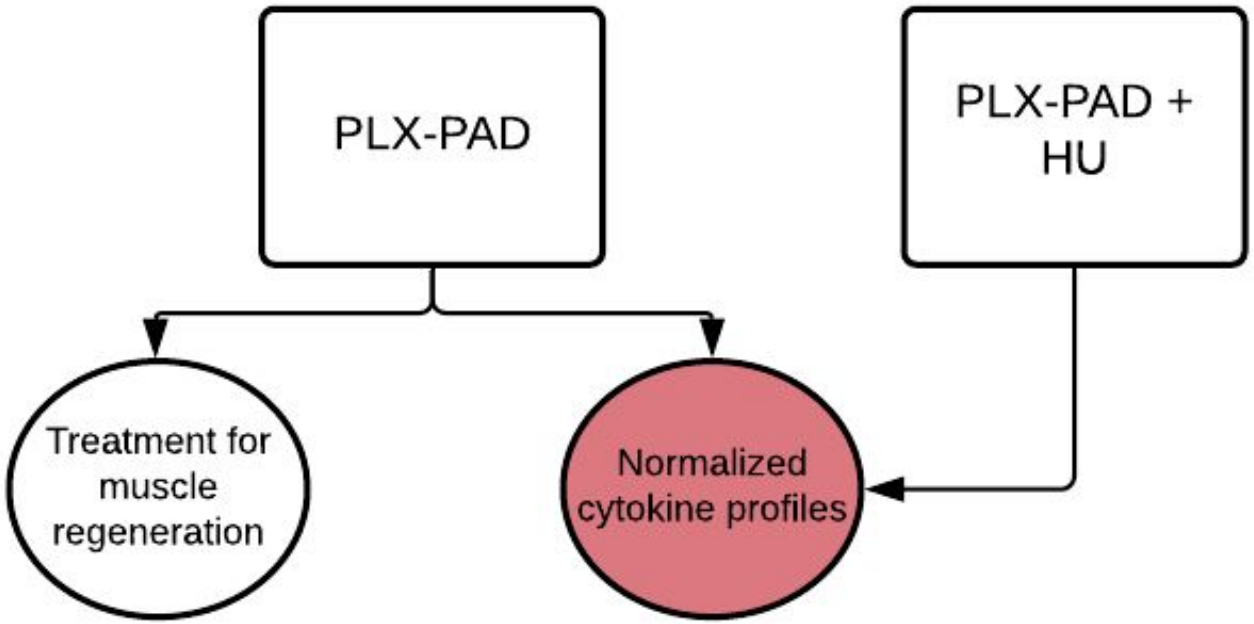
Links between physiological changes and behavioral changes!

The physiological consequences caused by spaceflight/microgravity overlap with those caused by HU[3,4,5]. Additionally, elevated levels of inflammation have been correlated to expression of anxiety and depressive-like behaviors in rodents[2,6]

What do we know from previous PLX-PAD studies?

PLX-PAD mitigates some of the negative physiological consequences caused by HU[1,2]

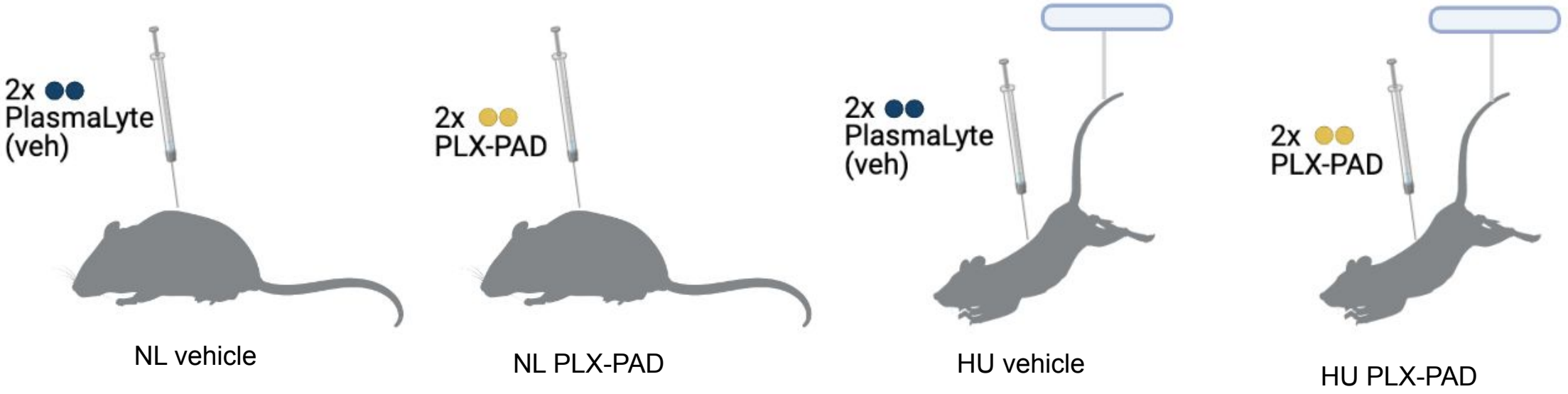
How might PLX-PAD treatment affect behavior and is there any correlation between behavior and cytokine profiles?



Methods

Experimental Design

- 4 month old female mice
- n = 7 per group (N = 28)
- Single housed

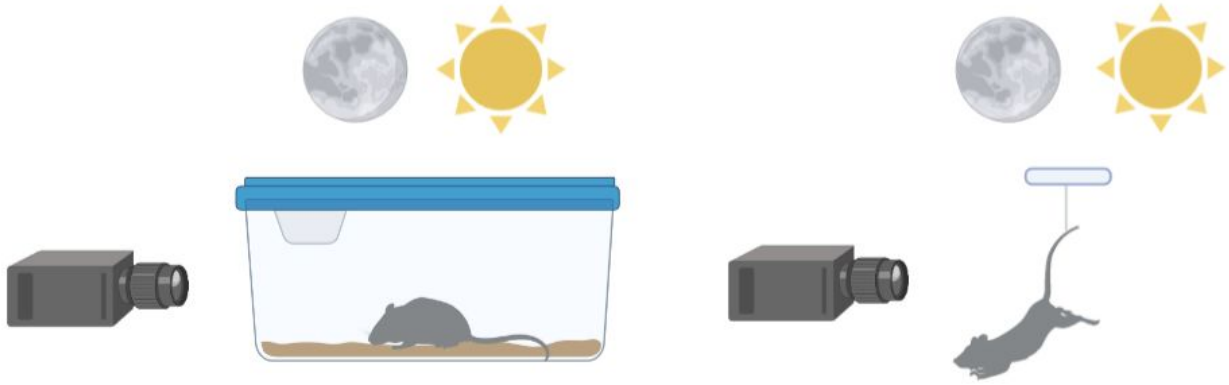


NL vehicle | NL PLX-PAD | HU vehicle | HU PLX-PAD

- **Behavioral Analysis:** scored videos of mice in the day and night (looked for 12 behaviors)
- **Cytokine Analysis:** Correlate different cytokines in the brain and plasma with behaviors seen in the mice

Video Analysis for Each Mouse

Cycle	Timepoint	Length of Video
Light	12 pm	10 minutes (600s)
Light	4 pm	10 minutes (600s)
Dark	12 am	10 minutes (600s)
Dark	4 am	10 minutes (600s)
		Total: 4 videos and 40 minutes content/mouse

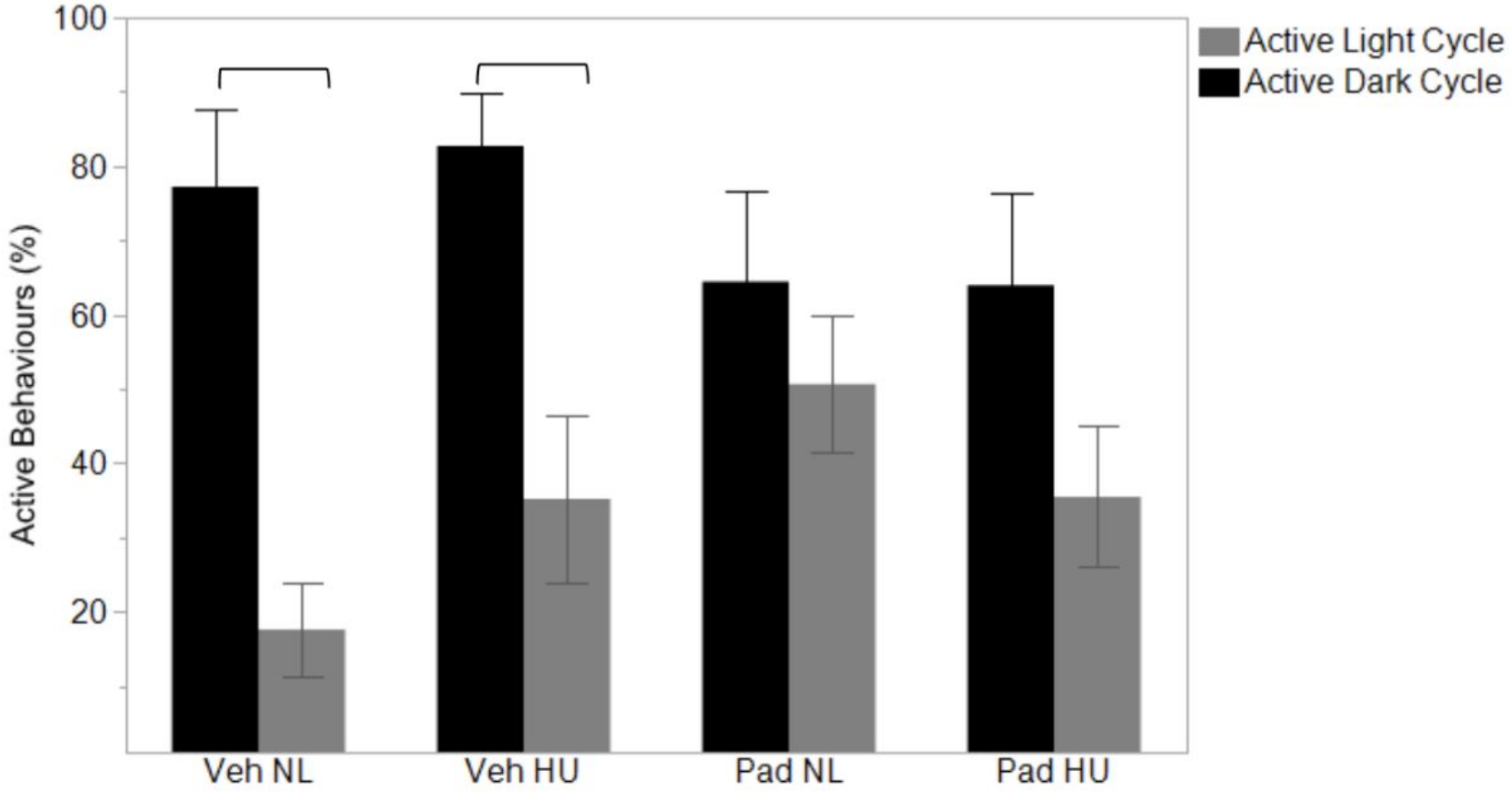


In-Cage Behaviors and Categories

Active	Inactive	Exploratory
Exploratory	Inactive	Exploratory
Eating	Involuntary Movement During Resting State (twitches)	Burrowing
Climbing	Rest	Climbing
Drinking		
Sniffing		
NesLet Manipulation		
Self grooming		
Ambulation		
Burrowing		

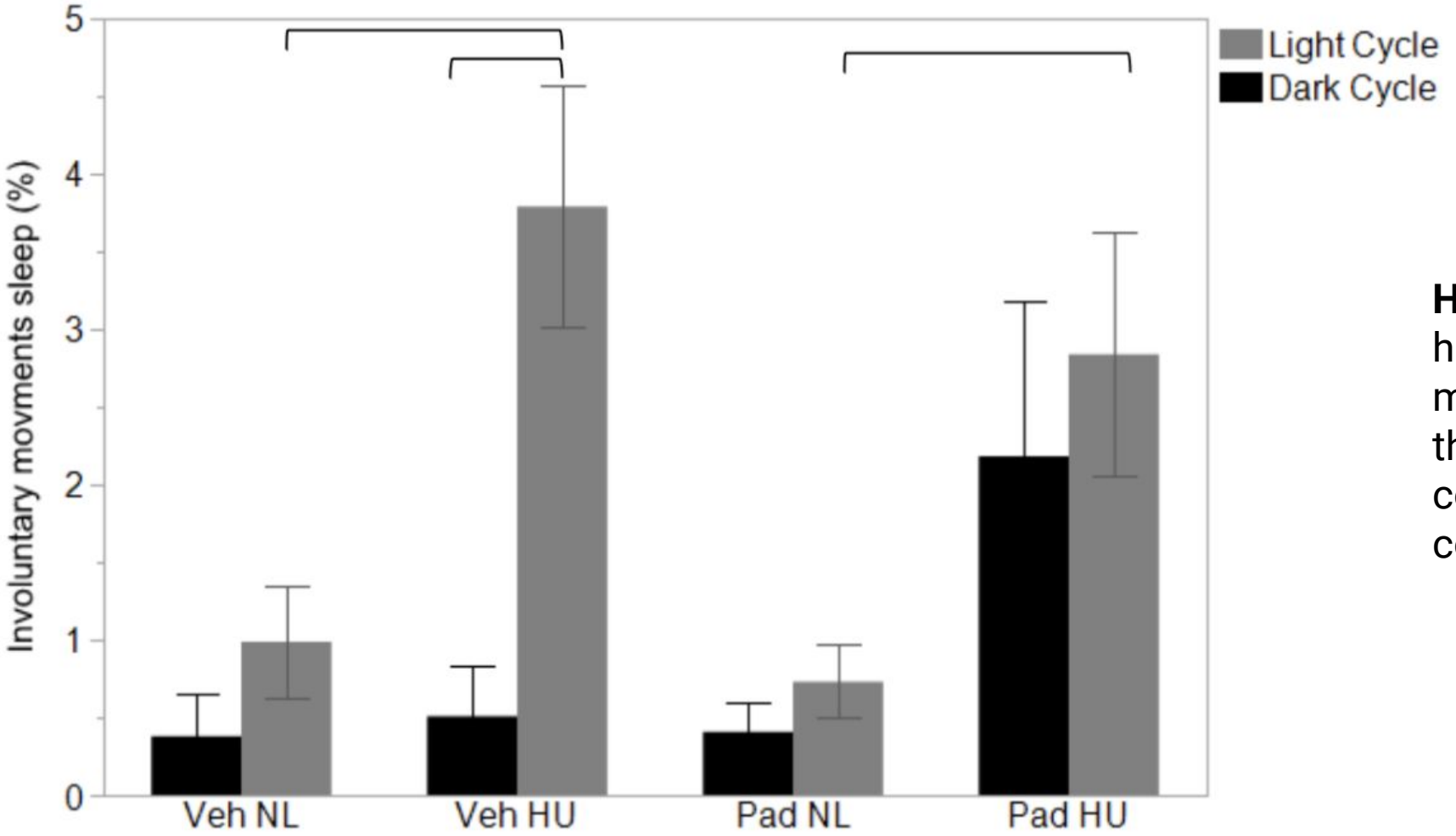
Results

Active Behaviors



Vehicle mice:
significantly higher % active behavior in the dark cycle than the light cycle

Involuntary Movement (During Sleep)/Sleep Twitches



HU mice: significantly higher % involuntary movement in the light cycle than the dark cycle compared to NL counterparts

Cytokine Results

	Brain	Plasma
Light	**Exploratory:** IL-3 **Involuntary movements:** IL-6,Mip-1b CD69+,CD3+/CD45+	**Active:** IP-10, EPO,Exodus2,IL-16,MCP-5, Mip-3b **Exploratory:** Eotaxin,MIG,Fractalkine,MCP-5,Mip-3b, Mip-2 **Rest/Sleep:** EPO,Exodus2, MCP-5, Mip-3b
Dark	**Involuntary movements:** IL-6, GCSF CD69+,CD3+/CD45+	**Exploratory:** IL-20,MCP-5, Mip-3b **Rest/Sleep:** MDC,EPO, MCP-5, Mip-3b

Blue – negative correlation
Red – positive correlation

Conclusions

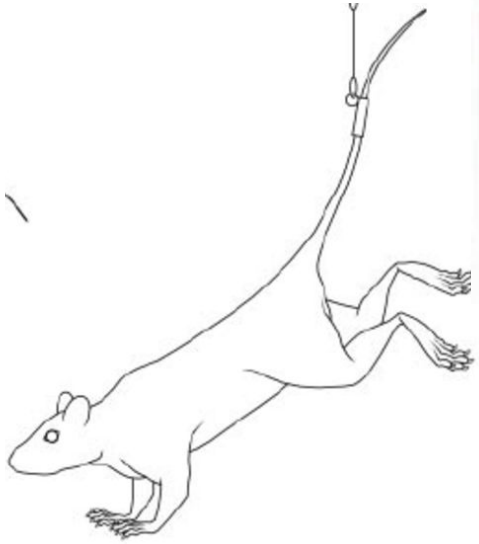
- **PLX-PAD may alter circadian patterns/sleep patterns in mice**
  - Based on no statistically significant differences between active behaviors in the light vs. dark cycle
- **HU disrupts sleep**
  - Sleep disruption has various negative physiological consequences
- **Involuntary movement/sleep twitches negatively correlates with with REM sleep cytokines**
  - Also indicative of sleep disruption in HU mice

Significance and Future Directions

Significance

WCCFtech

Pluristem Therapeutics Inc.



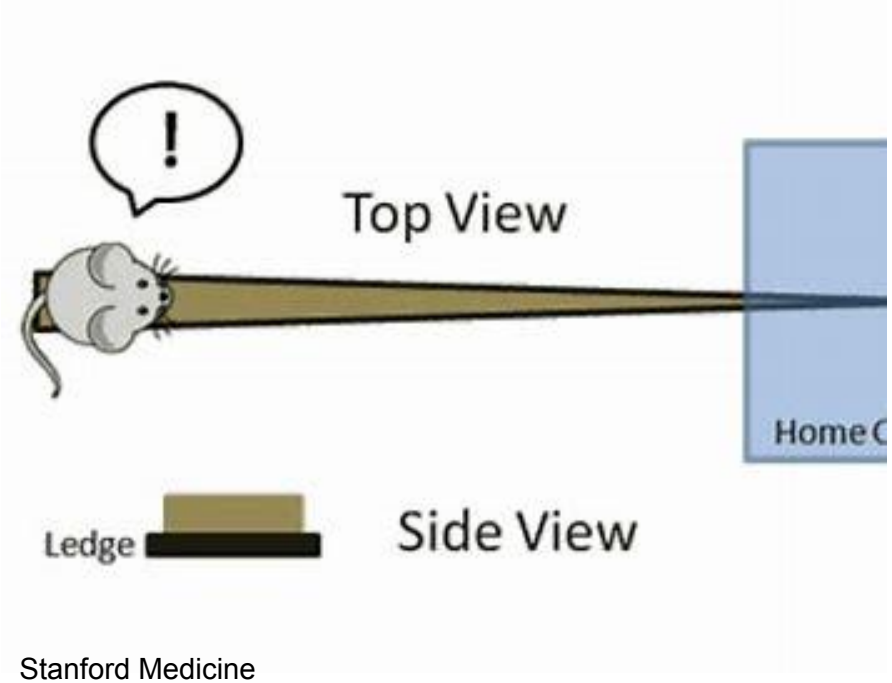
Research Gate

- Important to acknowledge the physiological and behavioral effects of PLX-PAD
  - Gain understanding of mechanisms behind its effects
  - Make clinical trials safer
- Solidify HU as an analog that can address behavior and physiology
- Gather data to help introduce PLX-PAD as an effective treatment for astronauts

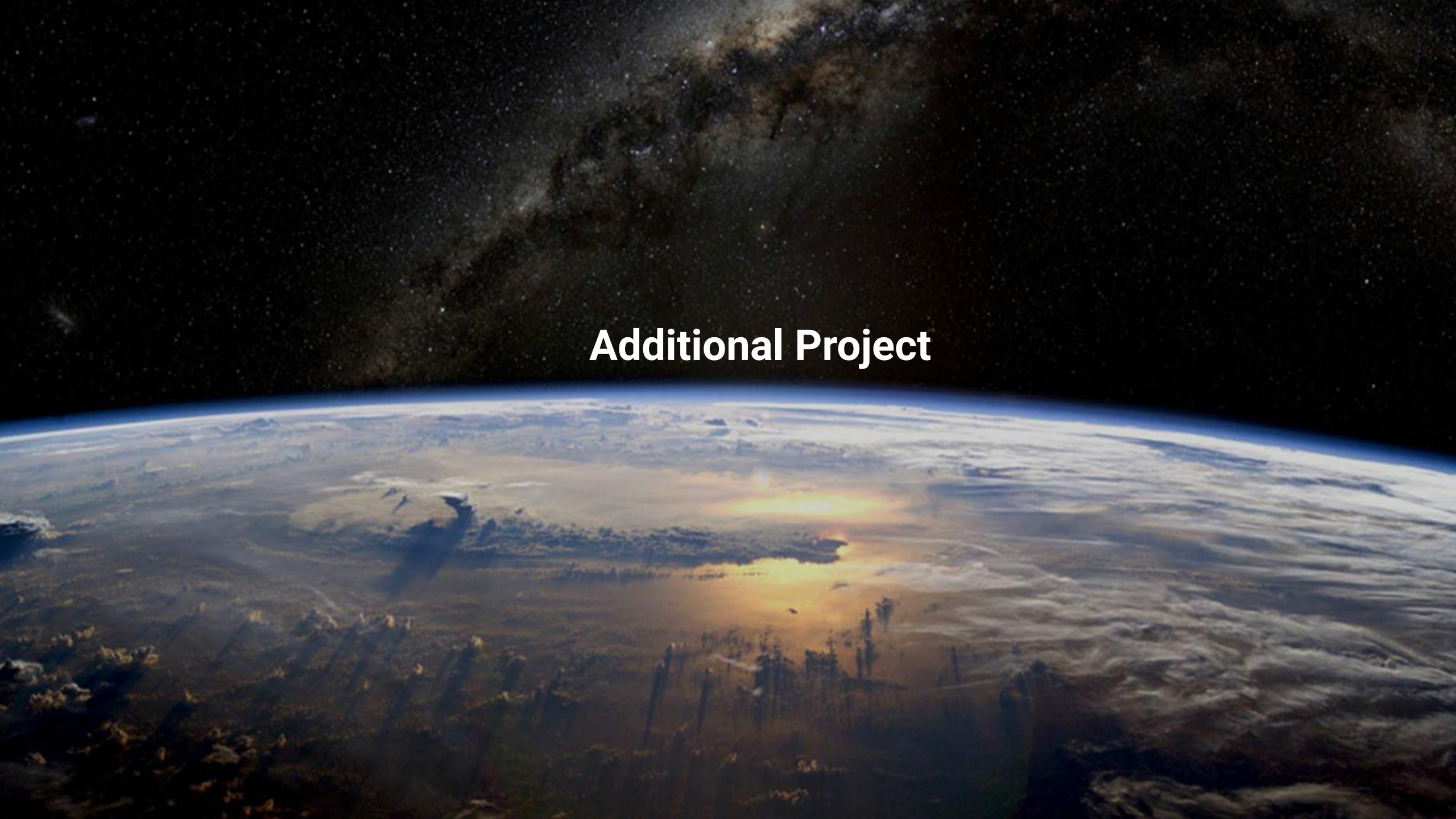
Future Directions

- Specific behavioral tests and sensorimotor tests for HU and PLX-PAD mice
- Monitor sleep in animals to investigate how PLX-PAD affects sleep
- Male and female mice (with a larger sample size)
- Implement PLX in actual space flight

Boston.com



Stanford Medicine



Additional Project

RadBREAD

- **Rad**iation **B**iology **R**esearch at an **E**levated **A**ltitude through **D**osimetry
- Recipient of the **2020 Ames Research Innovation Award (ARIA)**
- Collaboration with Swift Engineering Inc.
- Exploring the use of **Swift's High Altitude Long Endurance Unmanned Aerial Vehicle (HALE UAV) (right)** as a Mars analog and potential platform for conducting space biology experiments
- Implementing **M42 dosimeter provided by the German Aerospace Center (DLR)**
  - UV sensors?

Swift Engineering Inc.


Goals and Advice

My Goals

- **Inspire people to pursue space-related research**
- **Attend grad school**
  - PhD or masters in STEM
- **Work in the STEM industry doing my own research or project management**
  - Preferably space related, of course (:
- **Eventually, start a biotech and biology research company that is focused on commercializing and industrializing space biology**

Advice for those Interested in Space Research

- **Space isn't just for scientists and engineers**
  - Anyone can contribute!
- **Be creative and open minded**
  - There is no idea that is too crazy when it comes to space research or new technology!
- **Accept that we don't even know some of the challenges we will face when it comes to microgravity research**
- **Be excited!**
  - Space is fun and new and the opportunities for discovery really are endless

mars.nasa.gov

Acknowledgements

Dr. Ruth Globus PI on PLX grant
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- **Family and friends (:**


Questions?


Thank you, everyone!

References

- [1]Pluristem Therapeutics, Inc
- [2]Rubinstein et al. (in progress)
- [3]Crucian 2014
- [4]Vogelzangs et al 2012
- [5]Kohman and Rhodes 2012
- [6]Zhai et al. 2018

Graphic References

- Tahimic et al. 2019
- Pluristem Therapeutics Inc.
- News Medical
- WCCFtech
- Research Gate
- Boston.com
- Stanford Medicine
- Swift Engineering Inc.

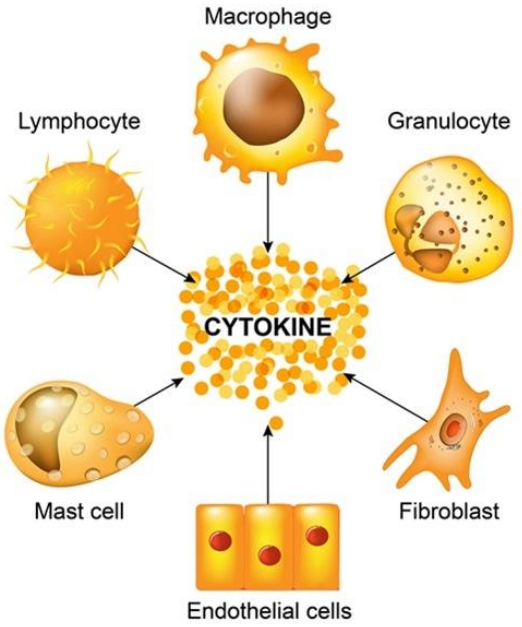
Supplementary Slides

Background Summary

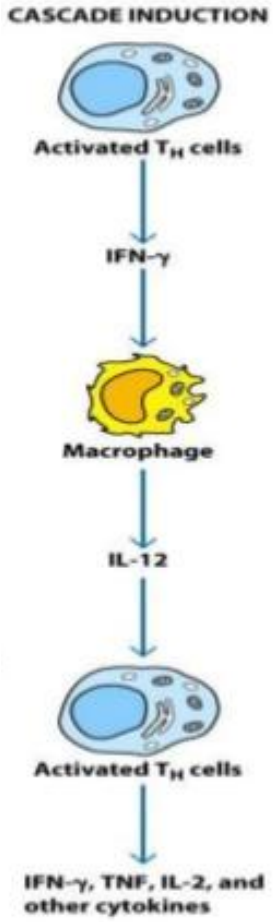
- HU, spaceflight, and microgravity = negative physiological consequences
- HU = known to increase anxiety and depressive like behaviors
- PLX-PAD = mitigates physiological consequences caused by HU
- How might PLX-PAD affect behavior and is there any correlation between behavior and cytokine profiles?

Physiology and Behavioral Detailed Background

- **Stress from spaceflight and microgravity** alters immune cell responses, inflammation, and gene expression of inflammatory markers (Crucian 2014)
- **Behavioral studies on earth**: links between elevated levels of inflammation and depressive like behaviors and inability to learn new behaviors (Vogelzangs et al. 2012; Kohman and Rhodes 2012)
    - **HU Behavioral Studies:** similar results
- **HU alone:** alters cytokine profiles in the brain and plasma and immune cell concentration in the plasma (Rubinstein, in press)
- **HU in combination with PLX:** normalized levels of cytokines in the brain and immune cell concentrations in plasma (Rubinstein, in preparation)



News Medical

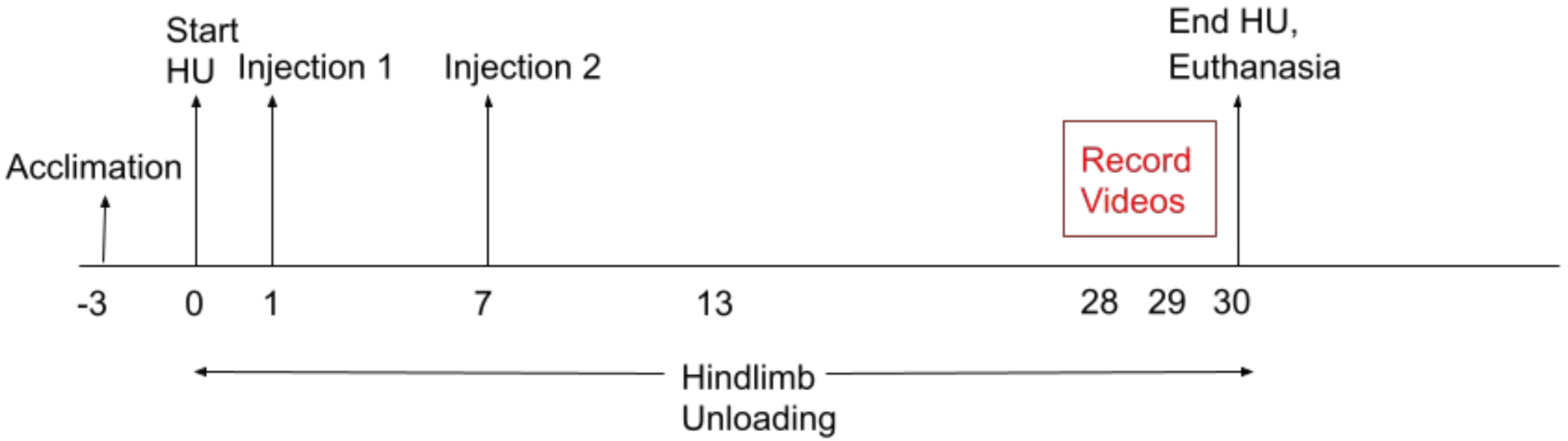


Soman 2016

Hypothesis

- After 4 weeks of HU, HU animals will exhibit different behavioral patterns than normally loaded (NL) animals
- In HU animals, PLX will mitigate some of the differences in behavioral patterns

Experimental Timeline



Calculation Example

Mouse ID #10

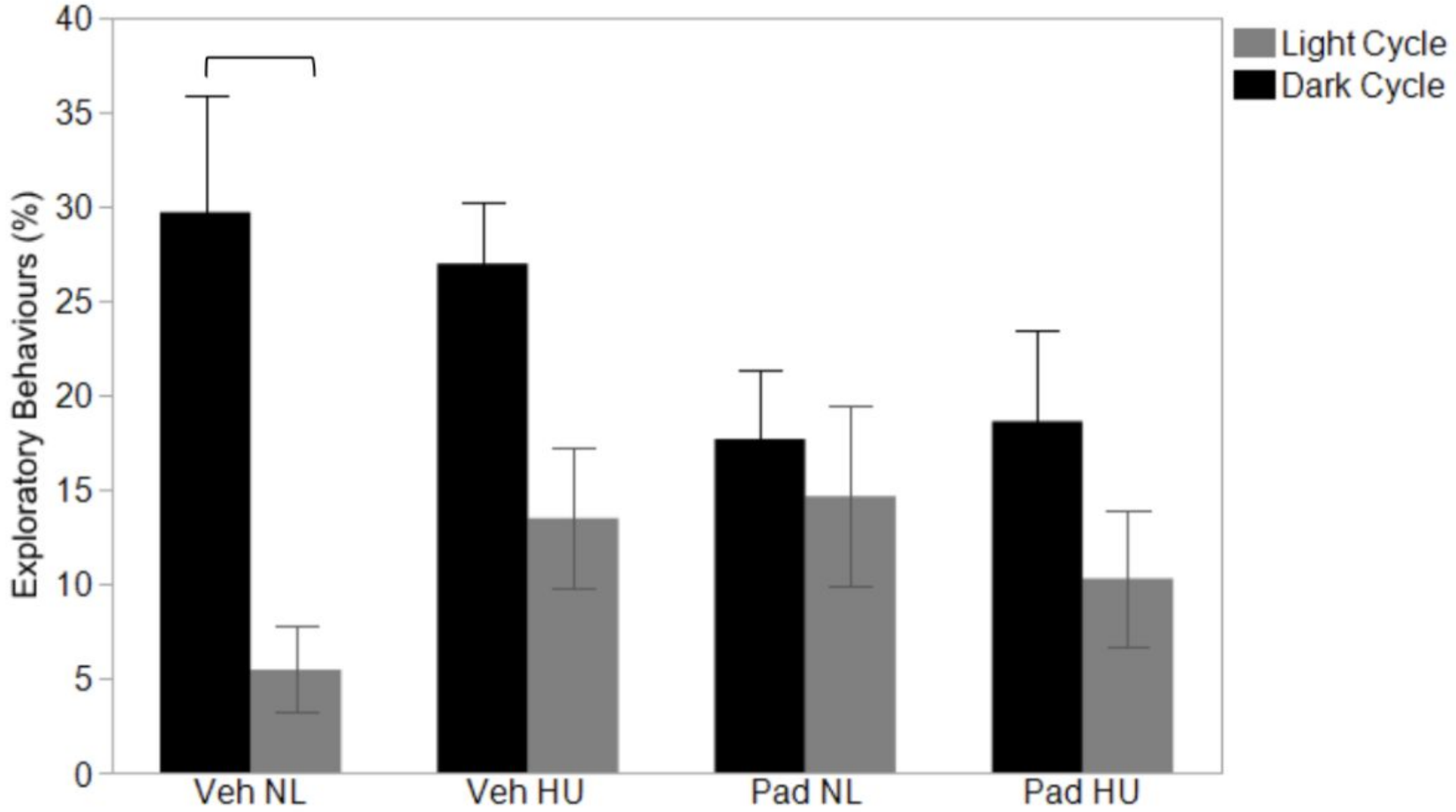
$$\frac{Time\ spent\ performing\ a\ behavior}{Total\ time\ of\ light\ or\ dark\ cycle\ videos} x\ 100\% = \%\ of\ time\ performing\ behavior$$

$$\frac{200\ seconds\ sleeping\ in\ light\ cycle}{1200\ total\ seconds\ of\ light\ cycle\ videos} x\ 100\%$$

$$= 16.67\%\ of\ time\ in\ the\ light\ cycle\ spent\ sleeping$$

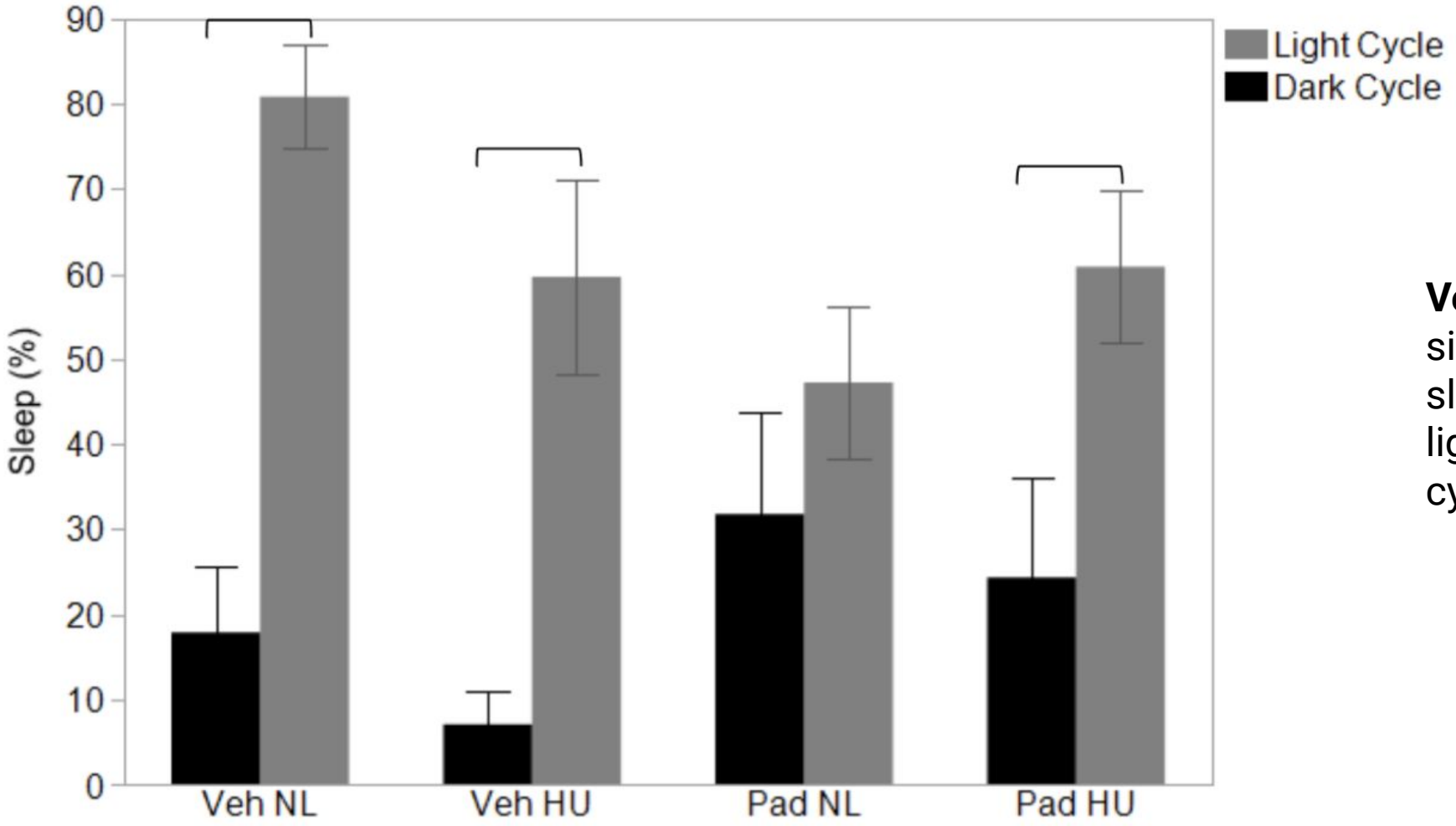
Repeat this for every behavior/behavioral category for every animal and then take the mean percentages of each group

Exploratory Behaviors



Vehicle NL mice:
significantly higher % exploratory behavior in the dark cycle than the light cycle

Sleeping Behavior



Vehicle and PAD HU mice: significantly higher % sleeping behavior in the light cycle than the dark cycle

Limitations

- Pilot study
  - HU single and pair-housed study should be done in the future
- Video quality during the night cycle was not always good
- Ideal experimental design: film before and after HU and injections
- Only basic in-cage behavior studied
- Only females
- Need more animals to decrease standard deviations