



National Aeronautics and
Space Administration



Simulated microgravity affects behavior and cytokine expression in the hippocampus of adult mice: influence of mitochondrial reactive oxygen species

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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

Working Hypothesis

Aspects of space environment increase ROS to regulate neuroinflammatory cytokines in the hippocampus

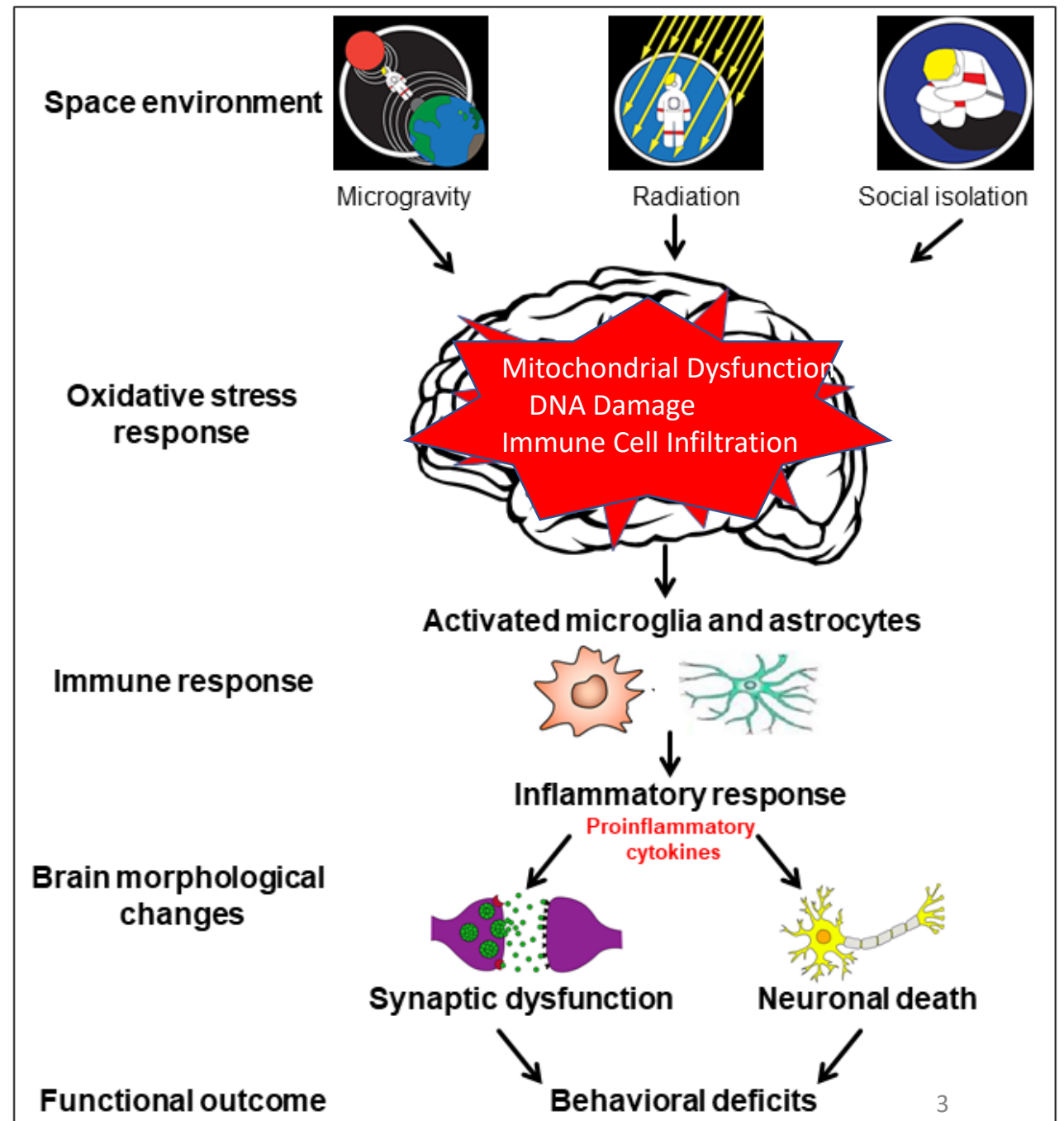
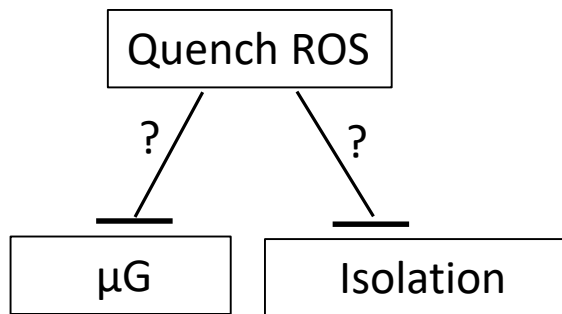


Figure credit: Siddhita Mhatre, Janani Lyer and Linda Rubinstein (Unpublished)

Effects of Microgravity on the Mammalian Immune System

- Both simulated and actual microgravity regulate cytokine expression
- Results in the literature vary due to different duration, methods and organs

Spaceflight
7-13 Days
Increased IFN γ
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-1 β increased
IL-2 decreased in spleen
Felix 2004

HU 4 Days
Increase in circulating IFN- α and IL6
Zhou 2012

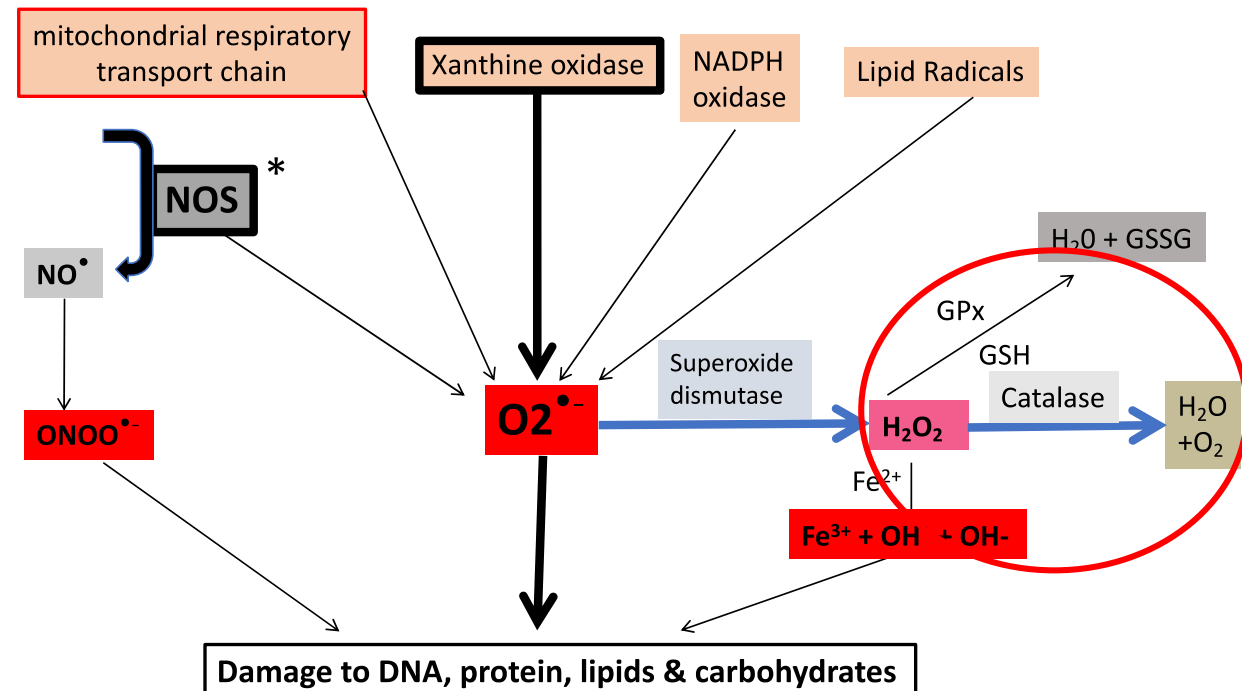
Astronauts
3 days
Elevated IL-6
Stein 1994

Astronauts
182 days
Reduced IFN γ , IL-10, IL-5, TNF α , IL-6
Crucian 2018

Approach: animal models

- Hind-limb unloading model to simulate weightlessness
- MCAT transgenic mice to manipulate mitochondrial ROS. Overexpress human catalase gene in mitochondria

Why catalase?
Catalase degrades hydrogen peroxide (ROS)



MCAT transgenic mice: quench mitochondrial ROS

Life span

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

CNS effects

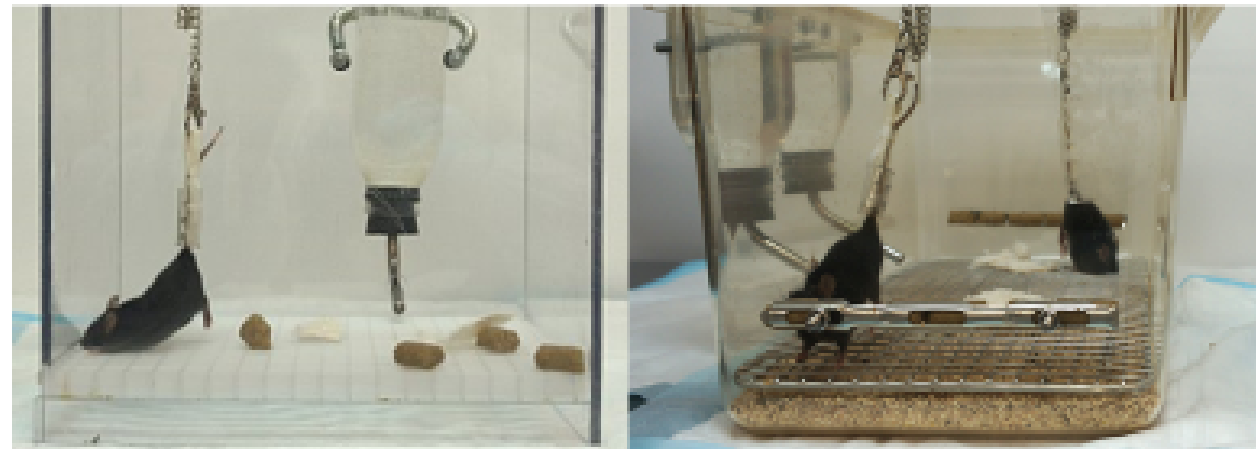
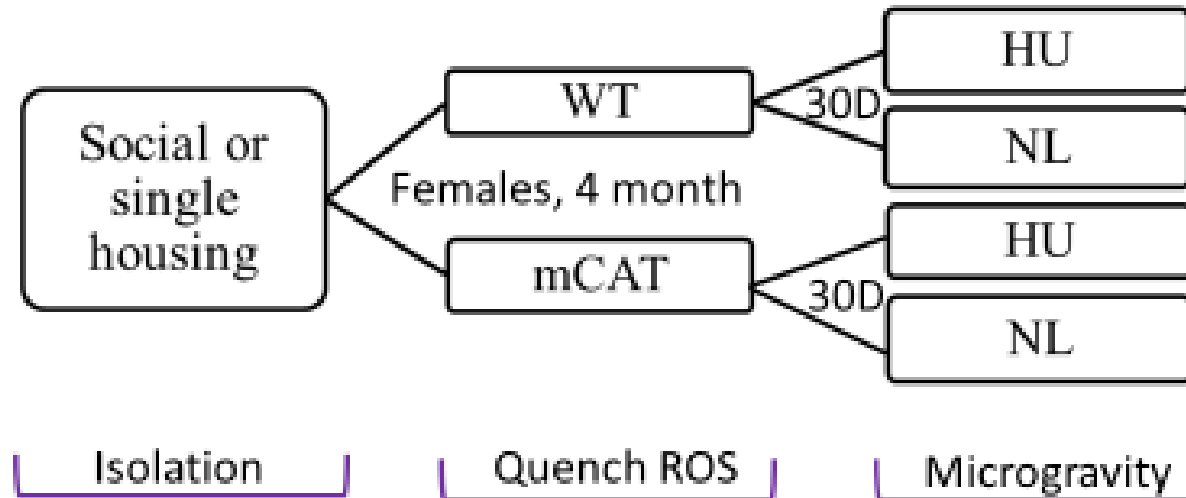
- Enhanced hippocampal spatial learning and memory, reduced contextual fear conditioning [*Olsen 2013*]
- MCAT mitigates radiation-induced deficits in behavioral performance (novel object recognition) and neuronal morphology [*Parihar 2015*]

Age-related disease

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



Experimental design



Standard single housed

Social housed (paired)

HU-model for microgravity and bedrest

Animal number: 8-12

Assays Performed

Inflammation:

- Cytokine protein expression
Multiplex assay (44 plex) in hippocampus and plasma
- Activated microglia (CD68 stain)
(Collaboration with Dr. Antino Allen)

Oxidative Stress:

- 4-HNE Elisa (lipid peroxidation)
- Park7 Elisa

HPA Axis

- Corticosterone Elisa

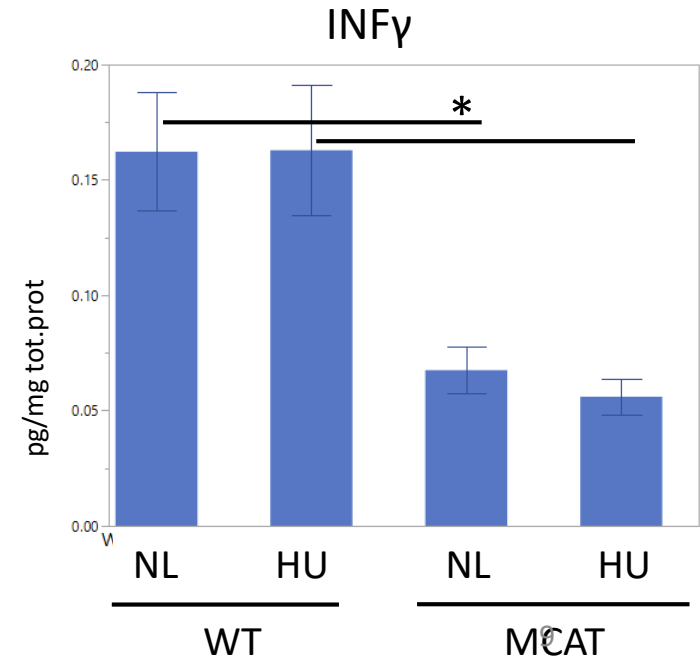
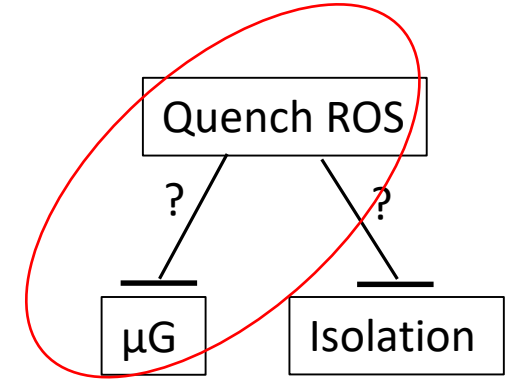
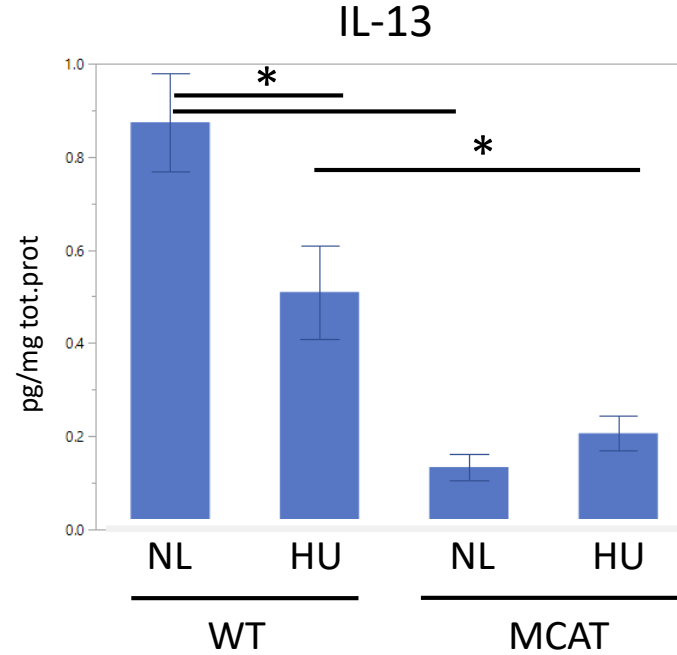
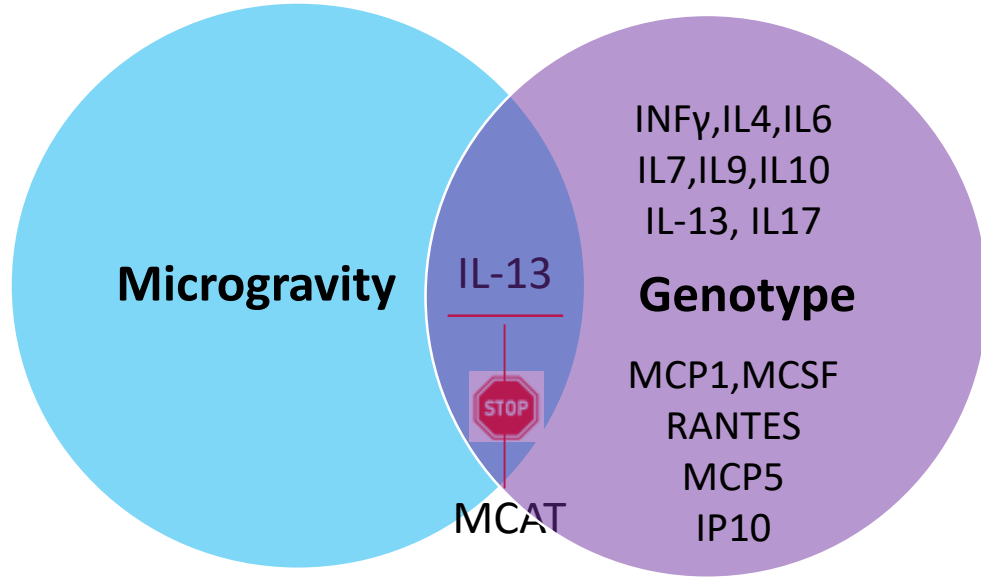
Behavior:

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

Other tissues collected: Heart, Bone, Soleus, Spleen, Adrenal, Aorta, Eyes
Immune assays (Dr. Amber Paul)

Statistics: parametric-One or Two-way Anova, non-parametric- Wilcoxon

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



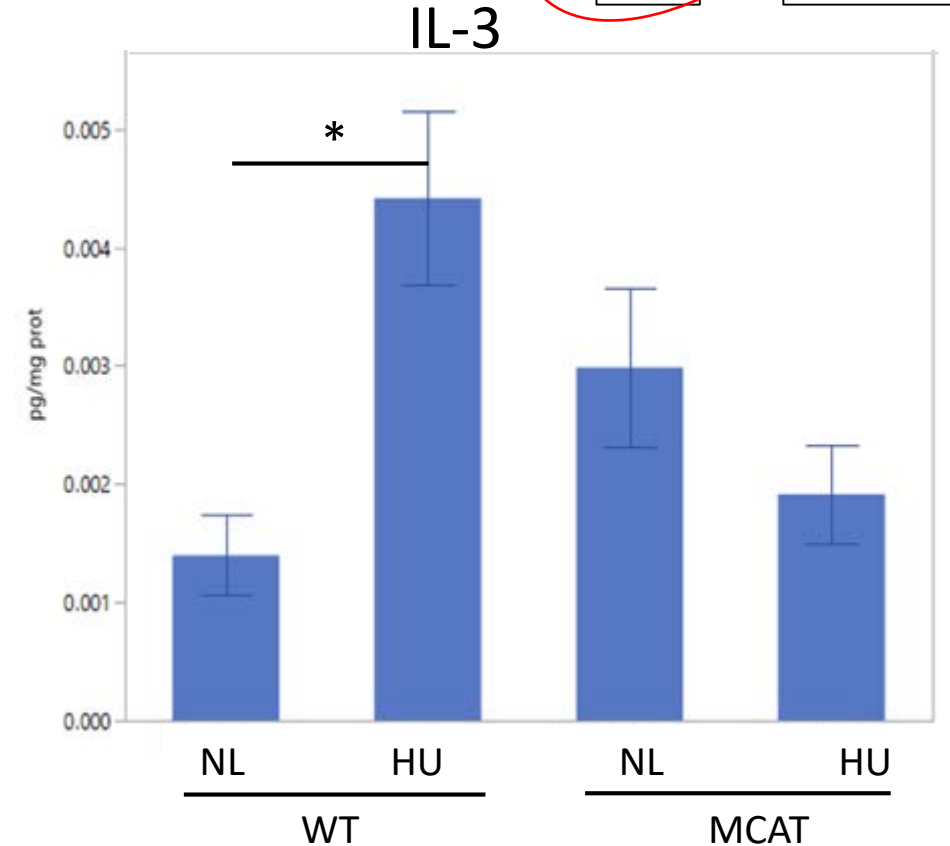
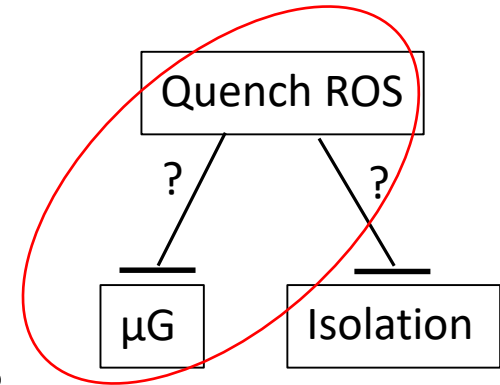
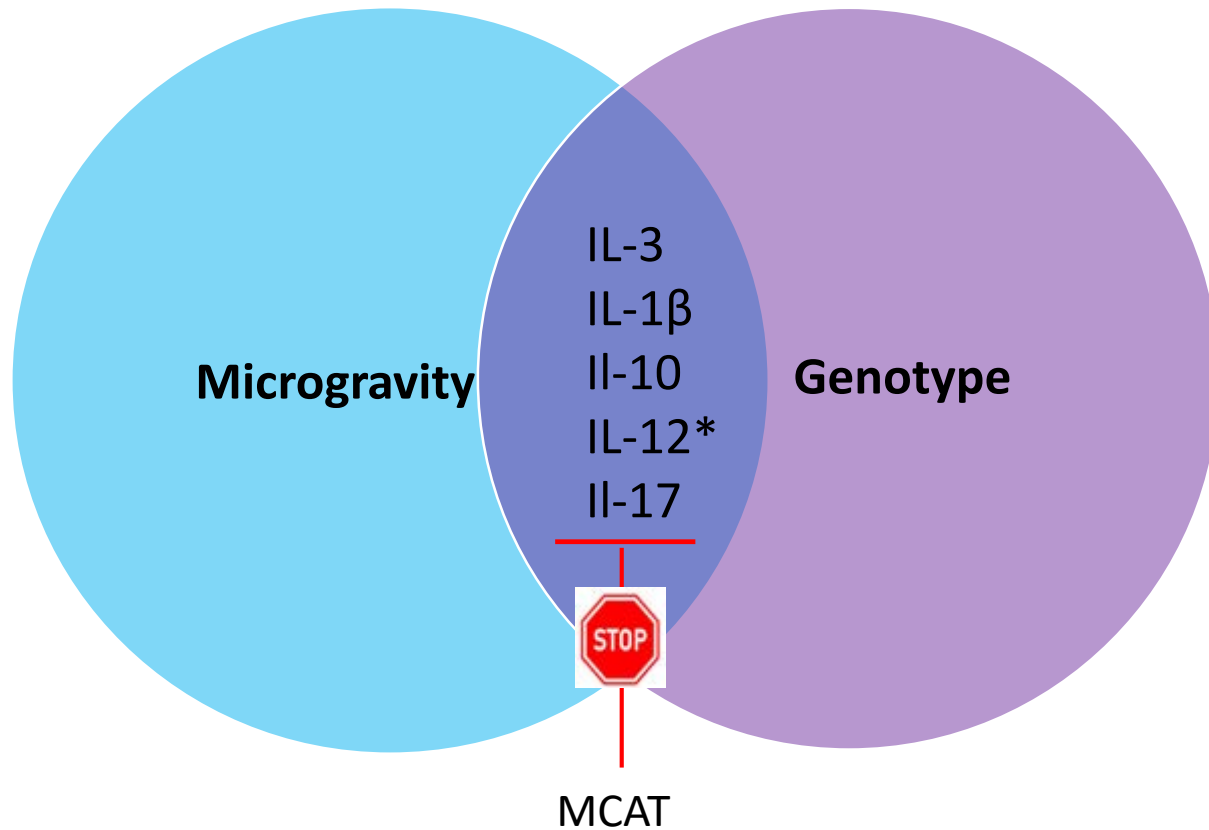
➤ Microgravity effect (HU vs NL) 1/44 differ
 ➤ MCAT mitigates

➤ 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

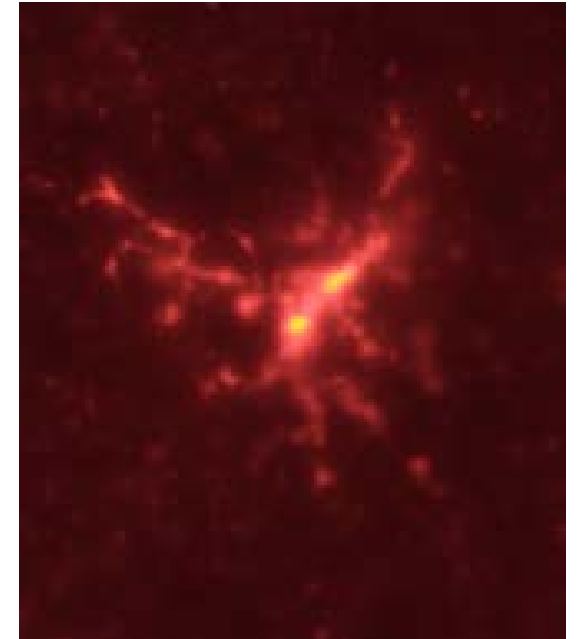
How does HU affect hippocampal cytokine expression? Does genotype mitigate? (In social housed HU model)



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

CD68 marker of activated microglia

- **Microglia** are glial cells located throughout the brain and spinal cord. Macrophage cells responsible for the active immune defense of the CNS (infectious diseases, inflammation, trauma, ischemia and neurodegeneration)
- **Microglia** are both a source and a target of cytokines
- **CD68** (Cluster of Differentiation 68) is a lysosomal protein highly expressed by activated microglia



CD68 expressing microglia
Rubinstein, Unpublished

HU increases CD68+ microglia in WT, but not in MCAT mice

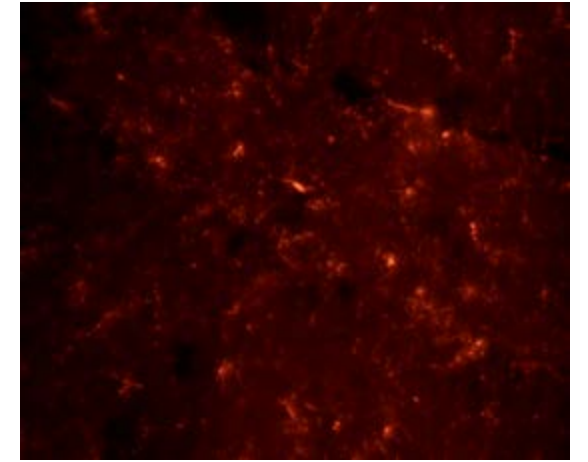
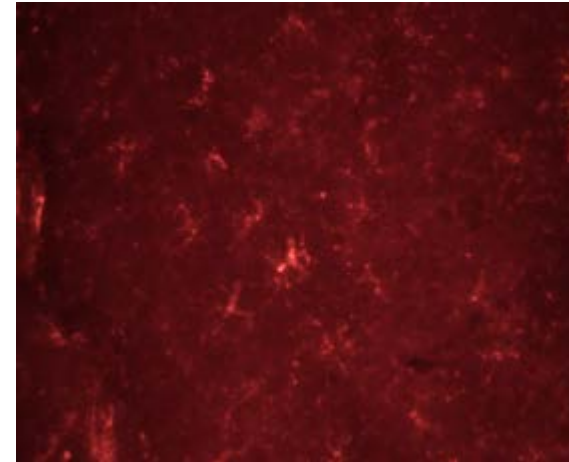
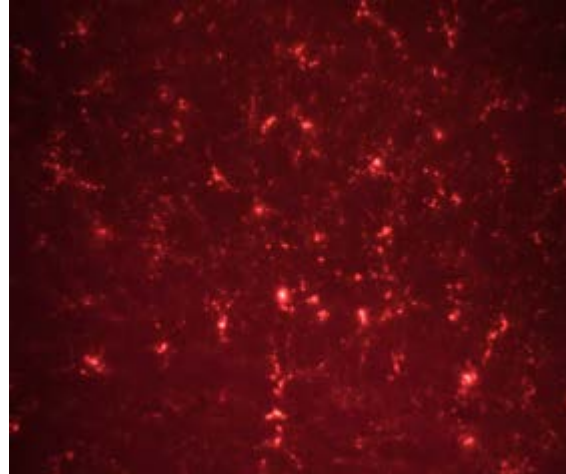
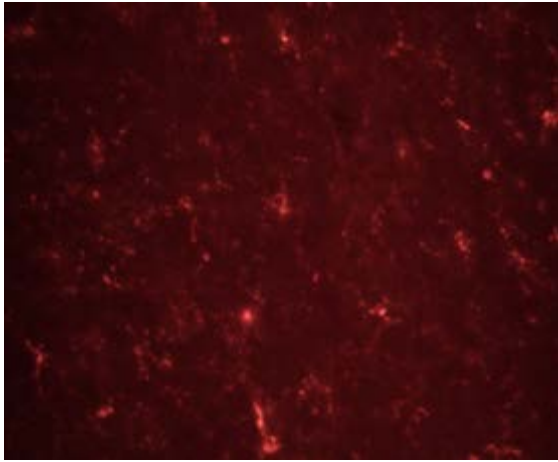
NL

HU

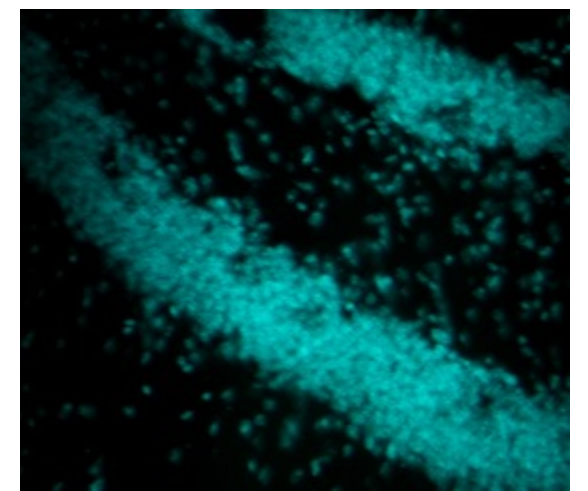
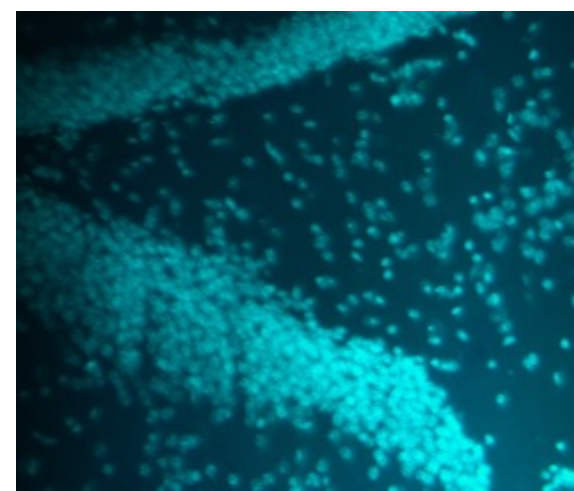
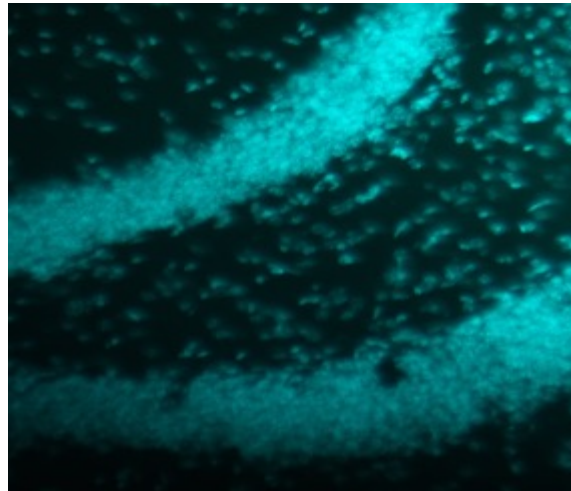
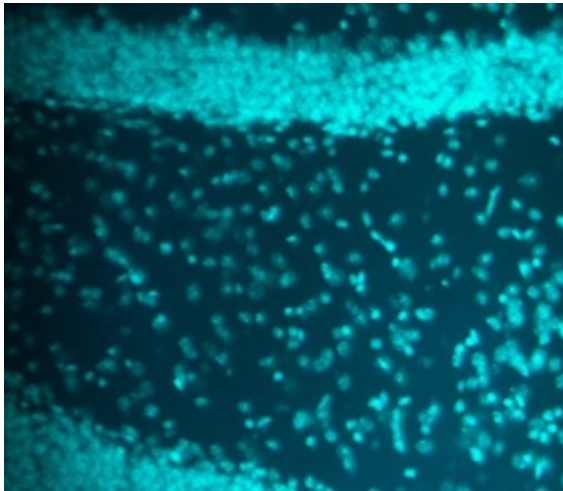
MCAT NL

MCAT HU

CD68



Dapi



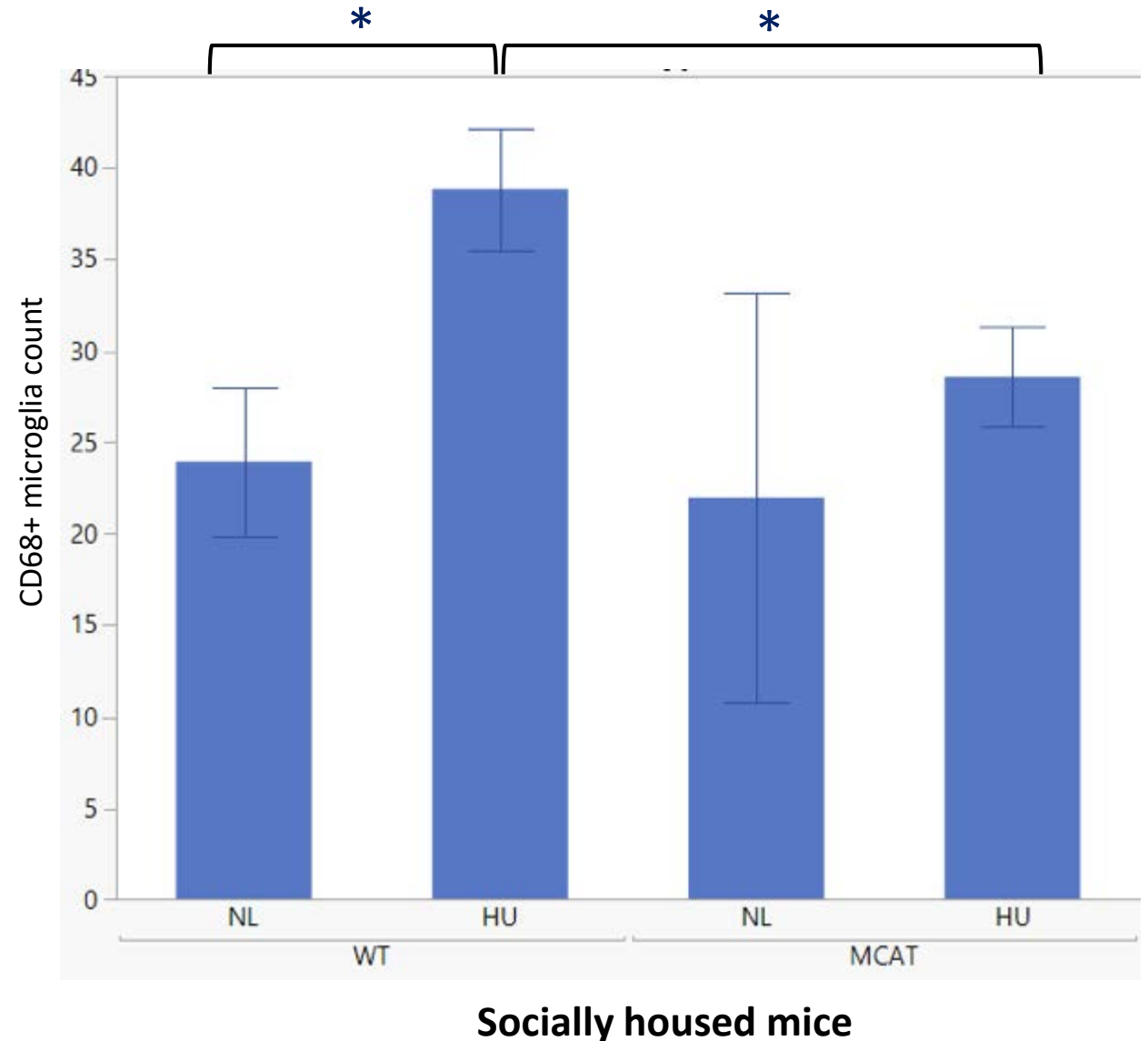
Dentate Gyrus
20X magnification

HU increases CD68+ microglia in WT, but not in MCAT mice

Simulated microgravity induces more microglia activation in the hippocampus and this activation is mitigated by quenching of mitochondrial ROS

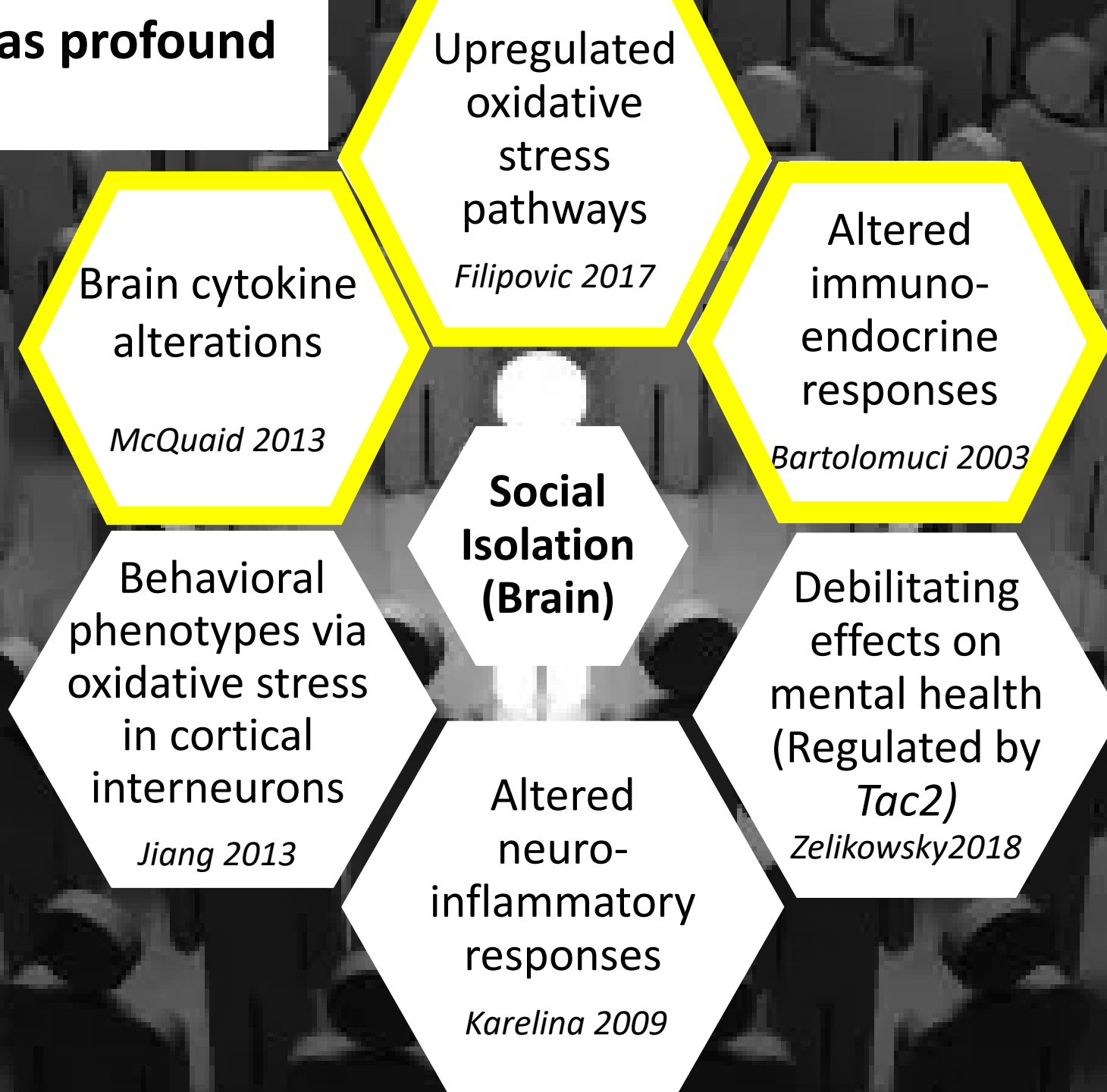
Collaboration with Dr. Antino Allen (UAMS)

Non-parametric Wilcoxon $p < 0.05$

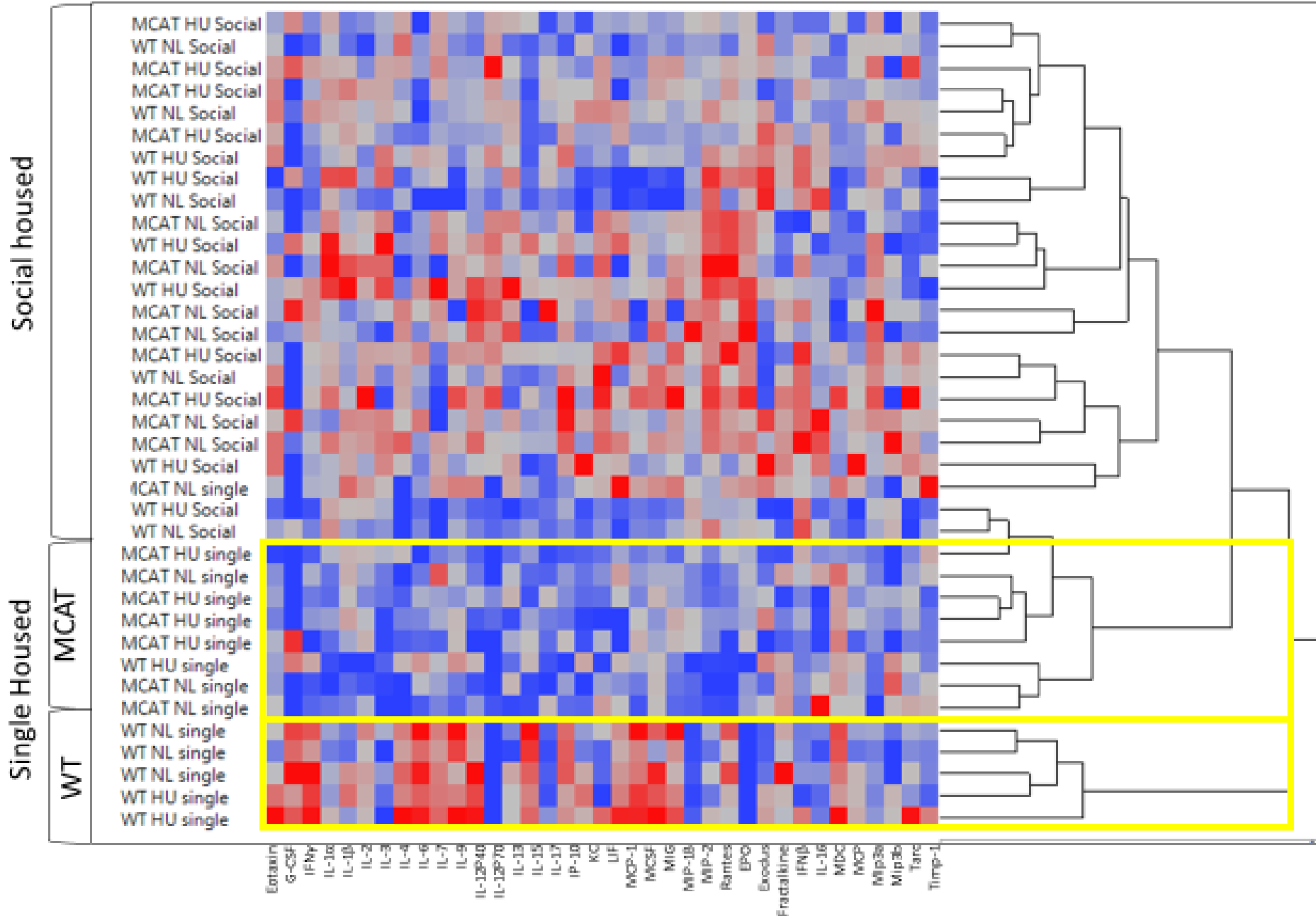


What is social isolation doing?

Social Isolation has profound impact on CNS

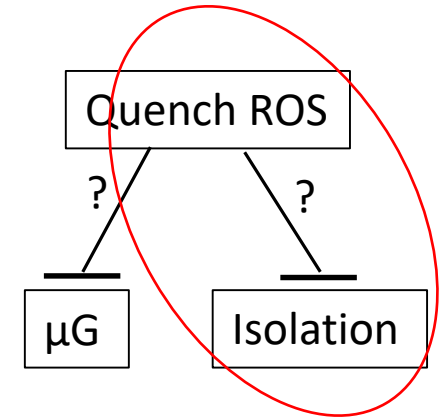
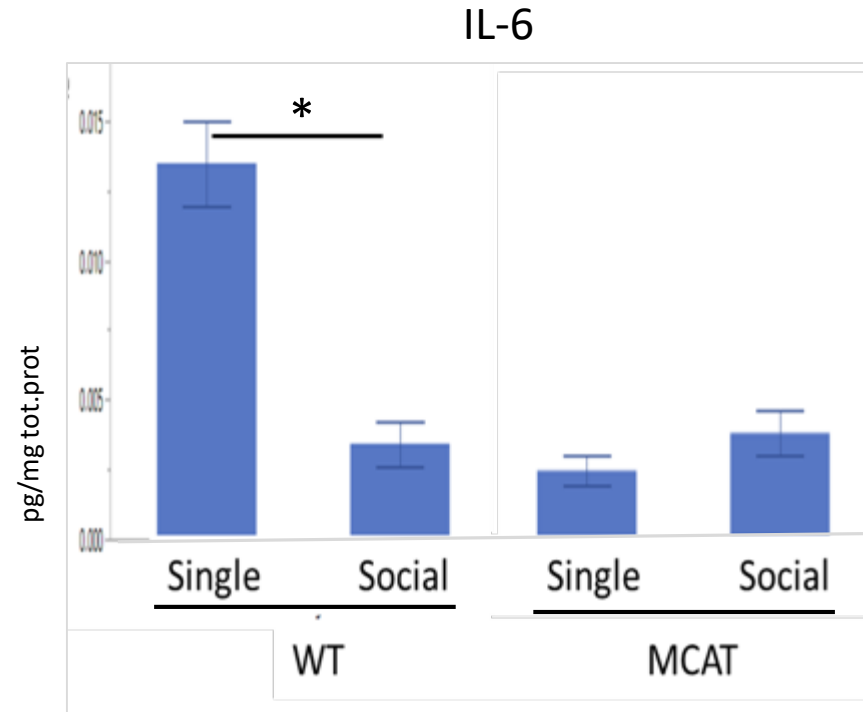
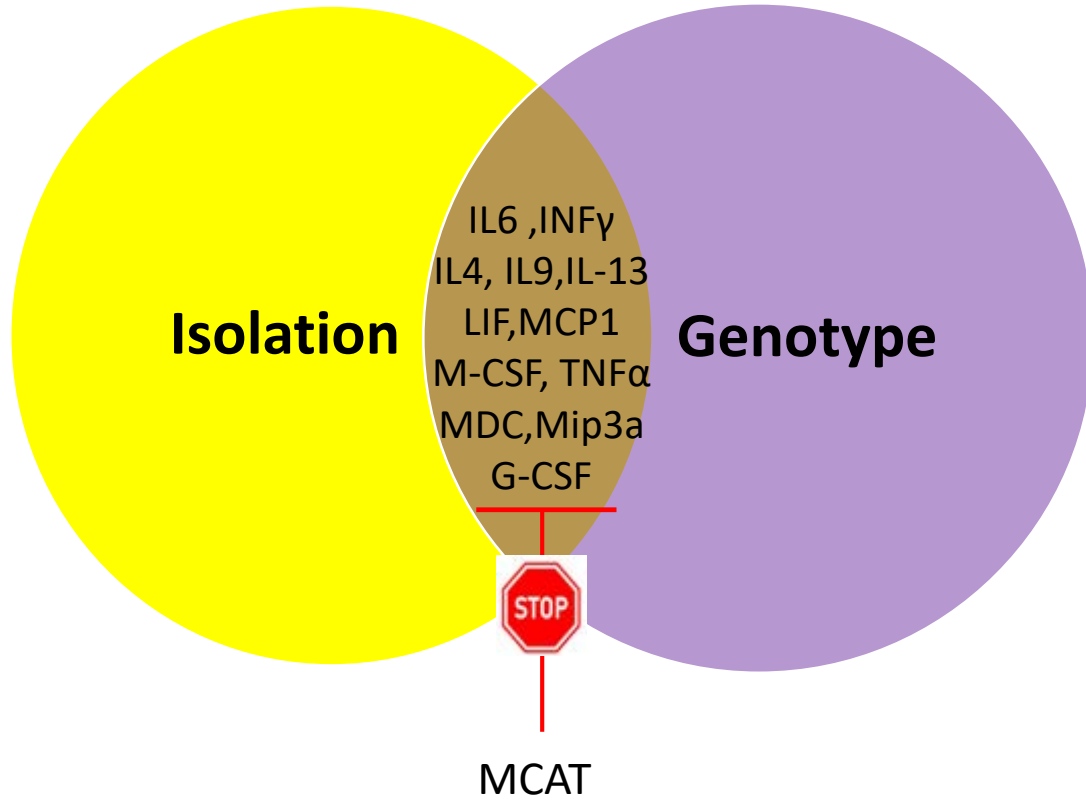


Hierarchical clustering of cytokine expression in the hippocampus



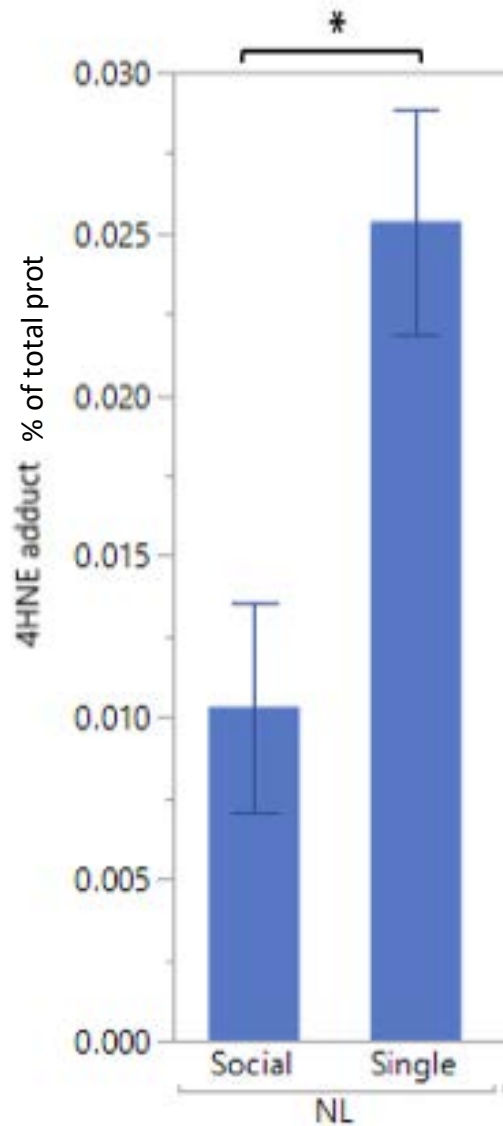
Single housed mice clustered separately from social housed and subclustered by genotype

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



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

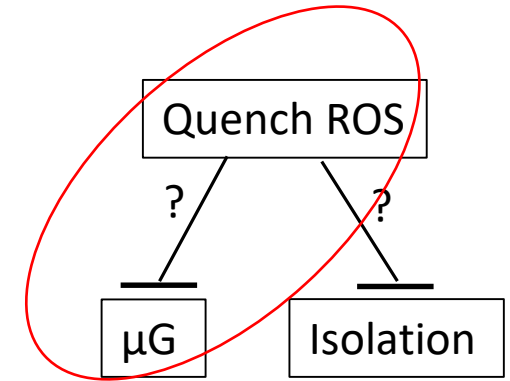
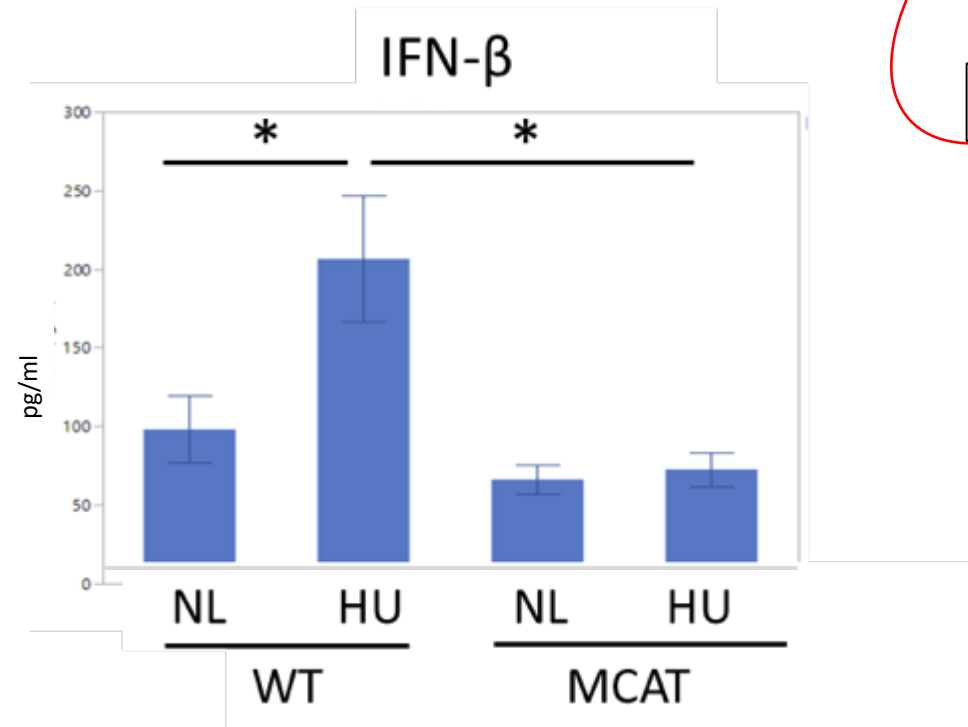
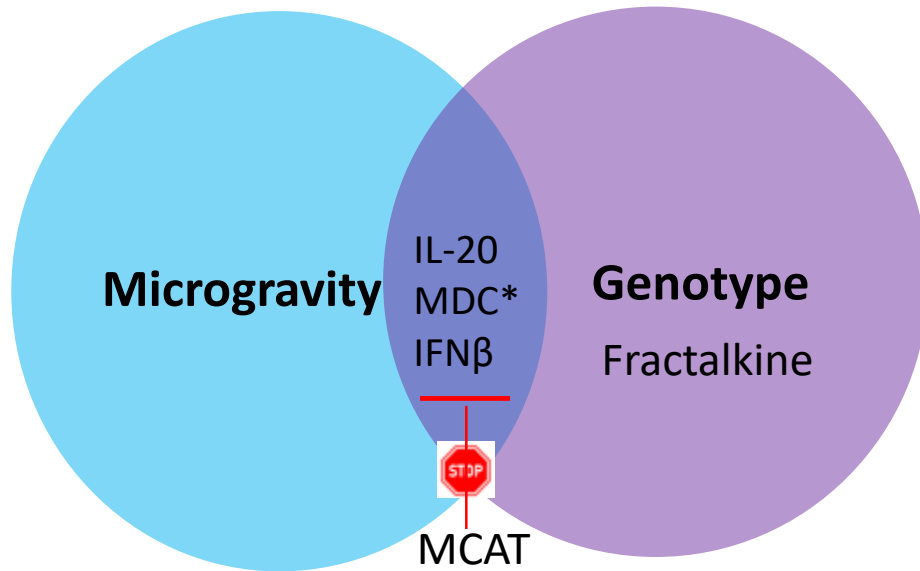
Social isolation causes oxidative damage



4HNE
Product of lipid peroxidation, marker of higher oxidative stress in tissues

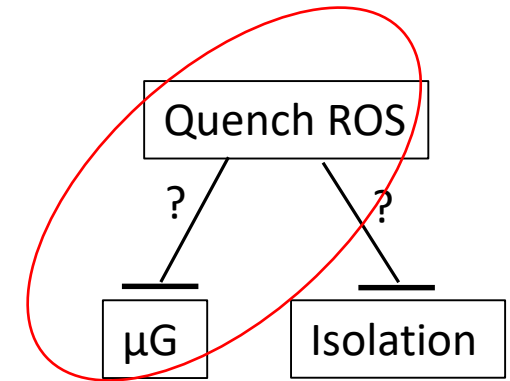
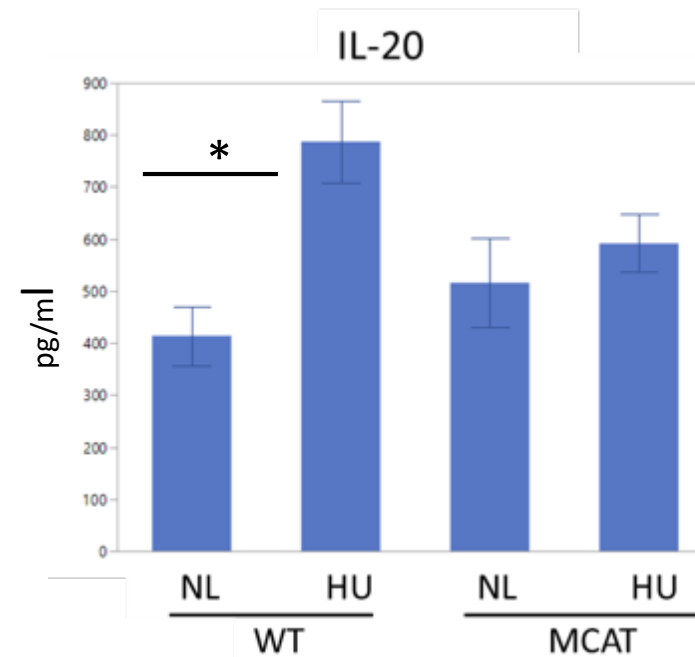
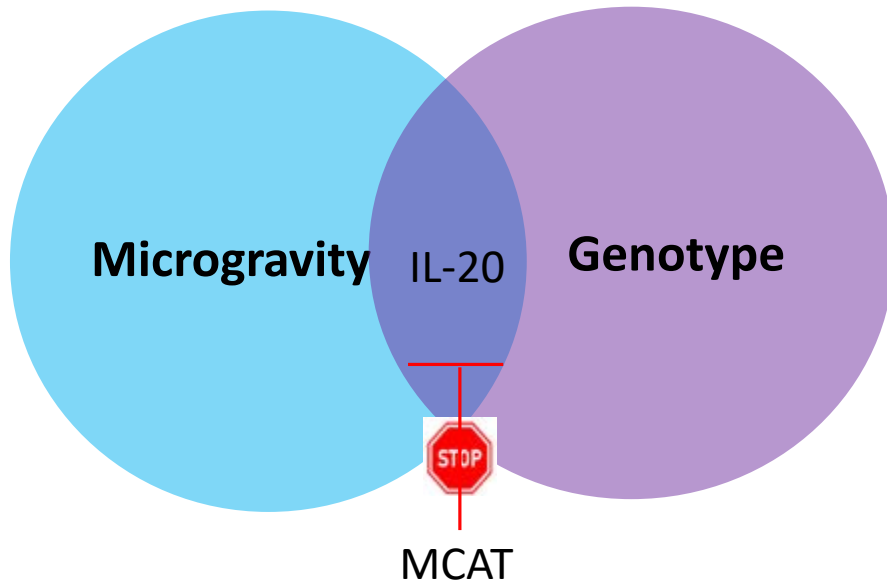
Are these effects local or systemic?

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



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

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

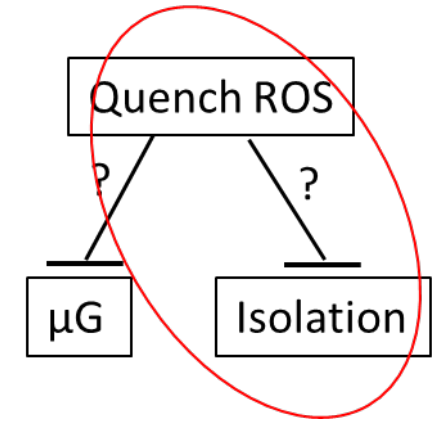
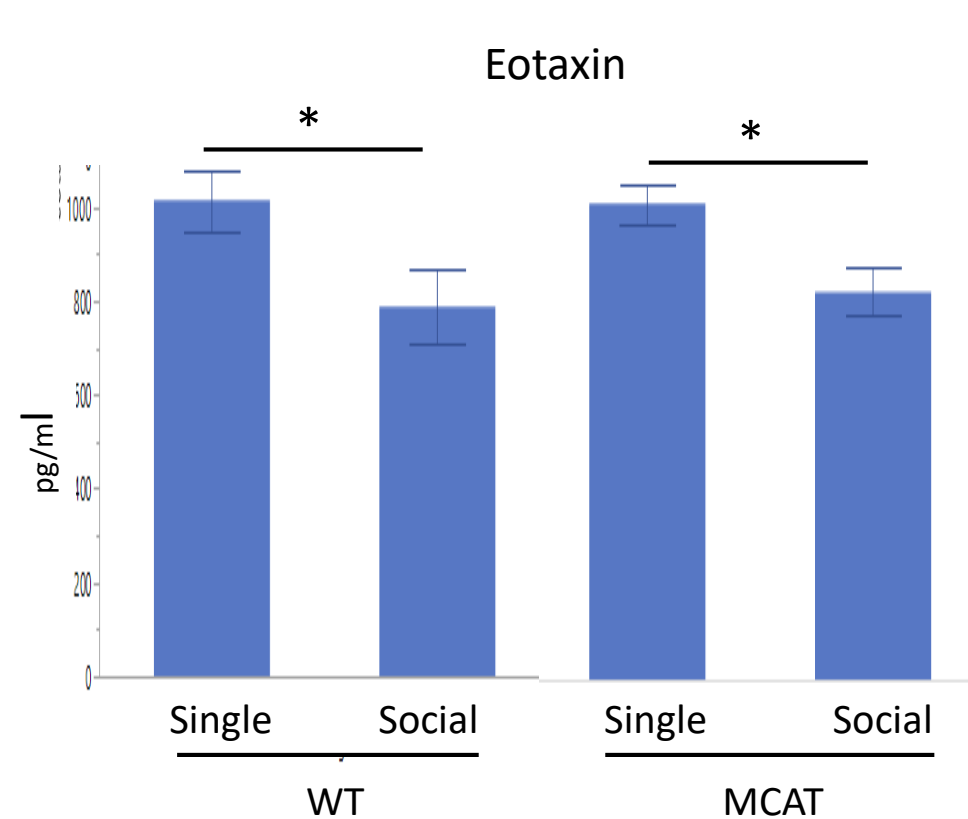
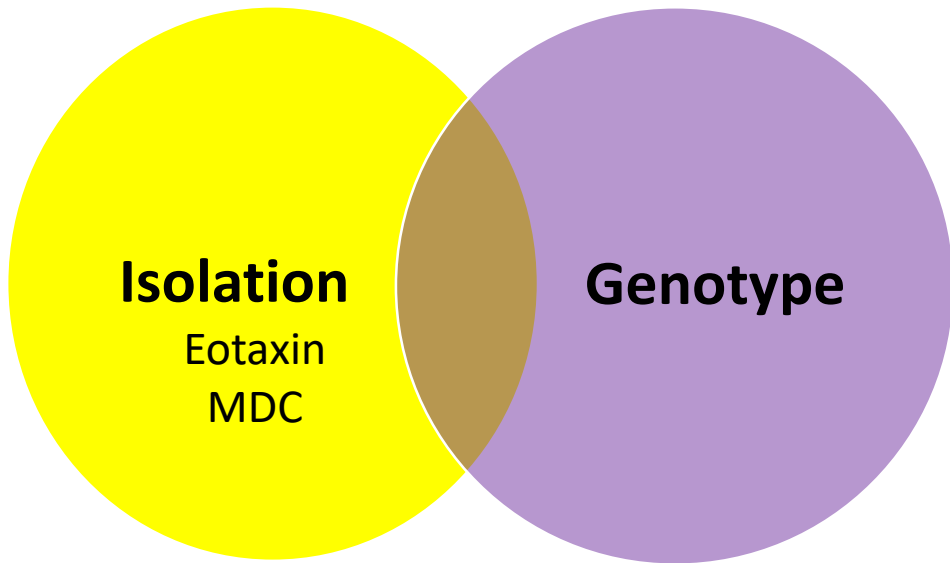


- Microgravity effect (HU vs NL) 1/44 differ
- MCAT mitigates

- IL-20 Biomarker for rheumatoid arthritis (*Kragstrup 2016*)
- IL-20 family is involved in vascular inflammatory diseases (*Autieri 2018*)

IL-20 could be used a possible biomarker

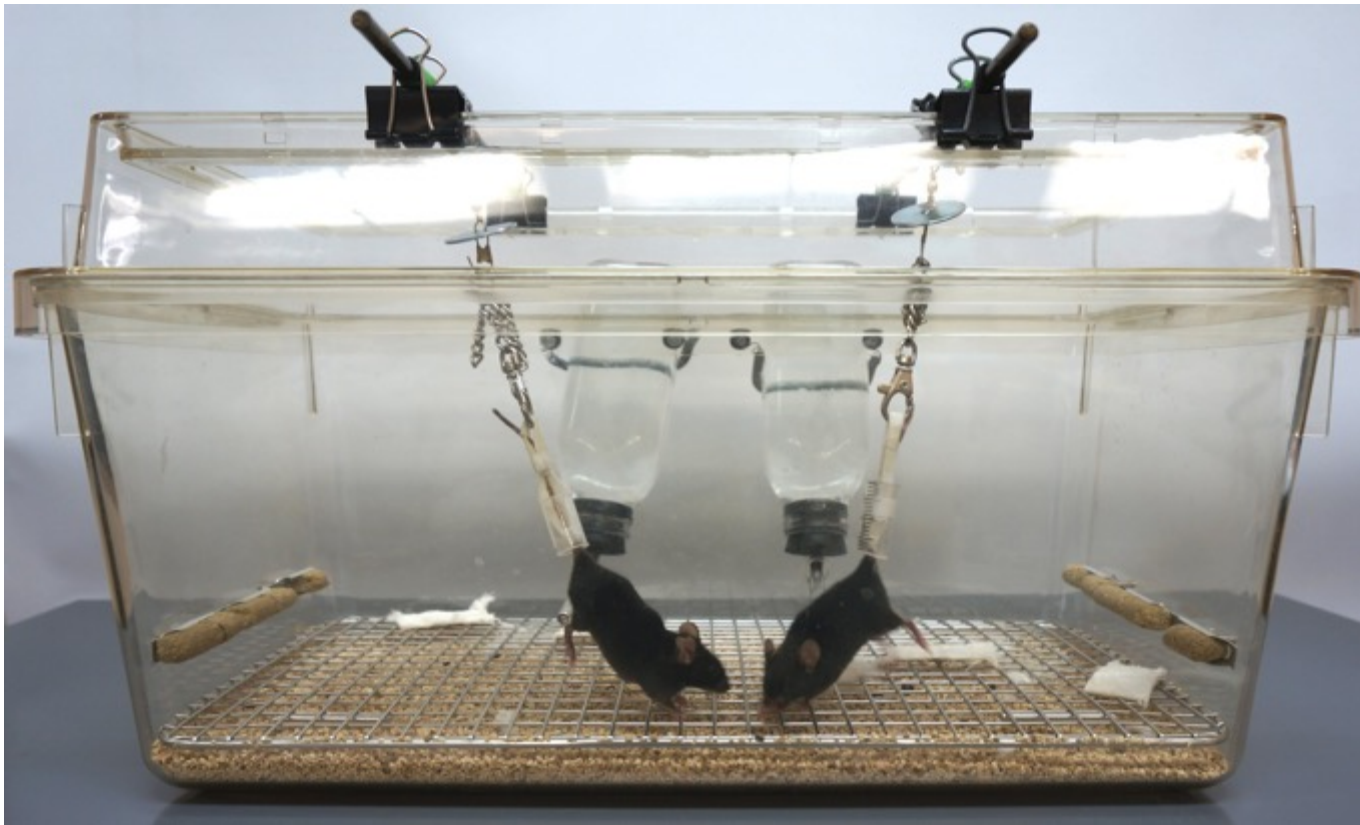
In plasma: How does social isolation affect cytokine expression? Does genotype mitigate?



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

Behavioral analysis in HU mice (social housed) model

- Data collected from 3 different dark cycle timepoints (15 minutes each)
- New social adaptive behavior to HU was discovered



Categories

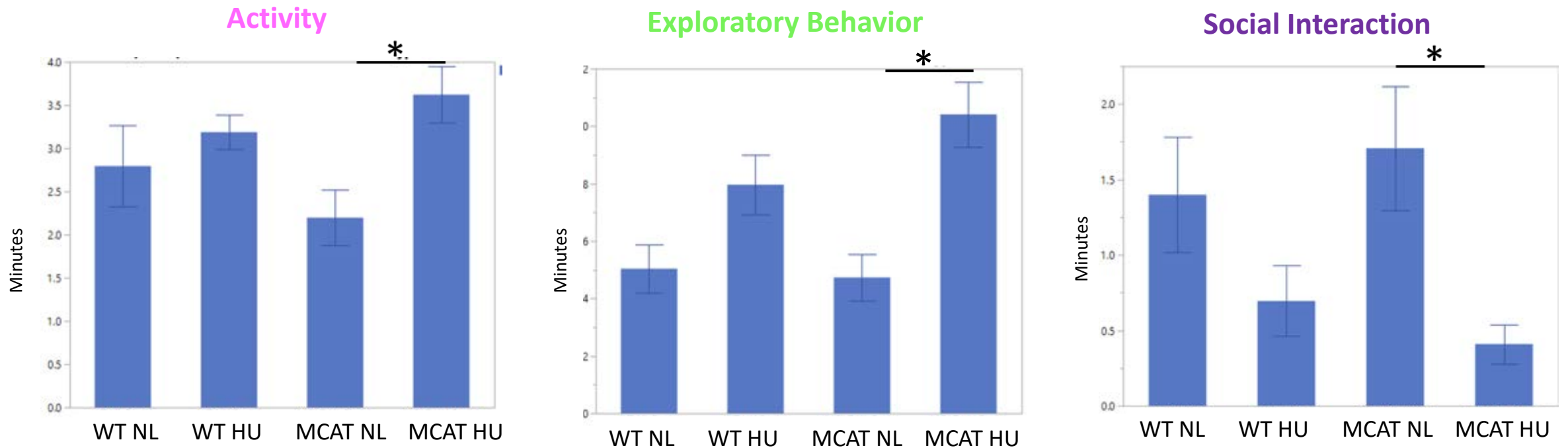
- Eating
- Drinking
- Allogrooming
- Nestlet Manipulation
- Sniffing
- Ambulation
- Self grooming
- Exploratory Behavior
- Burrowing in bedding
- Climbing
- Mounting chasing, sniffing other mouse
- **New**: Novel nestlet social engagement
- Inactive
- Nondirected movement

Active

Exploration

Social Interaction

HU affects several aspects of cage behavior in MCAT mice



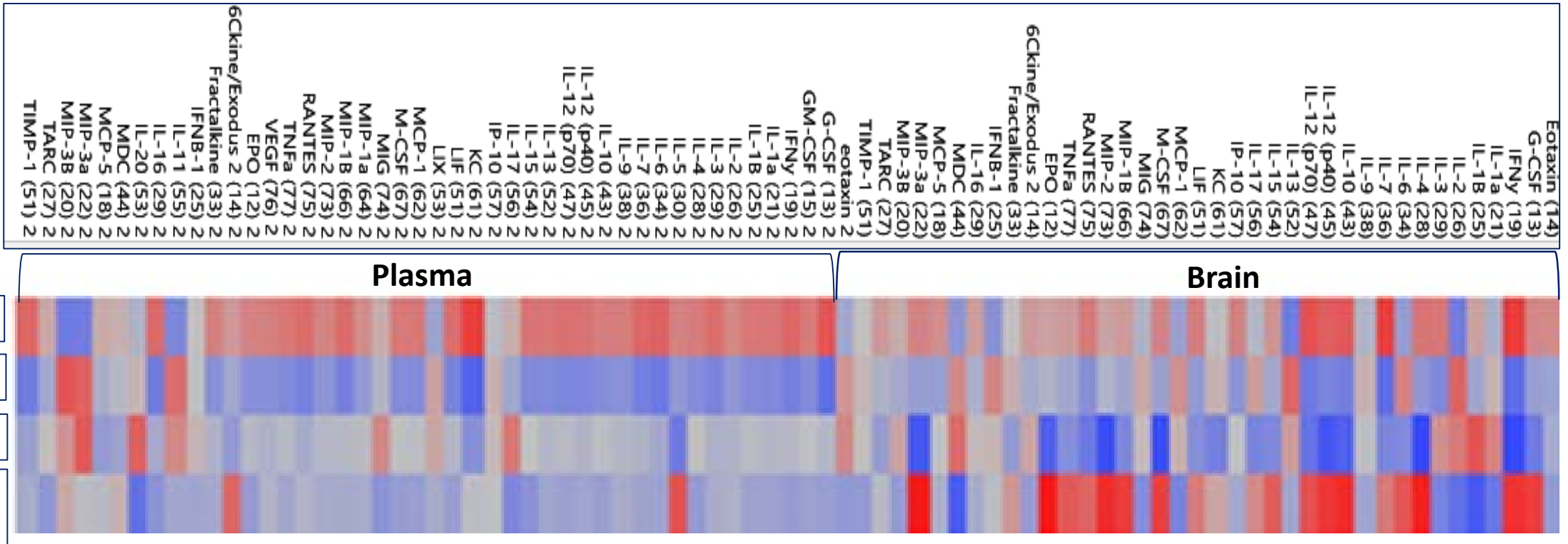
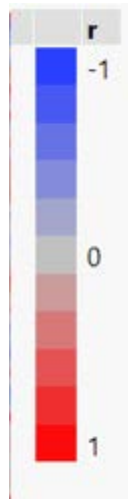
Microgravity effects: NL vs HU

- HU animals conduct more exploration and engage less in social interaction

Genotype effects: MCAT NL vs MCAT HU

- MCAT HU conduct more exploration, are more active and engage less in social interaction

Cytokine profiles in brain and plasma correlate with behavior



- Active behavior correlates with less cytokines in both brain and plasma
- Exploratory behavior correlates with less cytokines in brain
- Social interaction correlates with more cytokines in the brain

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-1 β	
IL-6	
Mip3-a	

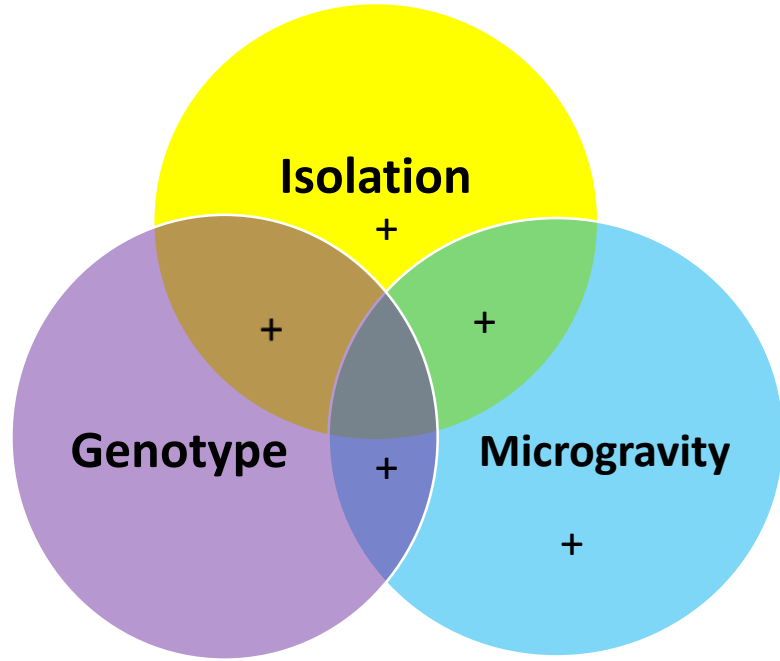
Eating	
Brain	Plasma
IL-2	Timp-1
MCP-1	

Inactivity	
Brain	Plasma
INF- γ	KC
	Timp1
	Mip3a

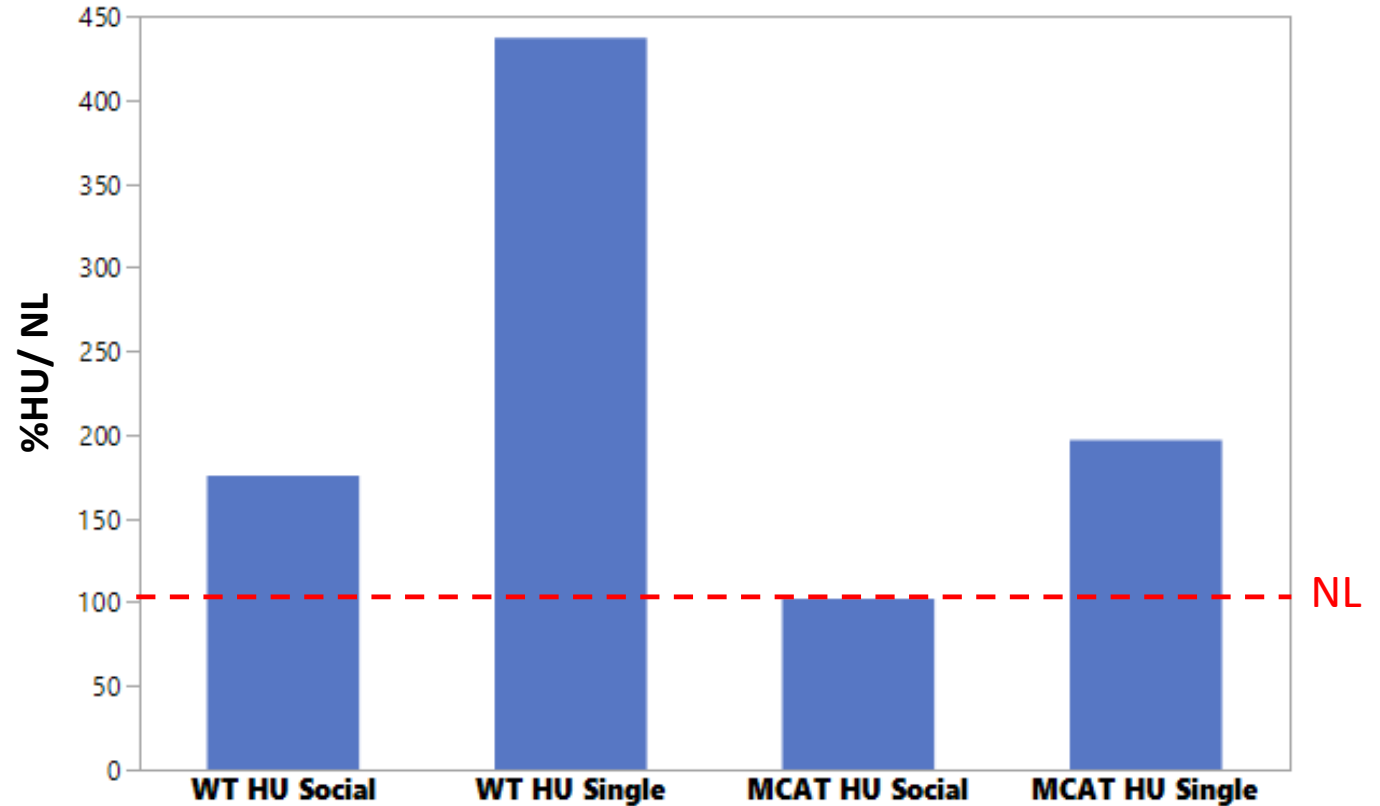
Active behavior	
Brain	Plasma
	G-CSF
	KC
	Timp1

Positive correlation **Negative correlation**

In WT mice, HU increased plasma corticosterone; this effect was exacerbated by social isolation.
 MCAT appeared to mitigate HU effects.



Corticosterone	
Plasma	Brain
Negative correlation	Positive correlation
MDC	Eotaxin
	IL-1 α
	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)

Conclusions

- Long term, simulated microgravity, altered cytokine expression levels in both the hippocampus and plasma
 - 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
 - Mitigated in MCAT mice
 - The cytokine responses to social isolation were more extensive in brain than in plasma
 - There was no overlap in cytokine responses to microgravity and isolation suggesting two separate mechanisms being involved in response to these stressors
 - **Implicates an important role for mitochondrial ROS in isolation stress**
- Simulated microgravity activated microglia in the hippocampus of WT mice
 - Mitigated in MCAT mice
 - **Therefore, hypothesis validated for neuroinflammation and ROS quenching in the hippocampus**

Bigger Picture

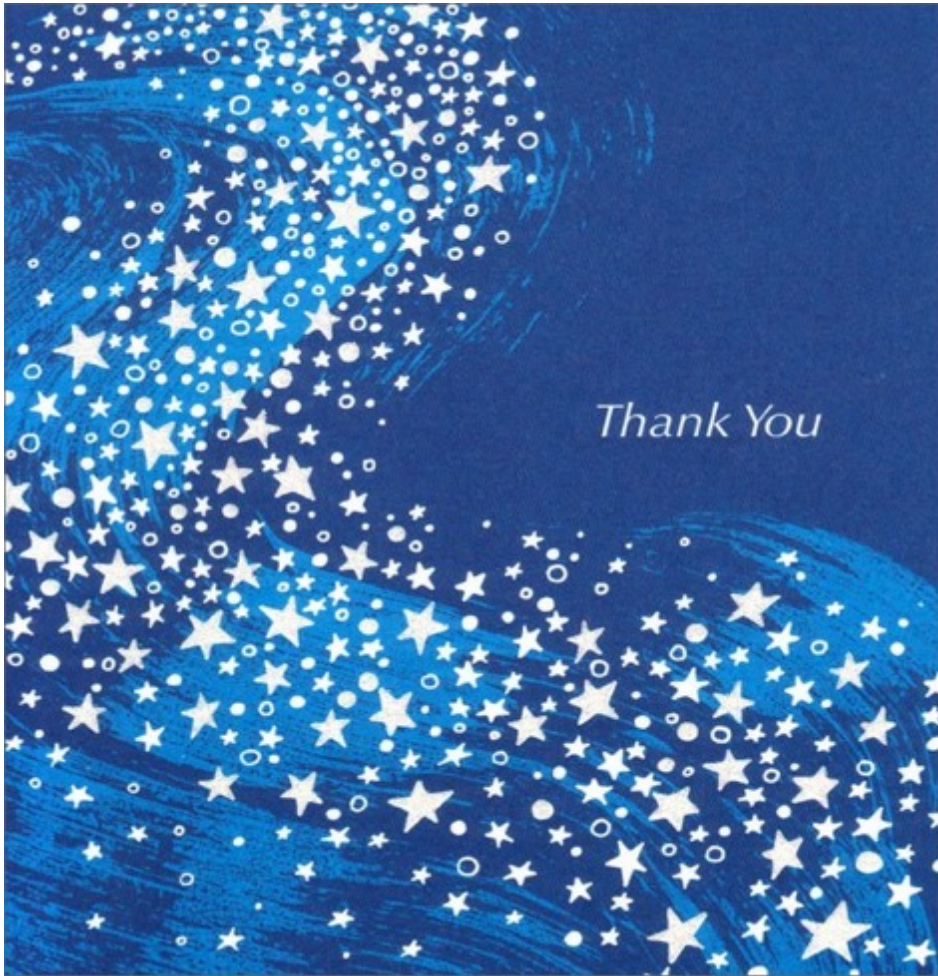
An iceberg floating in the ocean. The tip of the iceberg is visible above the water surface, while the much larger, submerged part is below. This visual metaphor represents the 'bigger picture' of the research findings.

From studying multi-organ changes due to microgravity and social isolation a different picture of response emerges for the muscle-skeletal vs immune/CNS systems

- The immune system and the CNS are more responsive to social isolation than other systems
- This stresses the importance of the social HU model
- These findings are highly relevant to space environment and advance the risk characterization in the CNS in long term space travel

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 and for sedentary lifestyle on earth
- Social engagement may mitigate “inflamm-aging” in space, as well as on Earth
- PLX cell therapy could be a candidate for mitigation of long-term effects of microgravity on the brain



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