Overexpression of Catalase in Mitochondria Mitigates the Effects of Simulated Microgravity and Social Isolation on Cytokine Expression in Mouse Hippocampus

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Space Biosciences
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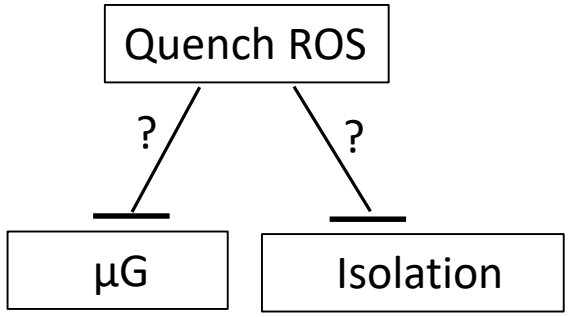
Problem

- Aging, sedentary lifestyle and spaceflight have similar degenerative effects on our body
- Chronic inflammation (via ROS generation) implicated in age-related pathologies, e.g. neurodegeneration (“Inflamm-aging”)
- Define contribution of ROS to neurodegeneration during exploration class missions

Specific Hypothesis

Aspects of space environment (microgravity and social isolation) increase ROS to regulate neuroinflammatory cytokines in the hippocampus



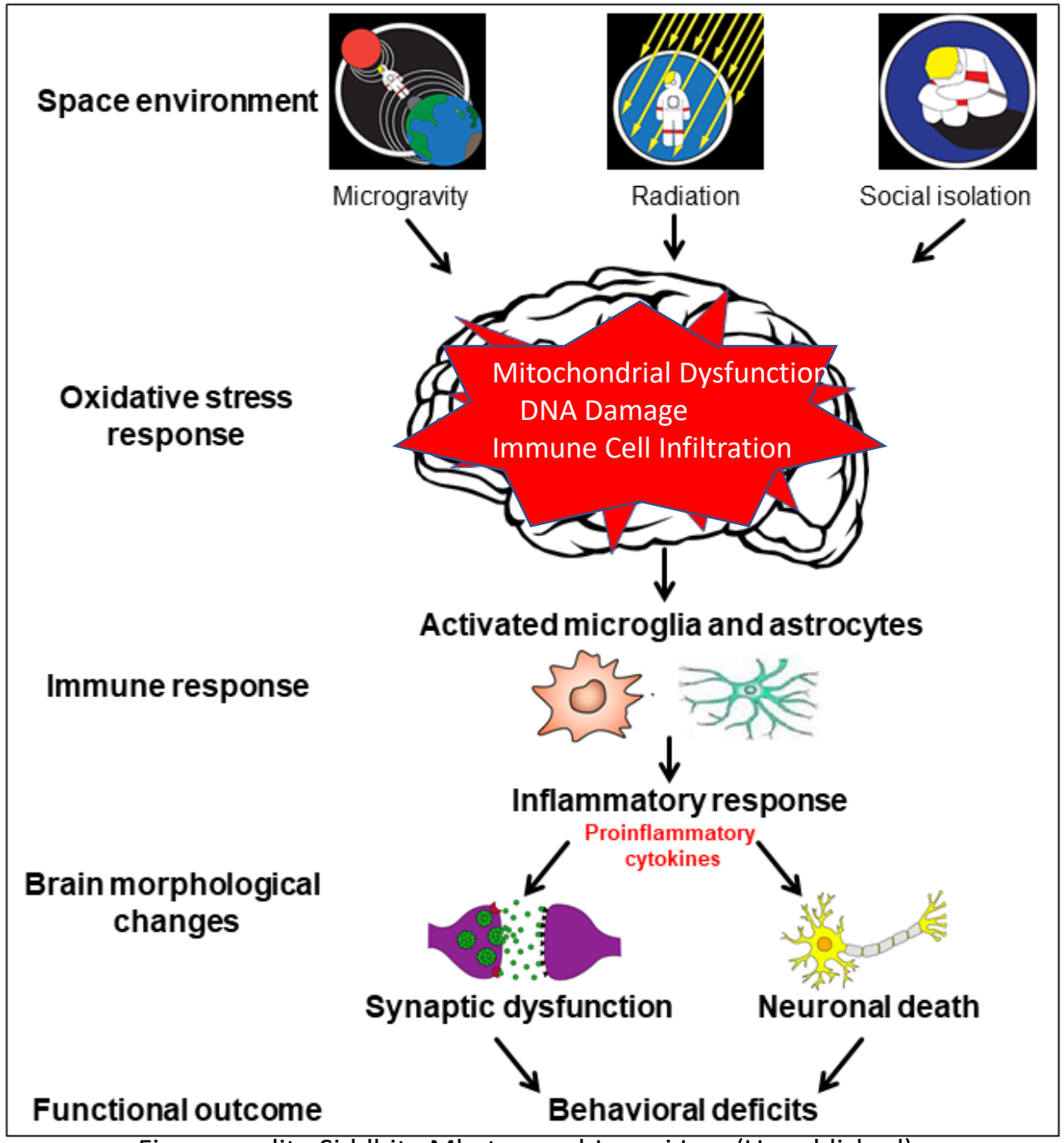
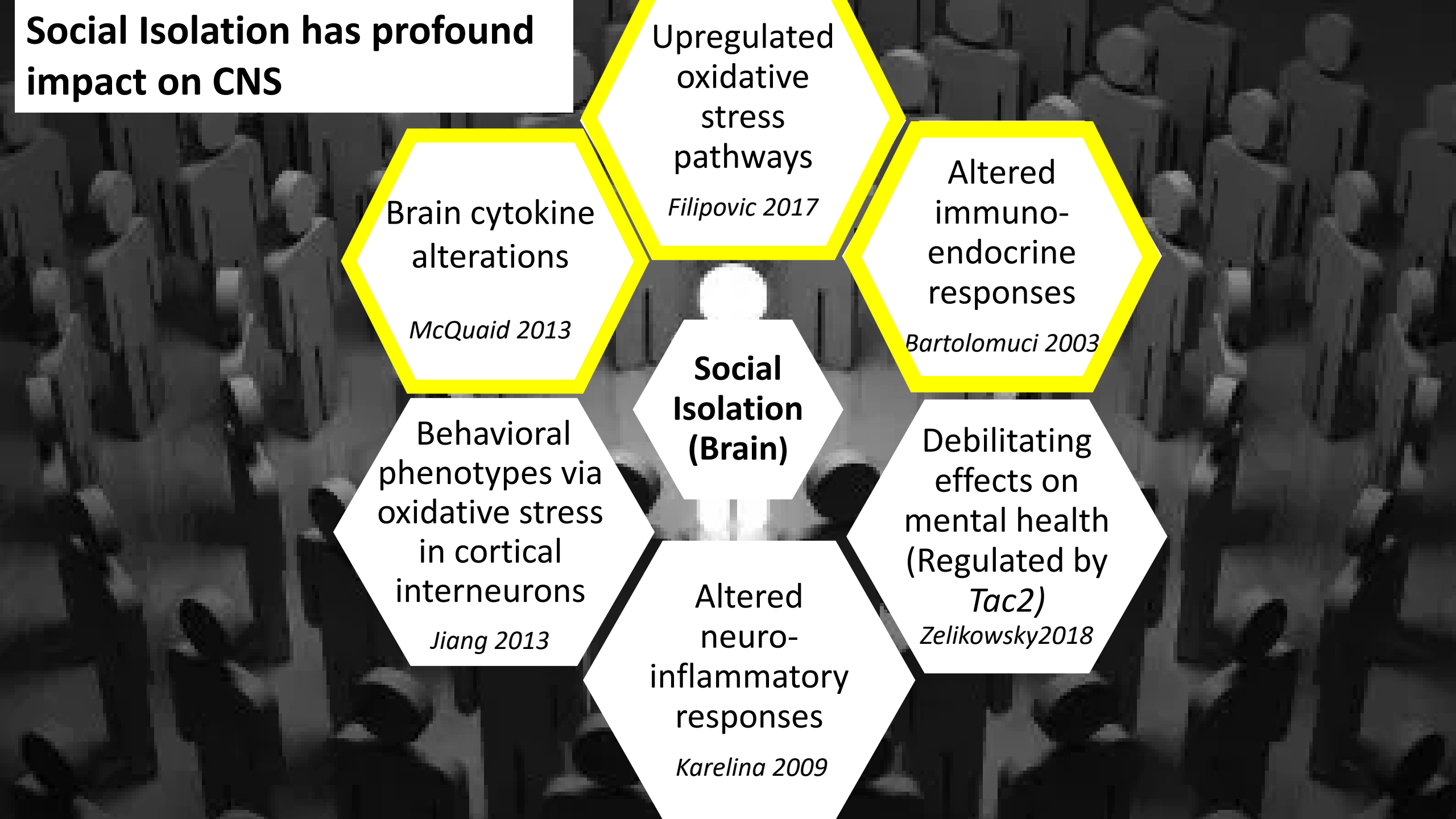


Figure credit: Siddhita Mhatre and Janani Iyer (Unpublished)

Social Isolation has profound impact on CNS



Effects of Microgravity on the Mammalian Immune System

- Both simulated and actual microgravity regulate cytokine expression
- Standard Hind limb unloading (HU) model entails single housing (social isolation stress)
- Distinguish between the effects of social isolation and microgravity

Spaceflight
7-13 Days
Increased IFNγ
Increased splenic ROS

Gould 1987
Pecaut 2016

HU 21 Days

Shifts from B to T (Spleen)

No cytokine changes

Gaignier 2014

HU 21 Days

Upregulation of cortisol, CRH, ACTH

Luan 2017

HU 7 Days
IL-1β increased
IL-2 decreased in spleen

Felix 2004

HU 4 Days

Increase in circulating IFN-α and IL6

Zhou 2012

Astronauts
3 days

Elevated IL-6

Stein 1994

Astronauts
182 days
Reduced
IFNγ, IL-10,
IL-5, TNFα, IL-6

Crucian 2018

MCAT transgenic mice: quench mitochondrial ROS

MCAT transgenic mice overexpress human catalase gene in mitochondria

Life span

- Increased mean and maximum life span [*Schriner 2005*]

CNS effects

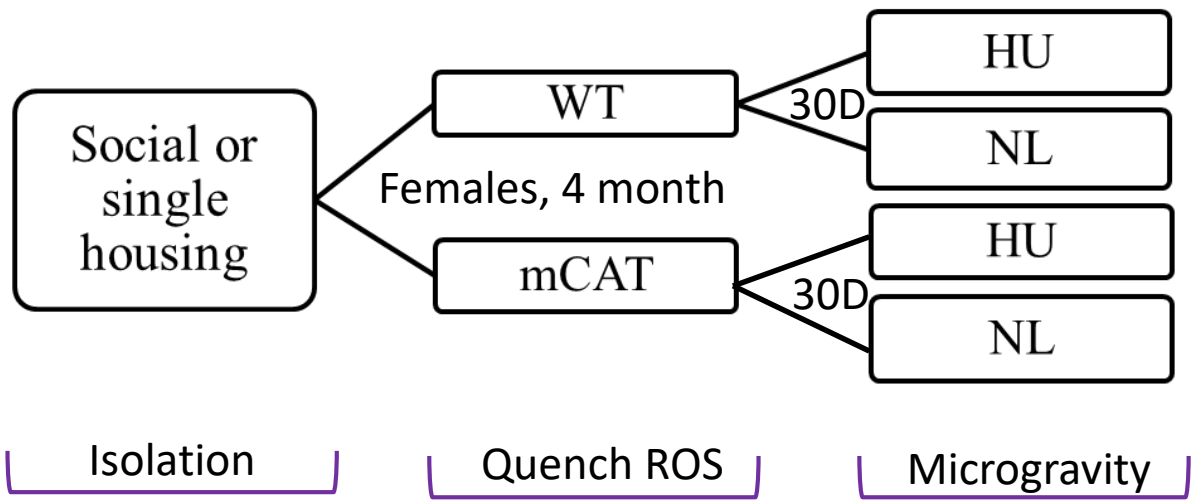
- Enhanced hippocampal spatial learning and memory, reduced contextual fear conditioning [*Olsen 2013*]
- MCAT mitigates radiation-induced deficits in behavioral performance (novel object recognition) and neuronal morphology [*Parihar 2015*]

Age-related disease

- e.g. Delayed cardiac pathology and cataract development [*Schriner 2005*]

Image: http://togotv.dbcls.jp/ja/togopic.2017.28.html

Experimental design



Standard single housed Social housed (paired)

Hindlimb Unloading (HU)

Assays Performed

Brain (Hippocampus):

- **Cytokine protein expression Multiplex assay (44 plex)**
- 4-HNE Elisa (lipid peroxidation)
- Park7 Elisa

Plasma:

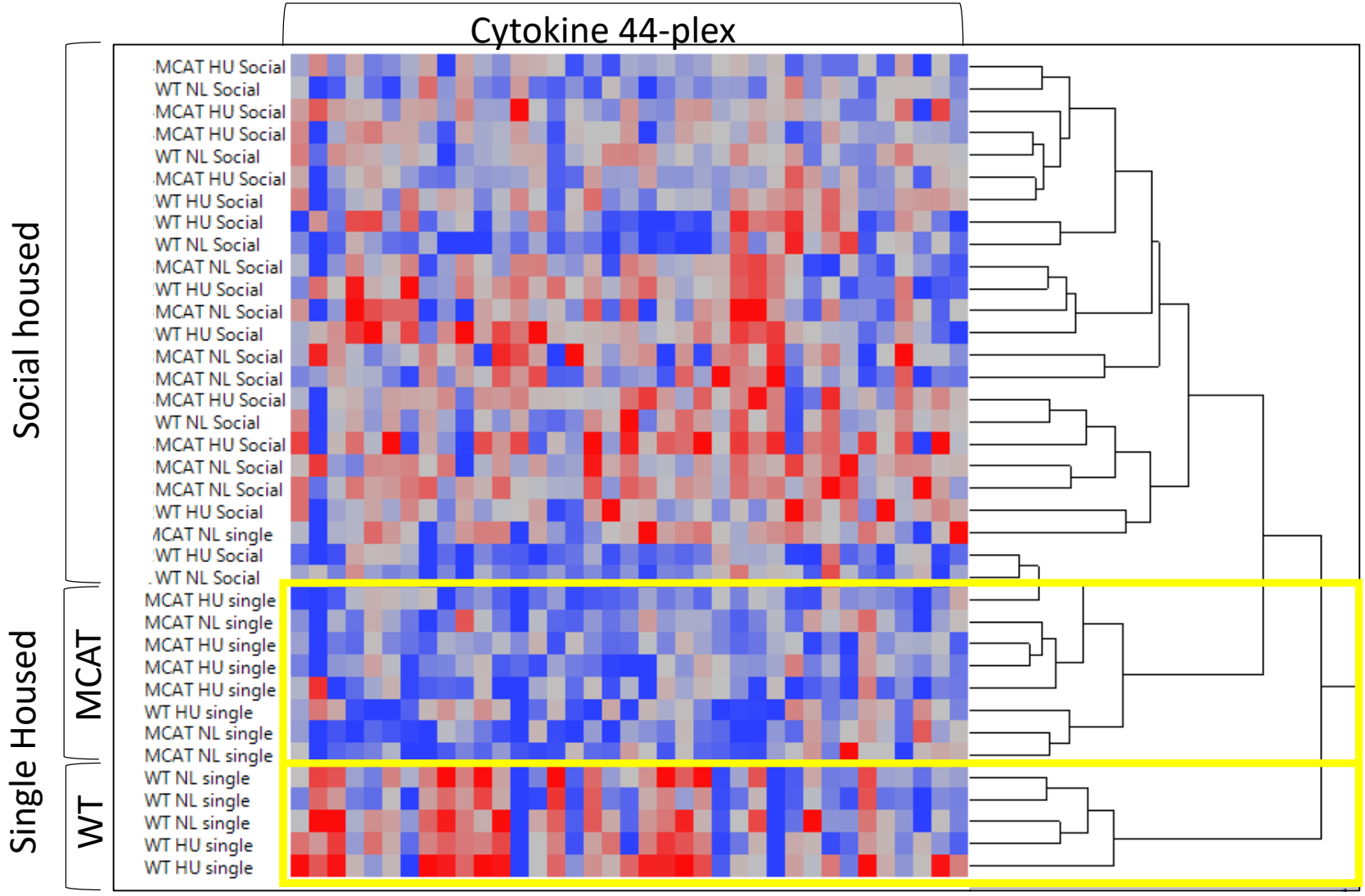
- **Cytokine protein expression Multiplex assay (44 plex)**
- **Corticosterone Elisa**
- 8-Hydroxyguanosine Elisa
- Immune assays (Dr.Amber Paul)

Behavior:

24 hour filming and behavioral analysis (Collaboration with Dr. April Ronca)

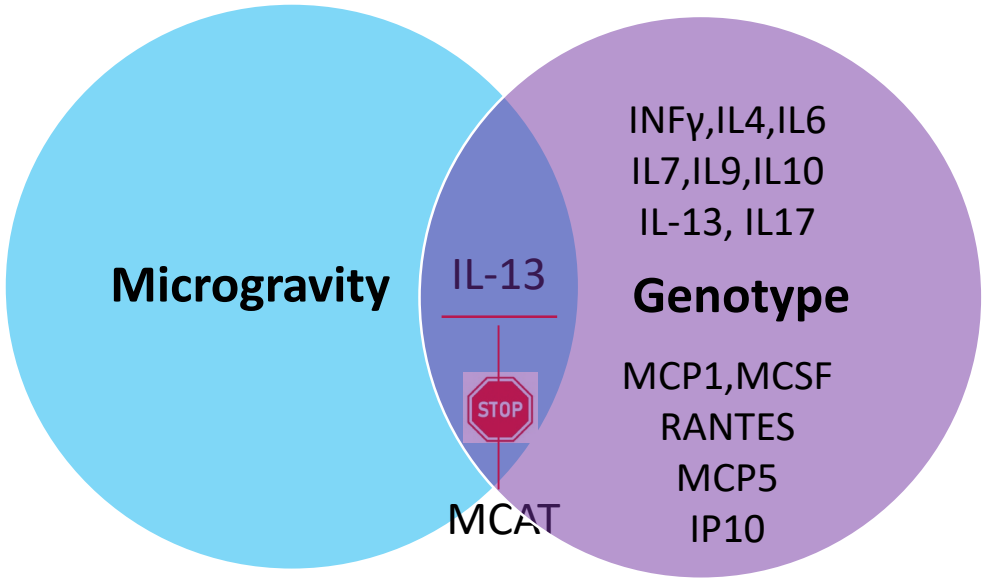
Other tissues collected: Heart, Bone, Soleus, Spleen, Adrenal, Aorta, Eyes

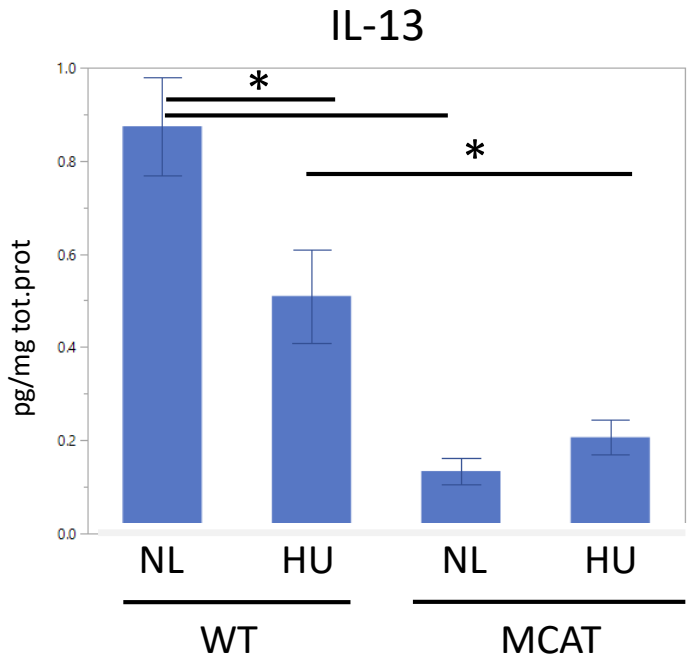
Hierarchical clustering of cytokine expression in the hippocampus

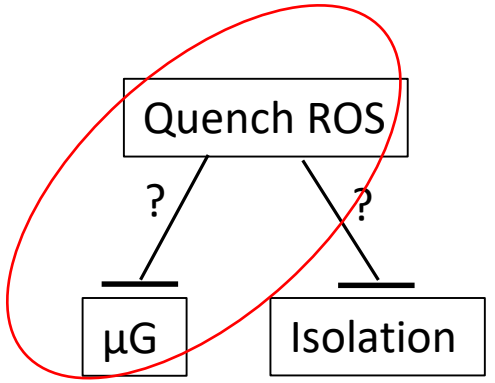


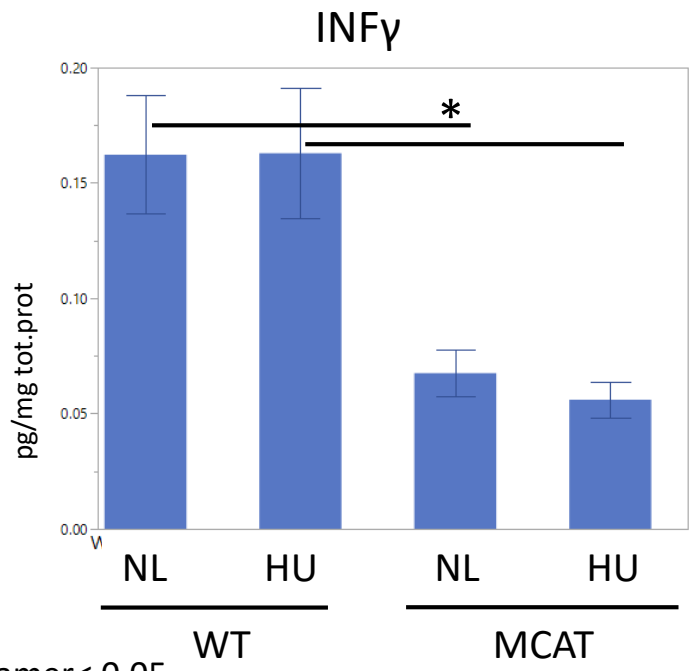
Single housed mice clustered separately from social housed and subclustered by genotype

How does HU affect hippocampal cytokine expression? Does genotype mitigate? (In single housed standard HU model)







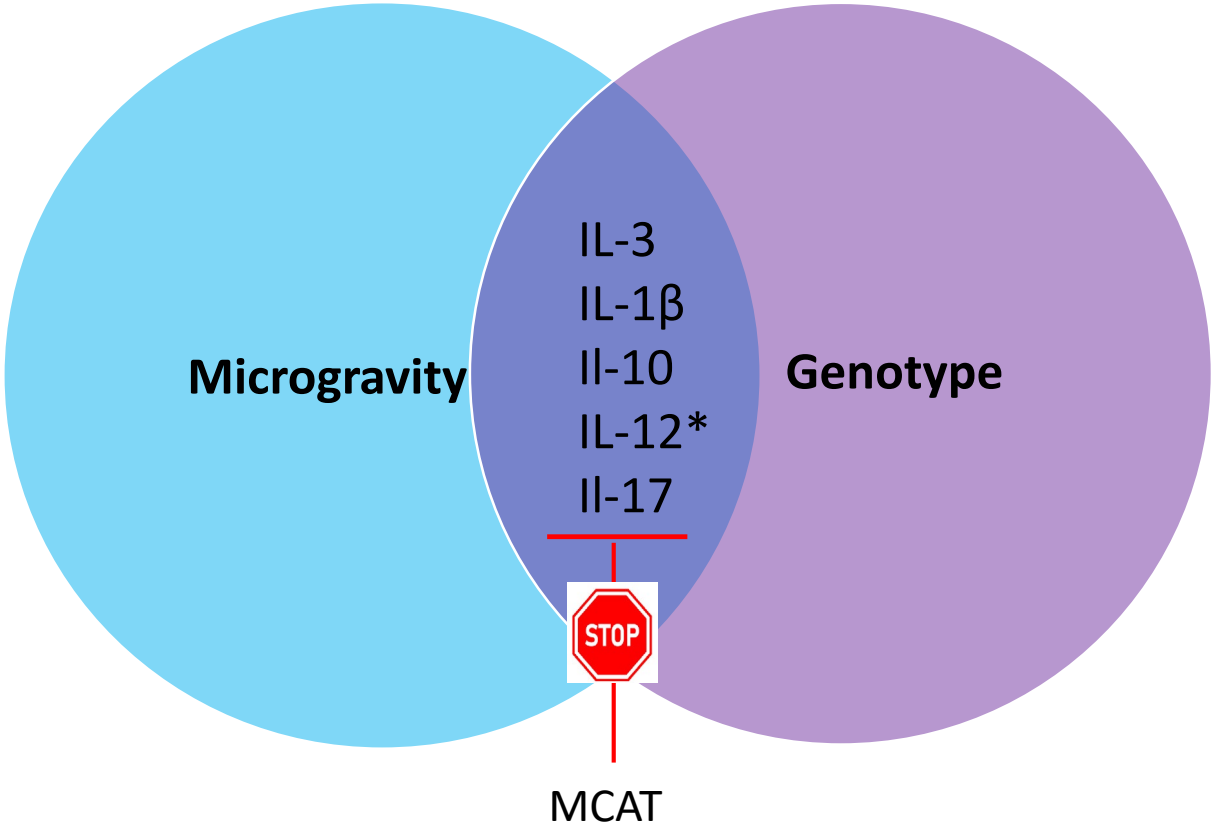


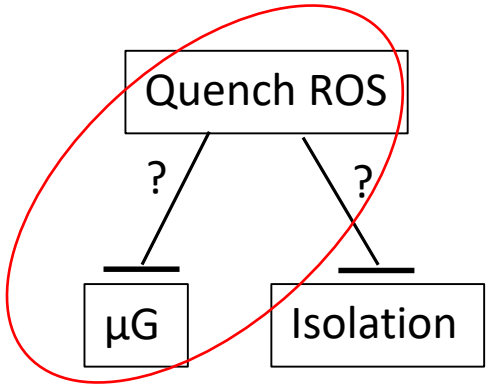
- Microgravity effect (HU vs NL) 1/44 differ
  - MCAT mitigates
- Genotype effect (WT vs MCAT) 13/44 differ

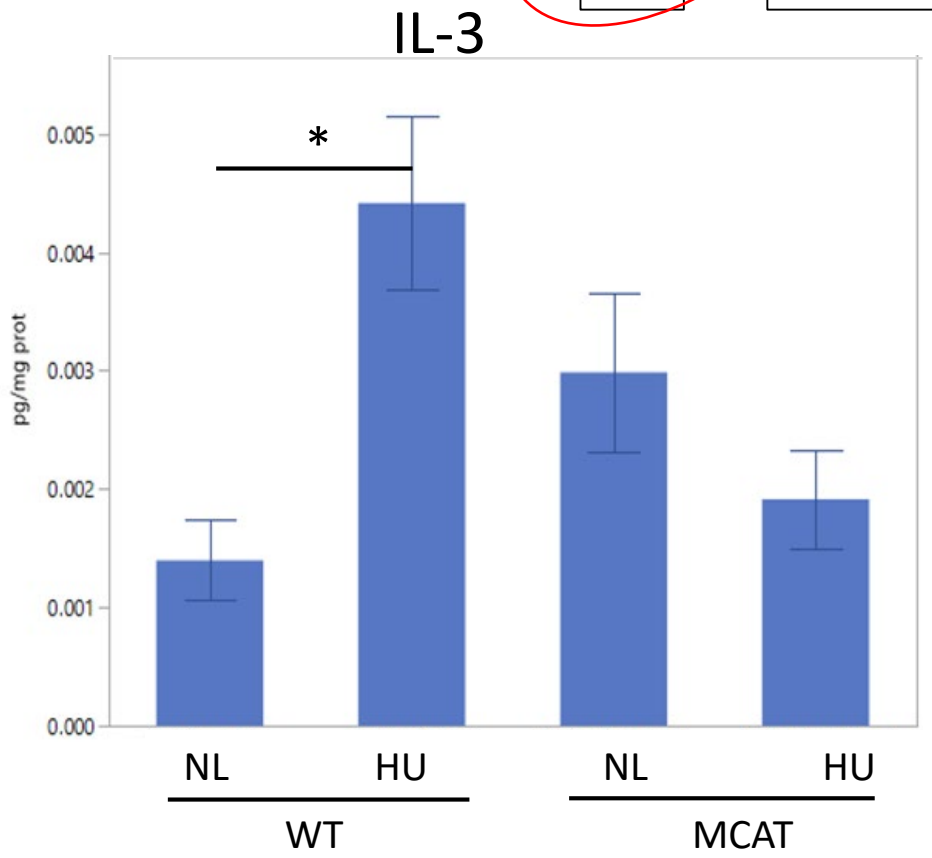
Do the same results obtain in socially housed mice?

MEAN ± SE 2-factor ANOVA.*Tukey Kramer< 0.05

How does HU affect hippocampal cytokine expression? Does genotype mitigate? (In social housed HU model)



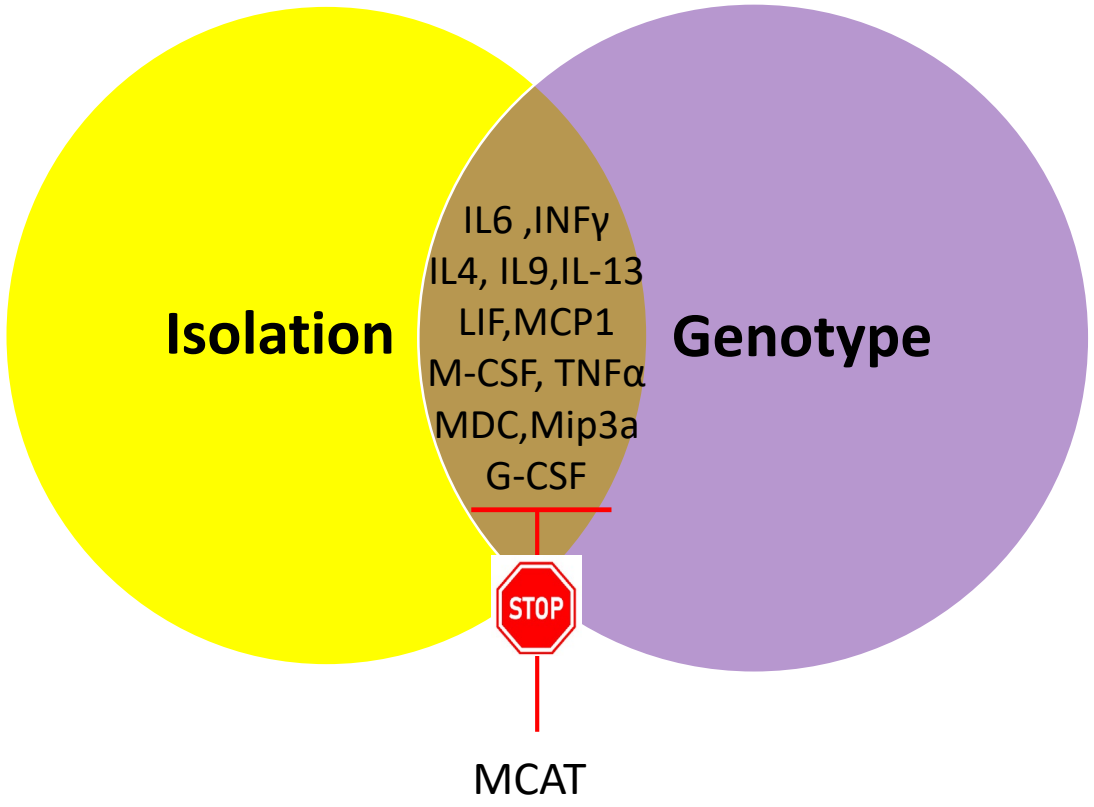


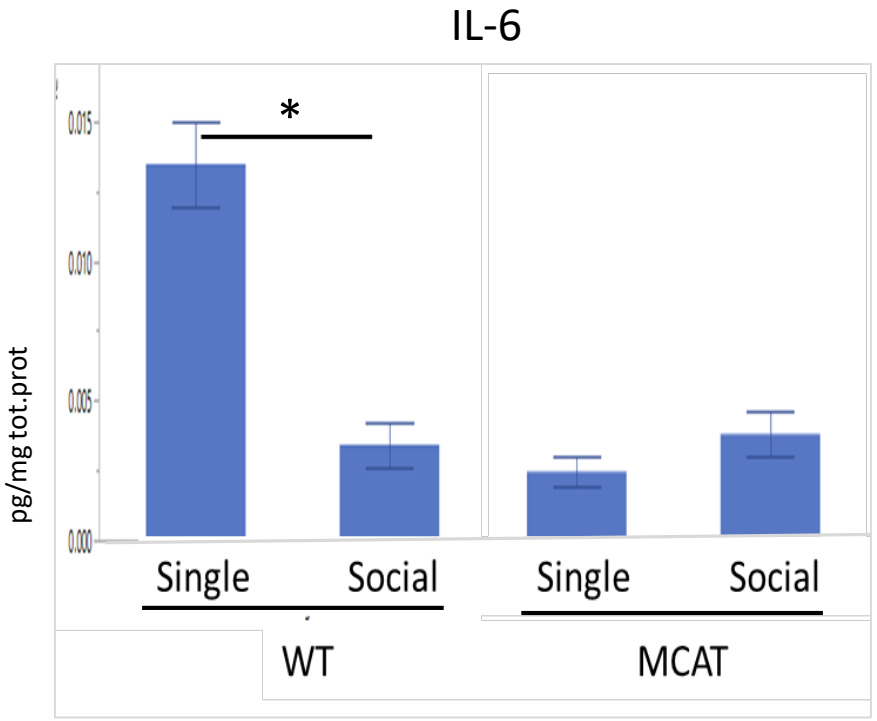


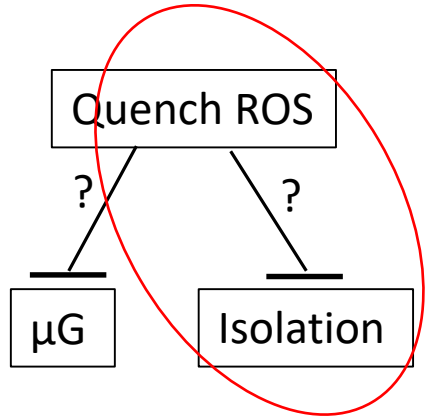
ANOVA*Tukey Kramer< 0.05

- Microgravity effect (HU vs NL) 5/44 differ
- MCAT mitigates

How does social isolation affect hippocampal cytokine expression? Does genotype mitigate?





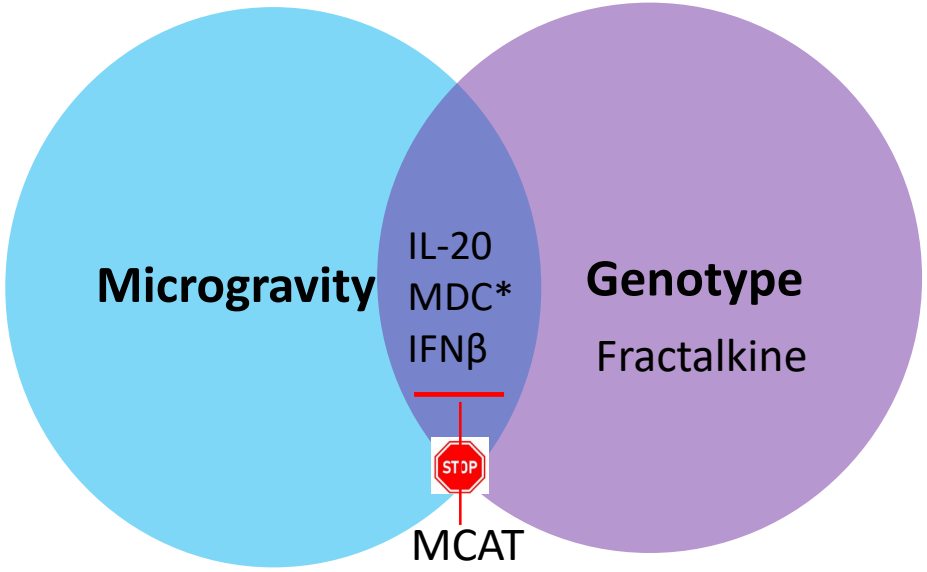


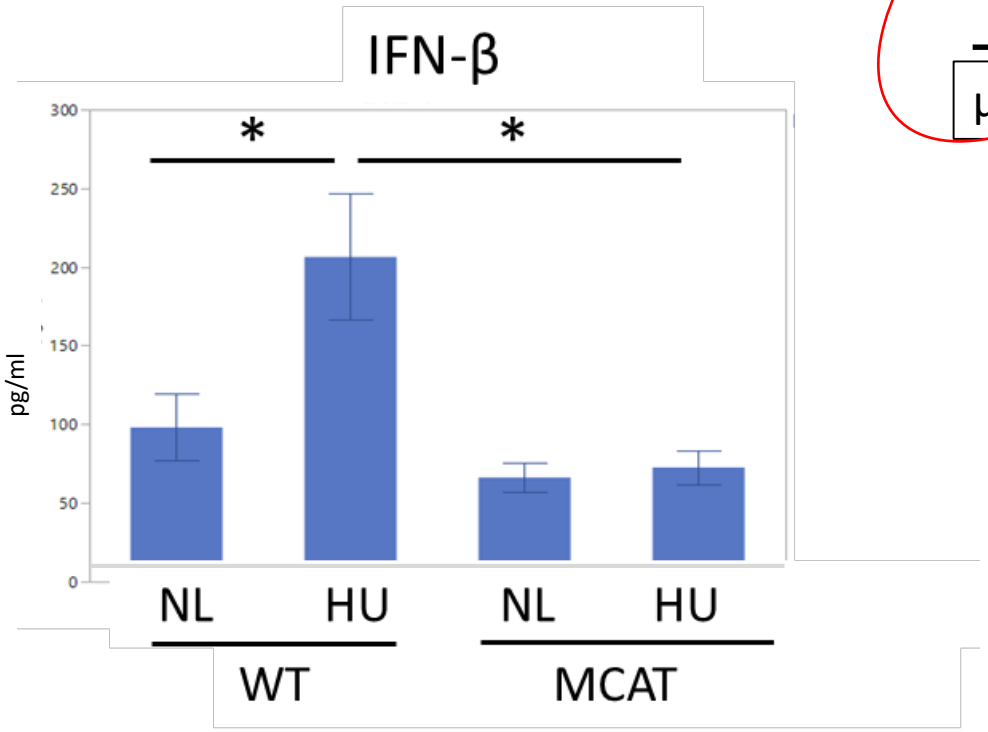
- Isolation effect (Single vs Social) 12/44 differ
  - MCAT mitigates

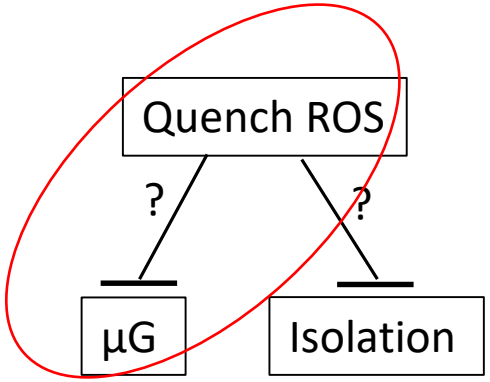
ANOVA*Tukey Kramer< 0.05

Are these effects local or systemic?

How does HU affect cytokine expression in plasma? Does genotype mitigate?
(In single housed standard HU model)



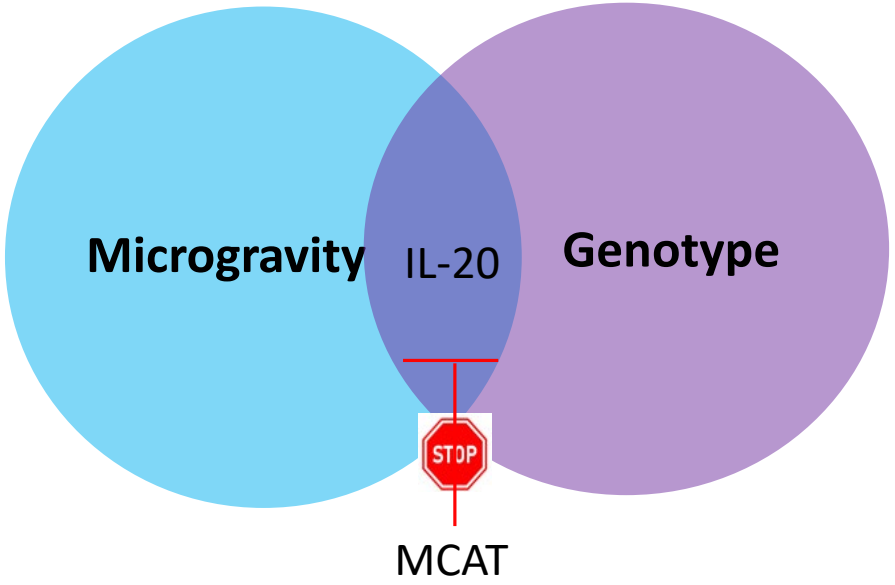


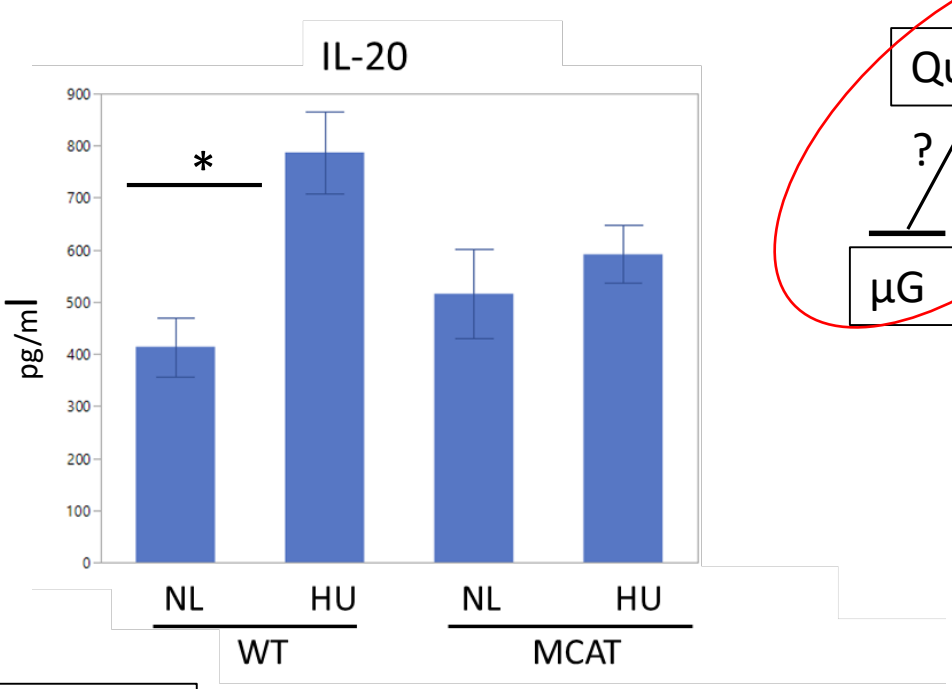


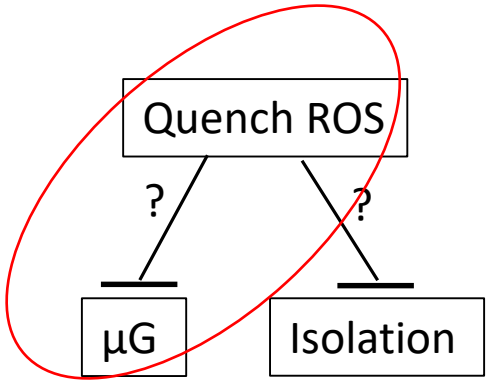
- Microgravity effect (HU vs NL) 3/44 differ
  - MCAT mitigates

ANOVA (Tukey-Kramer*p<0.05)

How does HU affect cytokine expression in plasma? Does genotype mitigate? (In social housed HU model)





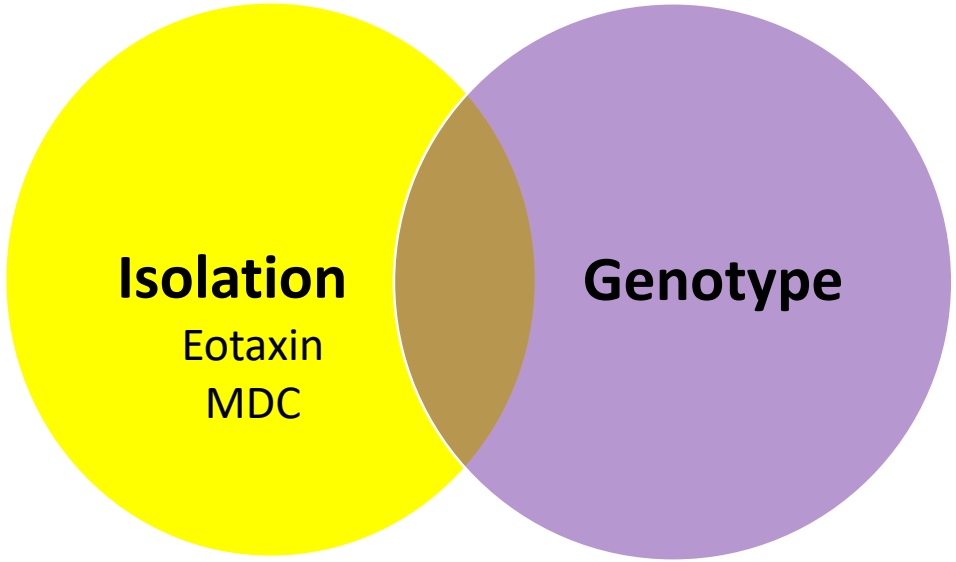


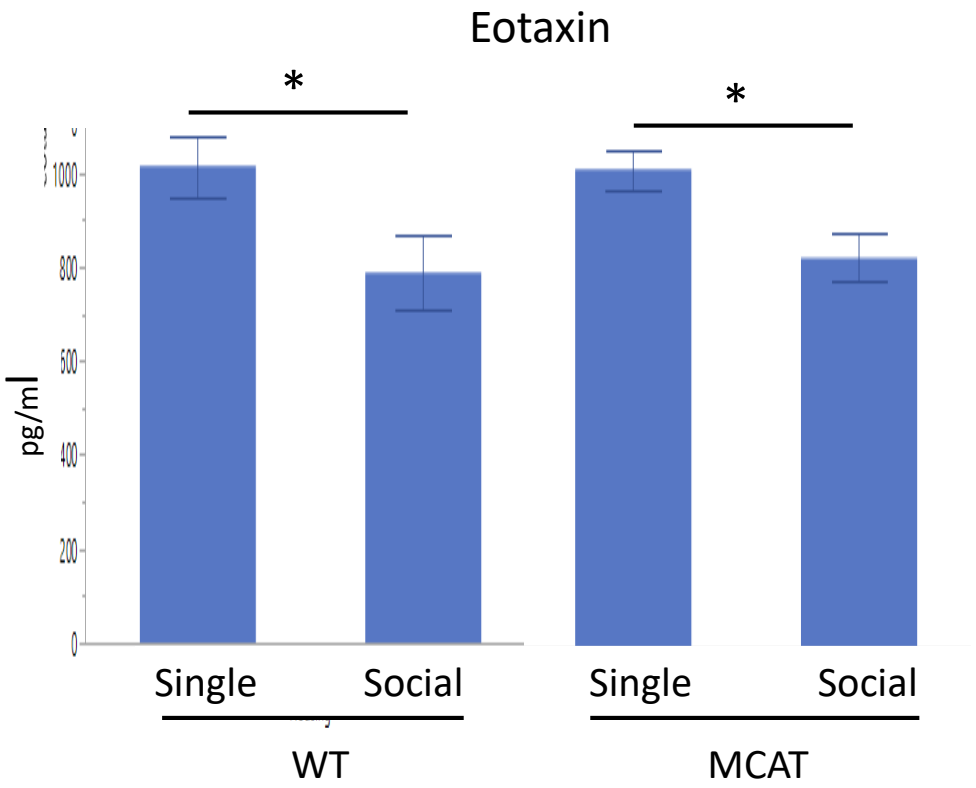
- Microgravity effect (HU vs NL) 1/44 differ
- MCAT mitigates

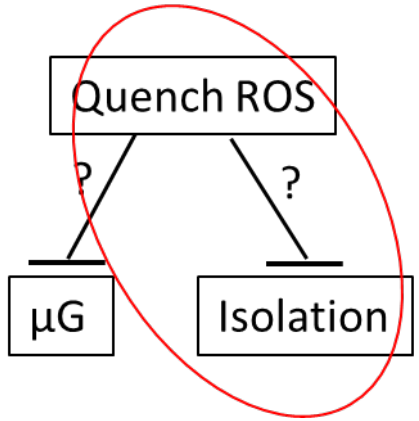
- IL-20 Biomarker for rheumatoid arthritis (*Kragstrup 2016*)
- IL-20R1-deficient mice -higher bone mineral density (*Hsu 2011*)
- IL-20 family is involved in vascular inflammatory diseases (*Autieri 2018*)

ANOVA*Tukey Kramer< 0.05

How does social isolation affect cytokine expression in plasma? Does genotype mitigate?



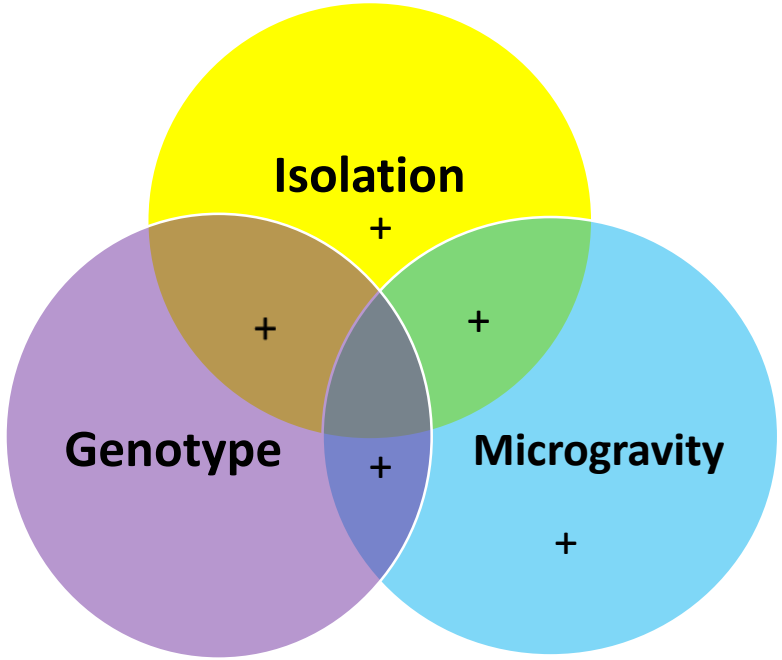




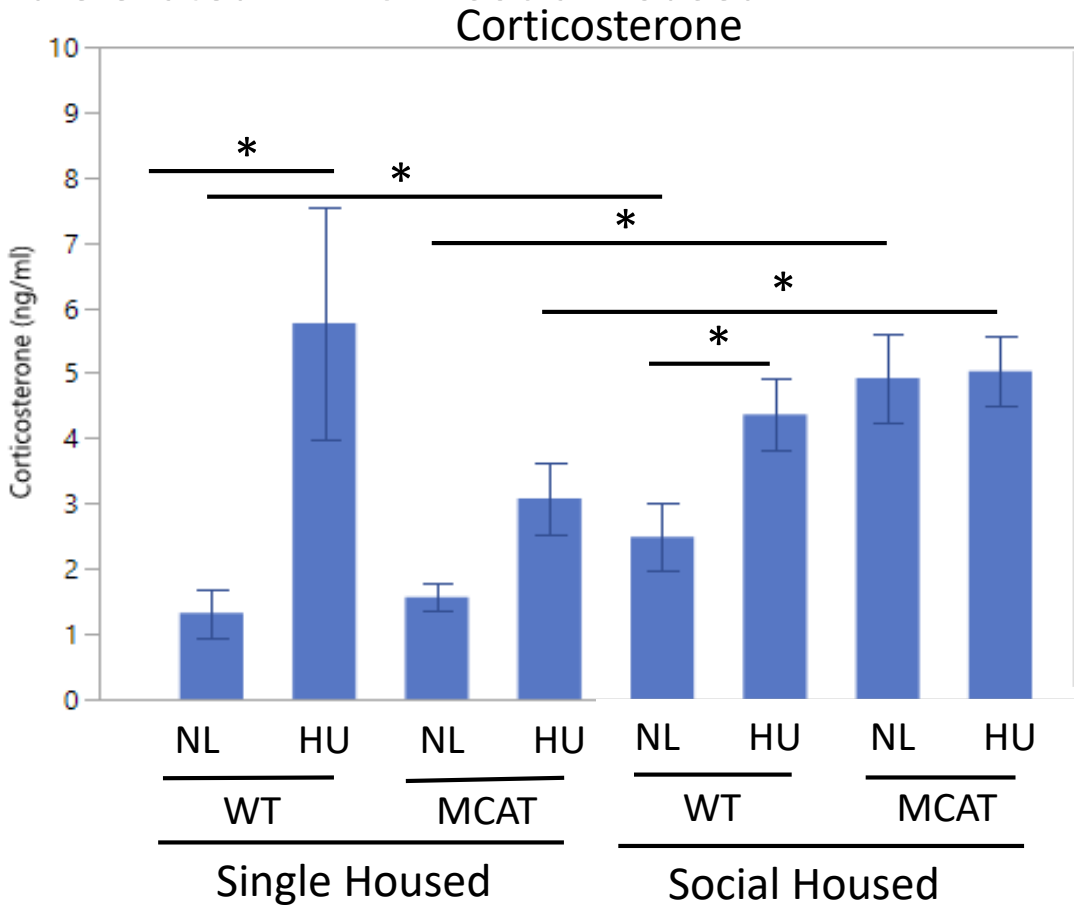
- Isolation effect (Single vs Social) 2/44 differ
- MCAT does **not** mitigate

ANOVA*Tukey Kramer< 0.05

Plasma Corticosterone was elevated due to microgravity in both single and social housed mice, mitigated in MCAT and elevated in MCAT social housed



Corticosterone	
Plasma	Brain
Negative correlation	Positive correlation
MDC	Eotaxin
	IL-1α
	IL-3
	IP-10



Correlation of higher cytokines and corticosterone levels was linked to smaller hippocampal volume in the elderly (IL-3 is linked to human brain volume variation)
(*Sudheimer 2014,Luo 2012*)

Non parametric Wilcoxon $p<0.05$

Correlation of behavior and cytokine profiles in long term space travel could help reveal potential biomarkers

Exploration	
Brain	Plasma
G-CSF	IL-20
Mip3-a	Timp1
INF γ	
IL-4	
IL-10	
IL-12p40	
IL-12p70	
M-CSF	
Mip2	
EPO	

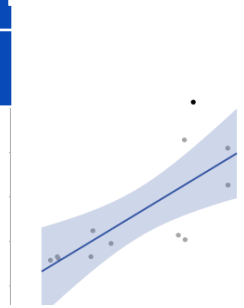
Social interaction	
Brain	Plasma
IL-4	
Mip2	
EPO	
G-CSF	
IL-1β	
IL-6	
Mip3-a	

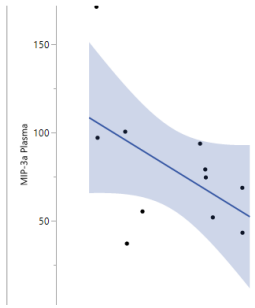
Eating	
Brain	Plasma
IL-2	Timp-1
MCP-1	

Inactivity	
Brain	Plasma
INF-γ	KC
	Timp1
	Mip3a

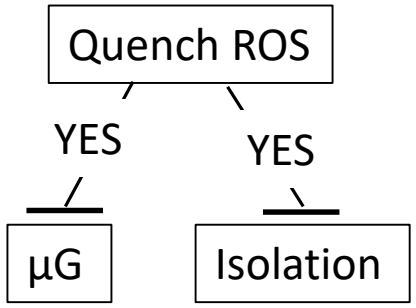
Active behavior	
Brain	Plasma
	G-CSF
	KC
	Timp1

Positive correlation	Negative correlation

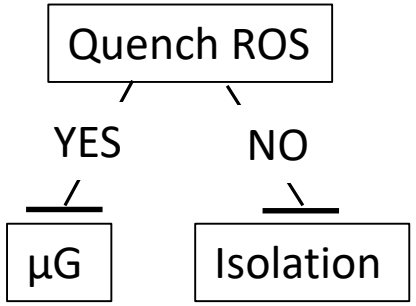




Inactive



Summary of results



Hippocampus

- Microgravity effect:
  - Single: 1/44 cytokines altered
  - Social: 5/44 cytokines altered
- Isolation effect:
  - 12/44 cytokines altered
- MCAT
  - Mitigated effects of both microgravity and isolation on cytokine expression

Plasma

- Microgravity effect:
  - Single: 3/44 cytokines altered
  - Social: 1/44 cytokines altered
  - Single: Corticosterone levels elevated
  - Social: Corticosterone levels elevated
- Isolation:
  - 2/44 cytokine altered
  - Corticosterone levels elevated in social vs single (except HU)
- MCAT
  - Mitigated effects of microgravity but not isolation on cytokine expression

Conclusions

- Long term, simulated microgravity altered cytokine expression levels in both plasma and hippocampus; this effect was mitigated in the MCAT mice implicating an important role for mitochondrial ROS in weightlessness

- Social isolation posed a strong stressor on the hippocampus with elevated cytokine expression; quenching mitochondrial ROS mitigated this effect, implicating an important role for mitochondrial ROS in isolation stress

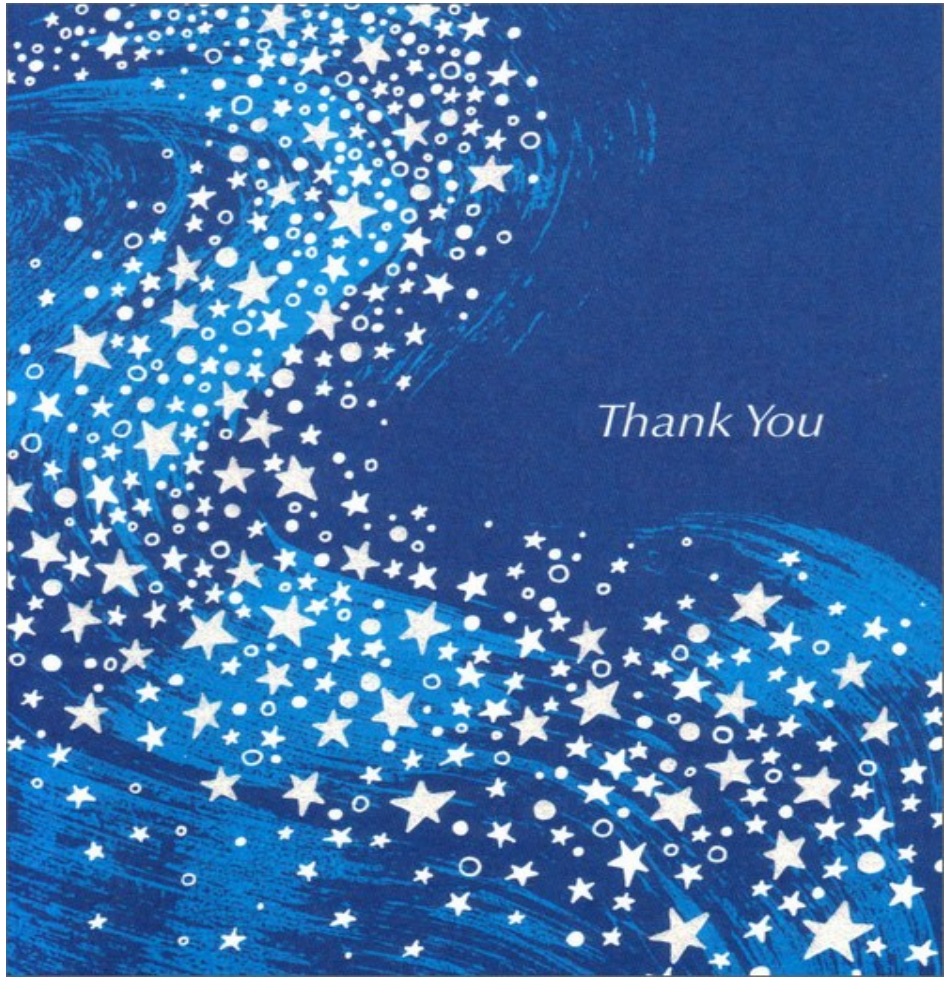
- The cytokine responses to social isolation were more extensive in brain vs plasma

Possible Future Countermeasures

- Cytokines/immune profiles may provide useful biomarkers for neuro-inflammation, and help predict behavioral deficits for long term space missions

- Antioxidants may be good candidates for mitigating the effects of long term microgravity and social isolation on the brain; both these stressors are highly relevant for long term space travel

- Social engagement may mitigate “inflamm-aging” in space, as well as on Earth



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Dr. Amber Paul (NASA Ames Research Center)

Future Plans

Underlying mechanisms

Brain:

Microglia:

- CD68 (activated microglia)
- Iba1 (all microglia)
- TRAP mice (Ribosomal RNA profiling in microglia)

BBB markers:

- AQP4, Occludin, Claudin5

Neurogenesis:

- Doublecortin

T-cells:

- CD3

Astrocytes:

- GFAP

Plasma:

HPA axis

Q-PCR/RNA aging arrays

Sex differences

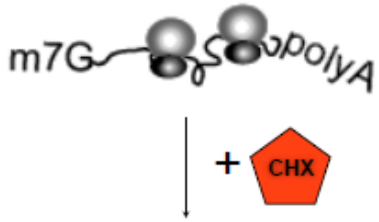
Provoked behavioral experiments (memory social interactions, stress-developed in HU settings)

Gut leakiness ,LPS

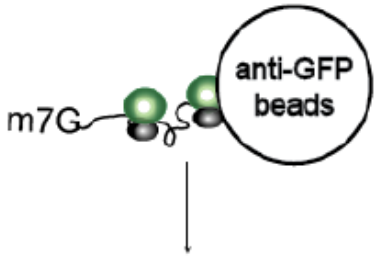
TRAP* mice (collaboration with Dr. Anne Schieffer, Mount Sinai NY)

* Translating ribosome affinity purification is a method initially developed for profiling mRNA from genetically defined cell types in complex tissues

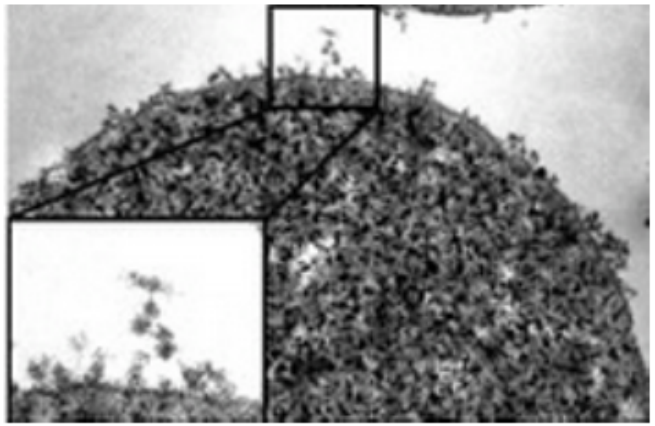
Translating Ribosome Affinity Purification (TRAP) captures ribosome-bound transcripts in complex tissues



I. chemical trapping of mRNA within polysomes



II. affinity purification



Heiman and Schaefer et al. 2008