Exosomes Derived from Human Stem Cells Differentially Impacts Brain and Heart Tissues- Two Primary Targets of Acute Stress Response:
Systems Biology Based Analysis

Nabarun Chakraborty

Nabarun.Chakraborty2.civ@health.mil

August 2026

Disclaimers

The views expressed in this presentation reflect the results of research conducted by the authors and do not necessarily reflect the official policy or position of the Defense Health Agency, Department of War, nor the U.S. Government.

The experiments reported herein were conducted in compliance with the Animal Welfare Act and per the principles set forth in the "Guide for Care and Use of Laboratory Animals," Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 2011.

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Acknowledgements

WRAIR

Rasha Hammamieh, PhD*

Aarti Gautam, PhD*

Alexander Lawrence

Routing Yang, PhD

Seid Muhie, PhD

James DeMar, PhD

Mital Patel, PhD*

Stacy-Ann Miller*

Allison Hoke

Ida Nela Crespo-Rosales

Stephen Butler*

Nicole Bozik*

Swapna Kannan*

Navya Goutam

Funded by: MOMRP

Integrative Systems Biology

Core Capabilities & Strategic Ecosystem

Deep Discovery

- Next-Gen Sequencing
- Single Cell Omics
- Spectral Assays (LC/MS, OLINK)
- Automated Liquid Handling

Advanced Models

- 3D Cell Culture
- Gnotobiotic Vivarium
- Space Biology/ISS

Data Maneuvering

- DoD HPC Access
- Custom Scripts/Libraries
- Big Data Management

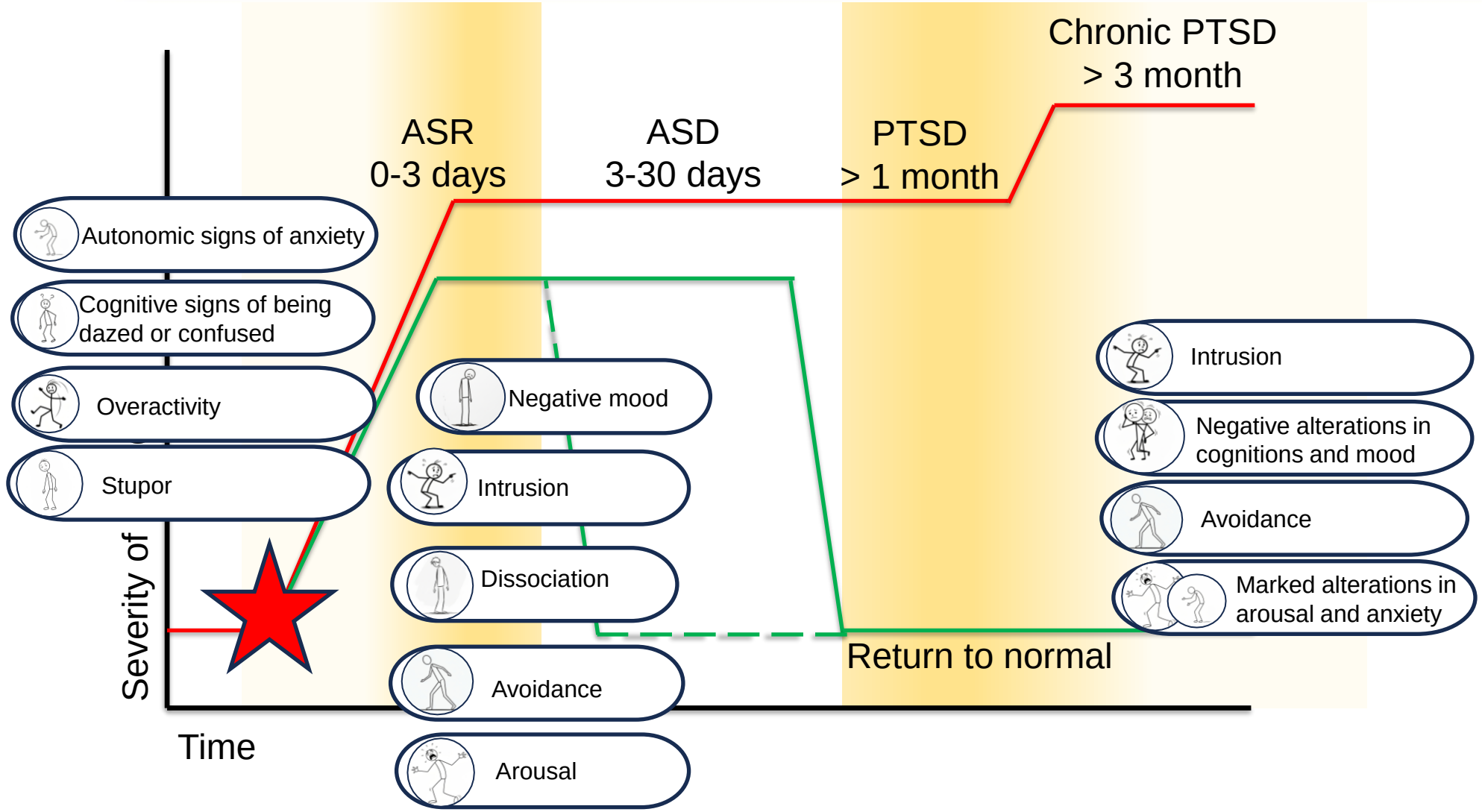
Nabarun.Chakraborty2.civ@health.mil
(301)-319-3398

Acute Stress Response (ASR)

- ASR is a short-lived disorder that is often overlooked, and if left untreated, a subset of this cohort eventually develops PTSD.
- Based on self-report, 17.2% of soldiers who have deployed to combat report having experienced a possible ASR.
- Currently, there is no objective treatment available for ASR; in fact, therapeutic options for PTSD are very limited.

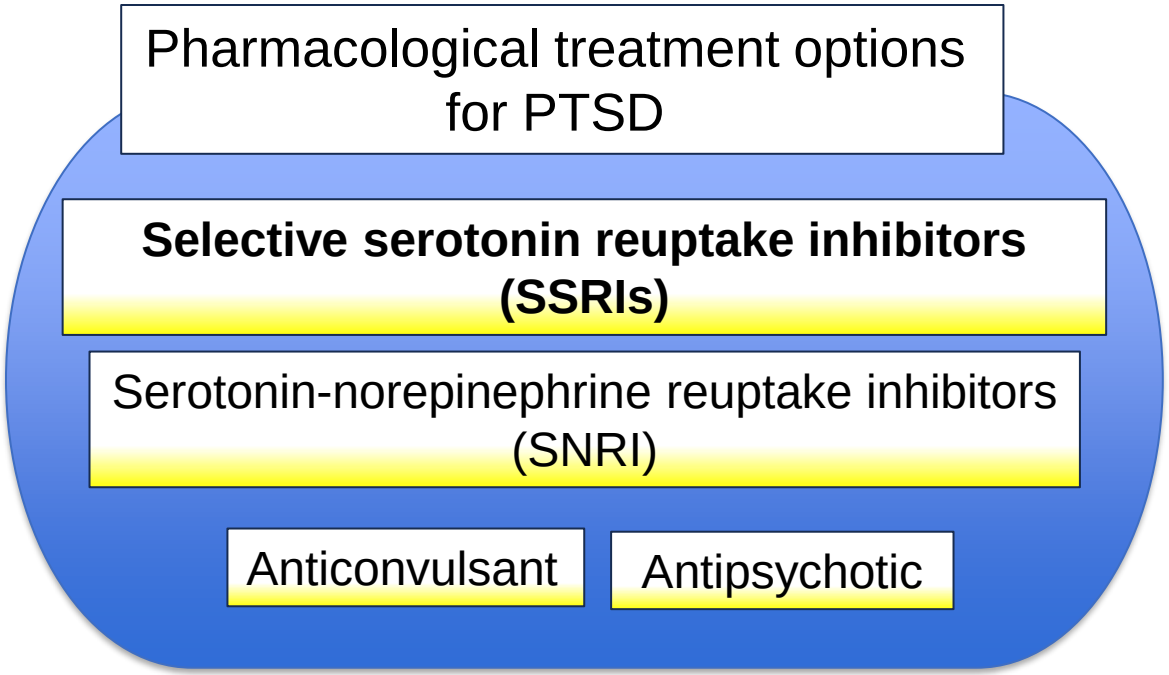
Ref: Adler, A. B. & Gutierrez, I. A. Acute Stress Reaction in Combat: Emerging Evidence and Peer-Based Interventions. *Curr Psychiatry Rep* **24**, 277–284 (2022).

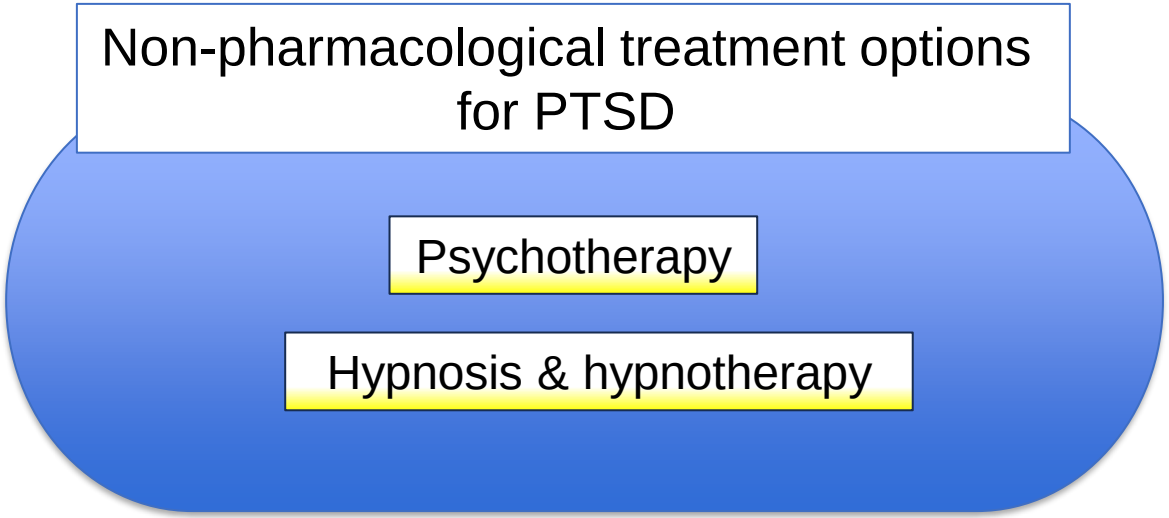
ASR → Acute Stress Disorder (ASD) → PTSD



ASR: Therapeutic option?

- Therapeutic options for PTSD is limited and for ASD, nearly none

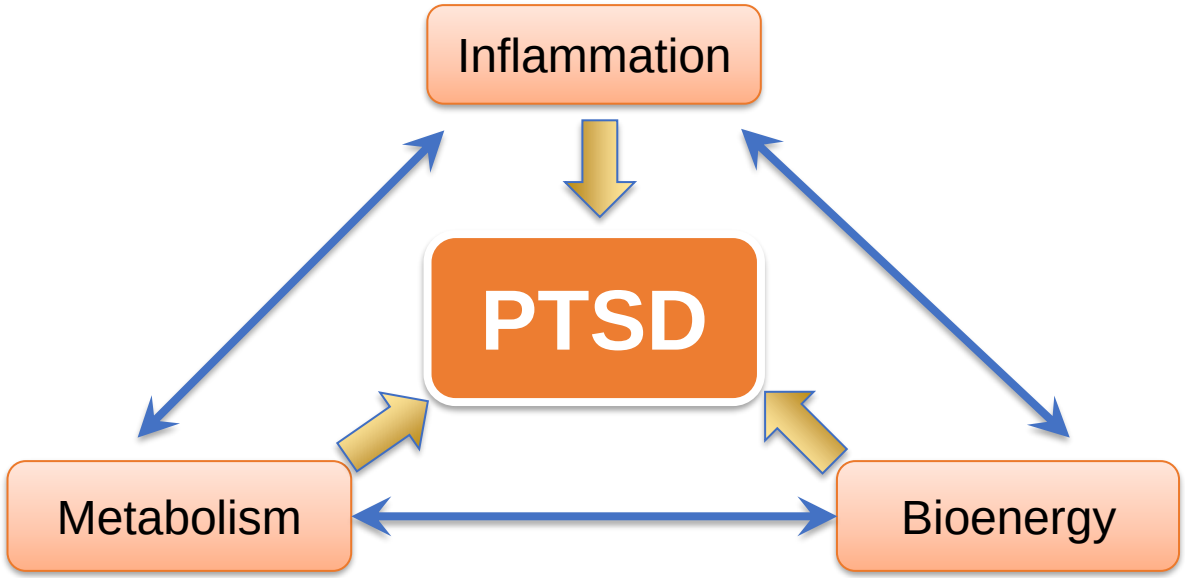




- ❑ Failure rates of SSRI approach a staggering 40%.
- ❑ 80+% of patients, who positively respond to SSRI, failed to achieve full remission.

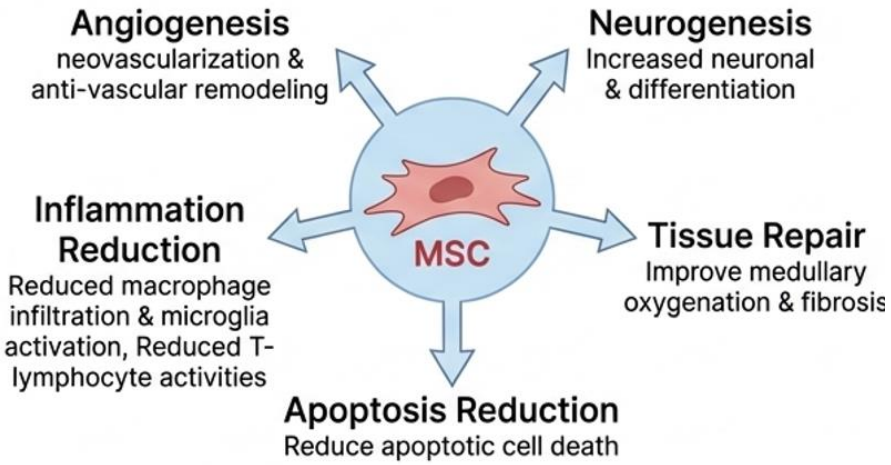
ASR:
Biomechanism and intervention target

- Following is a simple depiction of PTSD suggested by Bersani *et al,* 2020



- **Hypothesis:** Similar pathomechanism could elicit ASR.
  Therapeutic agents capable to target these eliciting mechanisms could be used as an early stabilizer.

Mesenchymal Stem Cells (MSC)



MSC demonstrated its capability to boost bioenergy, hence a potential countermeasure of ASR.

Limitations-

- High batch-to-batch variability in production
- Cold chain essential and limited shelf-life of cells
- Ethical issues

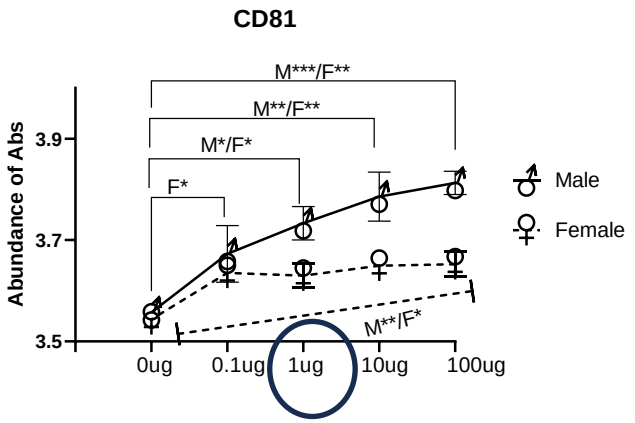
Solution:

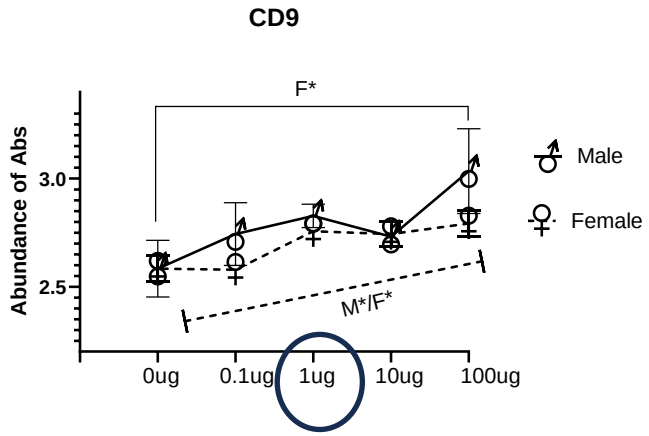
Exosomes derived from human MSC (hMSC-ex)

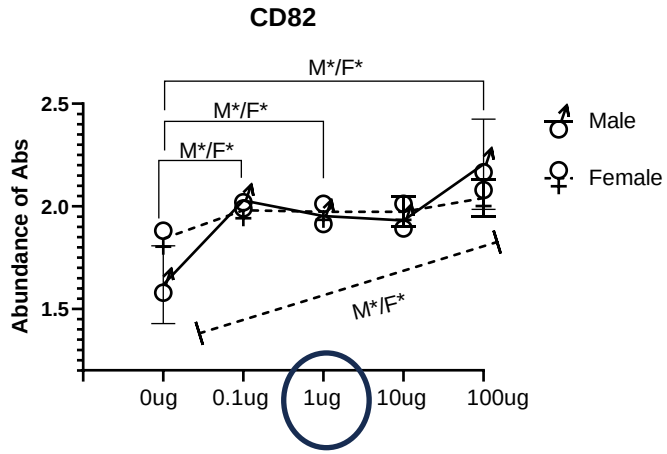
- Showed MSC-like efficacy
- Less batch-to-batch variability in production
- Less complicated archiving protocol

hMSC-ex: Dose determination

Estimation of bio-availability of exosomes in brain







hMSC-ex was purchased from RoosterBio, Inc., MD

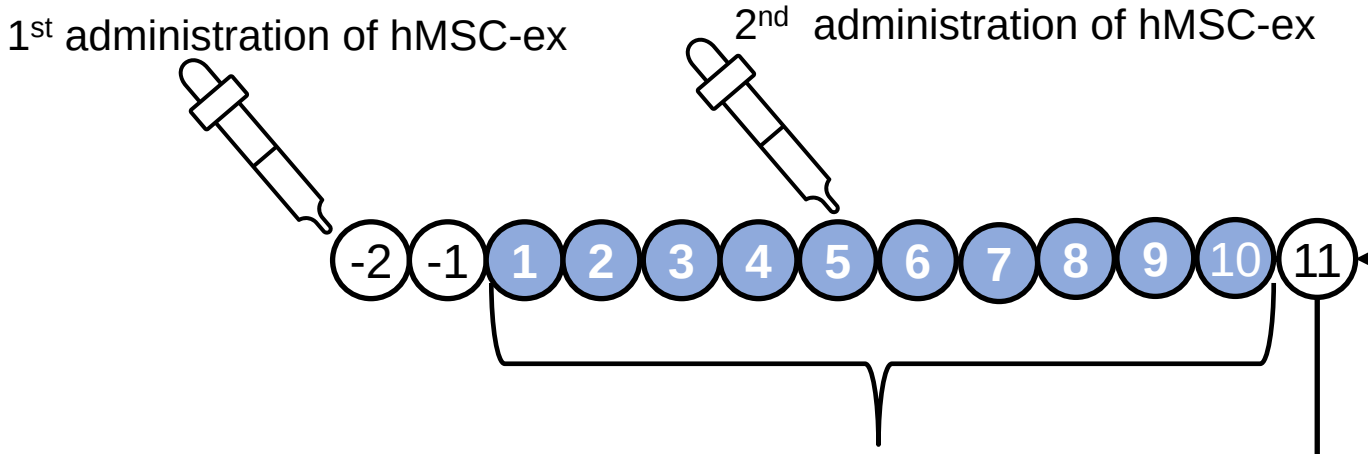
hMSC-ex: Dose determination

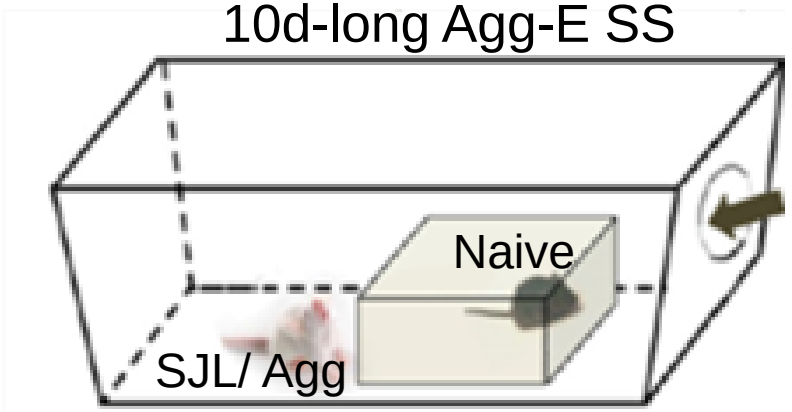
Blood inflammation profile:

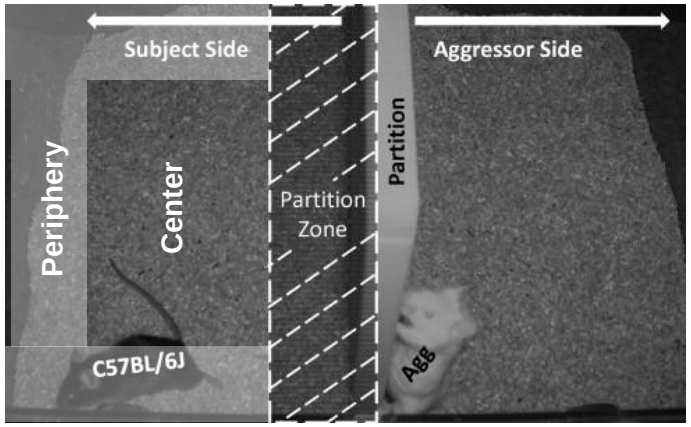
100ug/1.7e10 EV			10ug/1.7e9 EV			1ug/1.7e8 EV		0.1ug/1.7e7 EV	
Protein	logFC	Inflammatory	Protein	logFC	Inflammatory	Protein	logFC	Protein	logFC
Cyr61	1.13	Pro	Cyr61	1.32	Pro	None		None	
Tnfsf12	1.00	Pro	Tnfsf12	1.20	Pro				
Adam23	-1.02		Fli1	1.11	Pro				
Nadk	-1.07	Anti	Ccl2	1.05	Pro				
Itgb1bp2	-1.30		Ntf3	1.00					
			Gcg	-1.05	Anti				
			Nadk	-1.14	Anti				
			Itgb1bp2	-1.28					

Taking all in consideration, we decided to dose 1ug/1.7e8 EV in 1 week

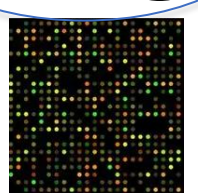
Study design:
Aggressor exposed social stress (Agg-E SS)







Barrier test

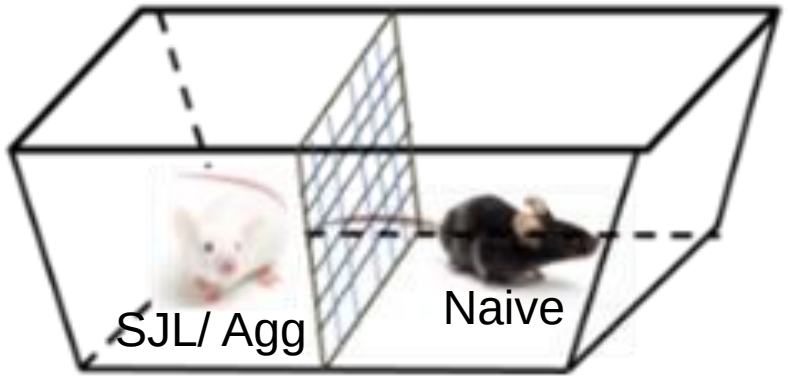


Whole Genome molecular assay

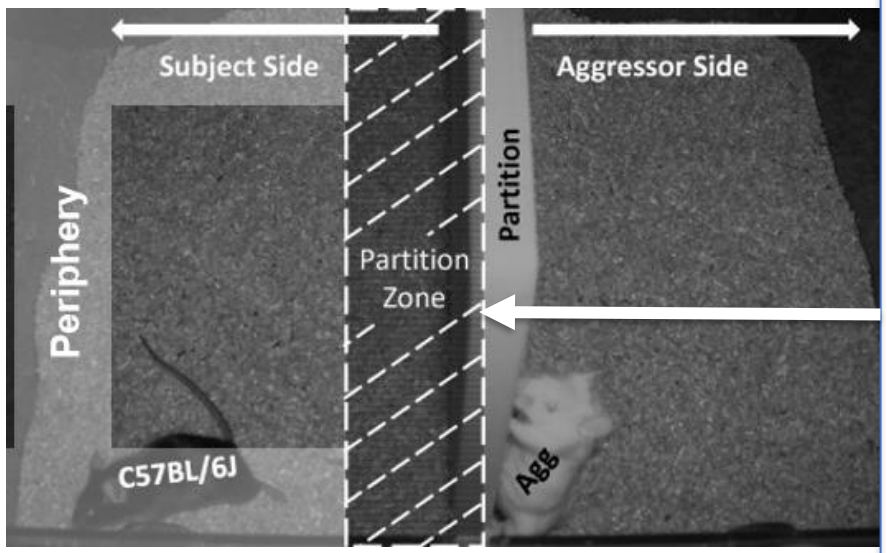
- Social stress in a cage-in-cage paradigm:
  Elicited PTSD-like behavioral and phenotypic features

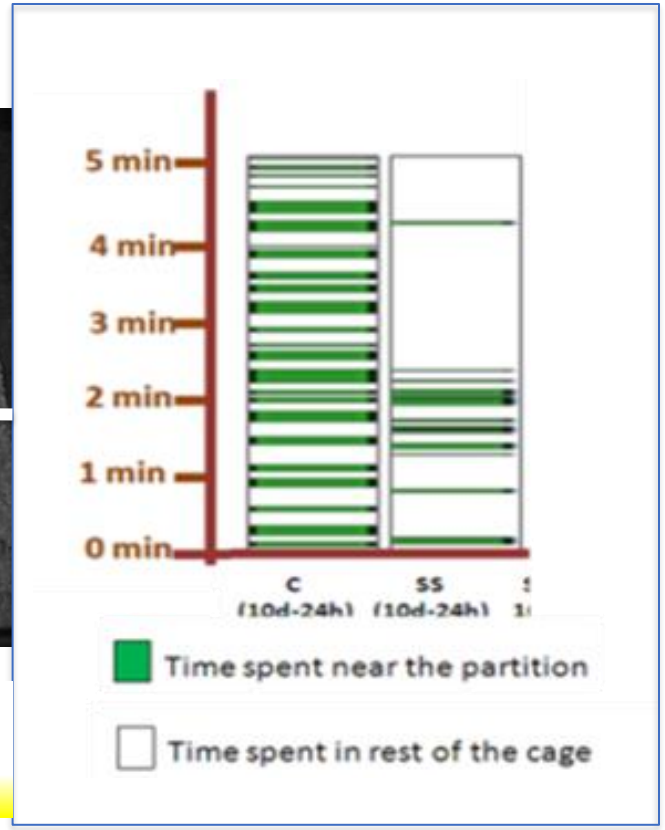
Agg-E SS model: Behavioral profile

- Barrier test to present context of past trauma



Cage outline:



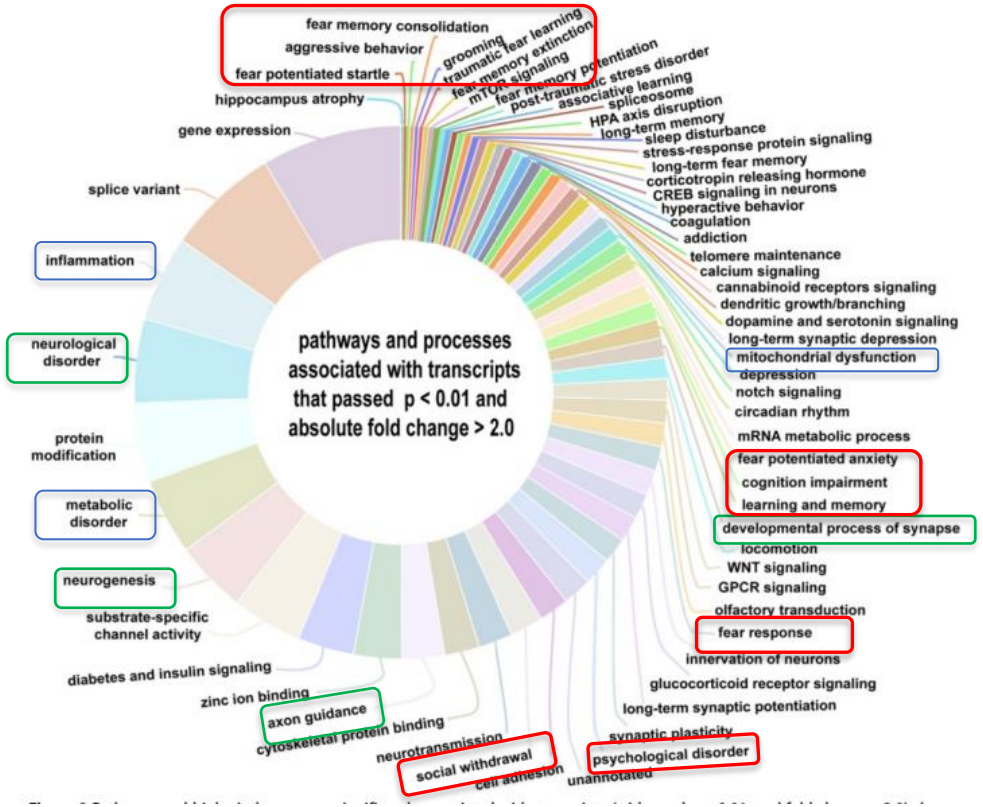


Barrier test demonstrated that Agg-E SS mice experienced fear at the presence of the contextual trauma and made significant effort to avoid it.

Ref: Hammamieh R, Chakraborty N, De Lima TC, Meyerhoff J, Gautam A, Muhie S, D'Arpa P, Lumley L, Carroll E, Jett M. Murine model of repeated exposures to conspecific trained aggressors simulates features of post-traumatic stress disorder. Behavioural Brain Research. 2012 Nov 1;235(1):55-66.

Agg-E SS model: Brain functions

Whole genome analysis of different brain regions, such as hippocampus, amygdala, medial prefrontal cortex has been conducted to show the adverse impacts of Agg-E SS on brain functions



Basic mechanisms linked to PTSD

Behavioral features linked to PTSD

Neurological mechanisms linked to PTSD

Ref: Muhie S, Gautam A, Meyerhoff J, Chakraborty N, Hammamieh R, Jett M. Brain transcriptome profiles in mouse model simulating features of post-traumatic stress disorder. Molecular brain. 2015 Feb 28;8(1):14.

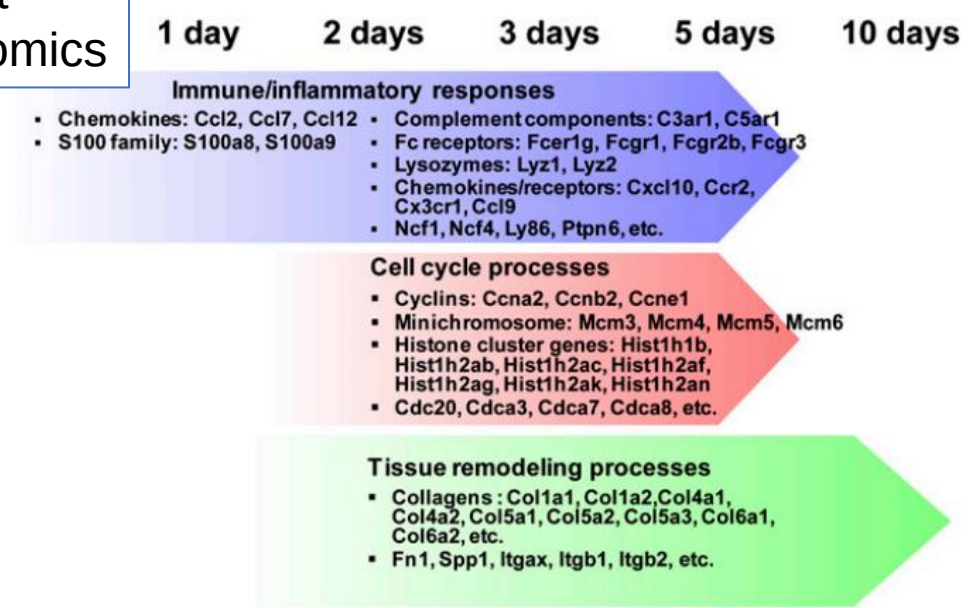
Agg-E SS model and cardiovascular disorder

Whole genome analysis of heart tissues and its histopathological data showed acute impacts of Agg-E SS and its residual effects lasted for long time

Heart histopathology

		10-day Agg-E stress (%)	
		1 d delay	4 w delay
C57BL/6j	Myocardial Degeneration	36.4	20.0
	Lymphohistiocytic Myocarditis	45.5	None

Heart Genomics

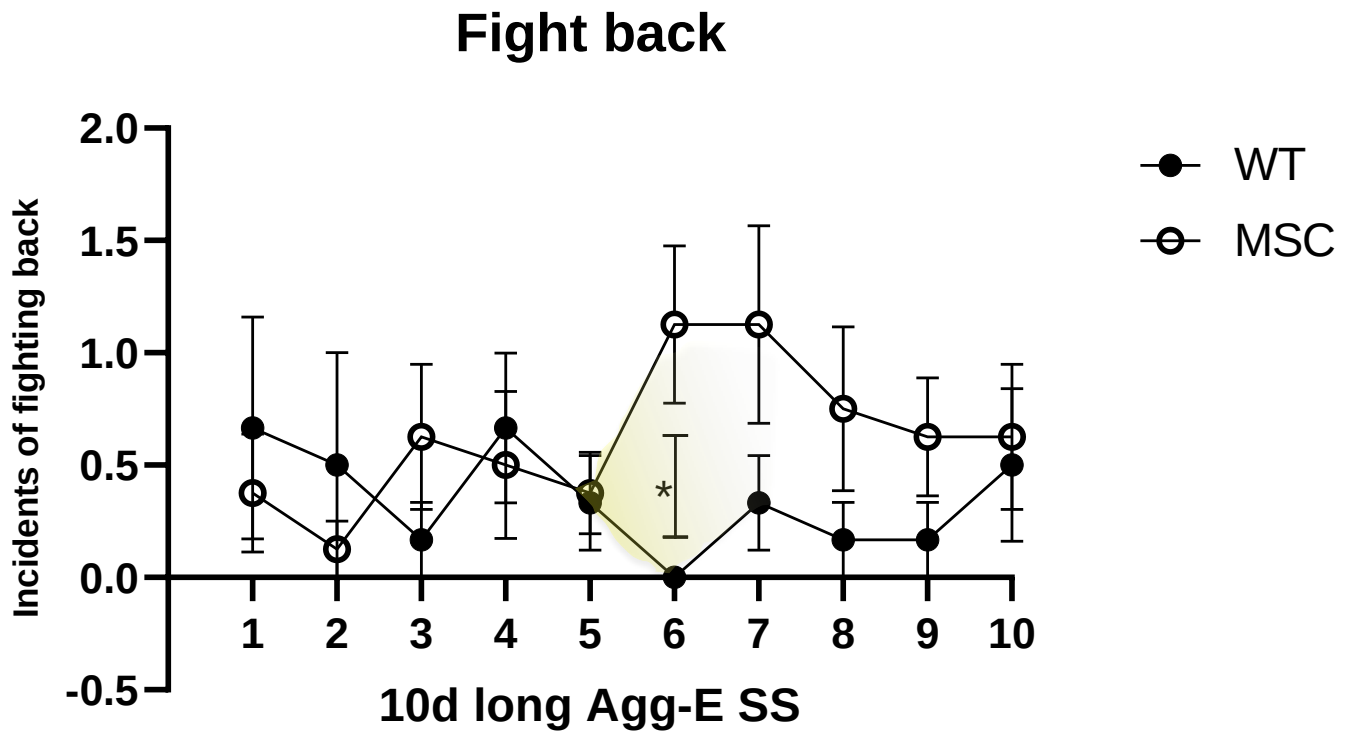


References:

1.Chakraborty N, Meyerhoff J, Gautam A, Muhie S, Jibitu M, De Lima TC, Hammamieh R, Jett M. Gene and stress history interplay in emergence of PTSD-like features. Behavioural brain research. 2015 Oct 1;292:266-77.

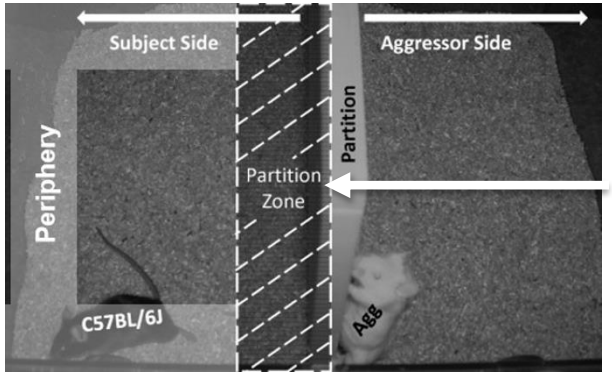
2.Cho JH, Lee I, Hammamieh R, Wang K, Baxter D, Scherler K, Etheridge A, Kulchenko A, Gautam A, Muhie S, Chakraborty N. Molecular evidence of stress-induced acute heart injury in a mouse model simulating posttraumatic stress disorder. Proceedings of the National Academy of Sciences. 2014 Feb 25;111(8):3188-93.

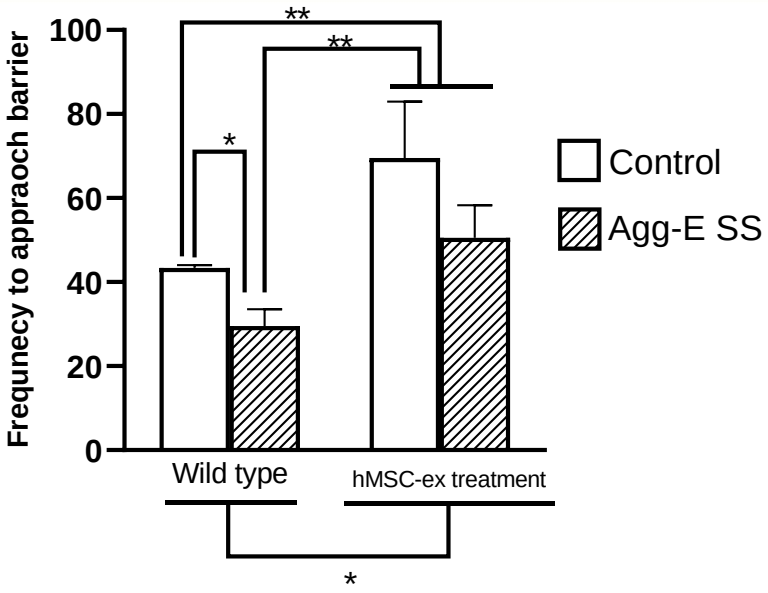
Behavioral profile during 10d Agg-E SS session



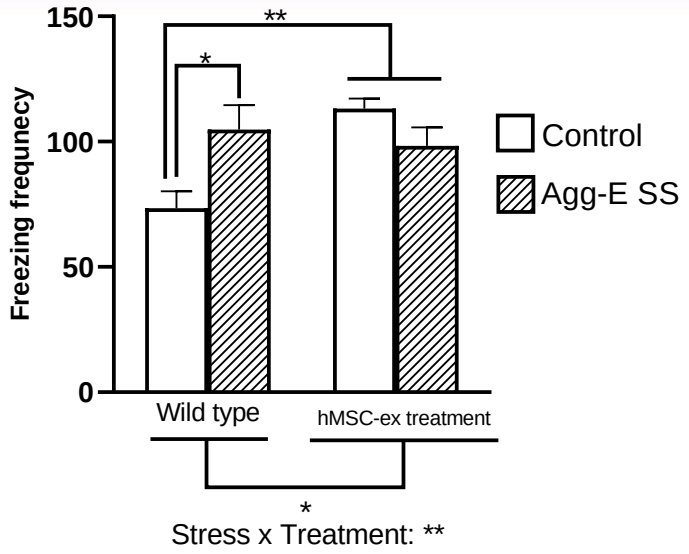
MSC-mice showed increased fight back to Agg mice after day 5,
which coincide with the 2nd round of hMS-ex administration.

Behavioral profile during the barrier test: Fear markers





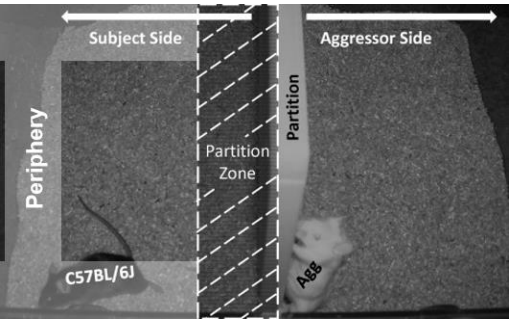
Increased frequency to approach barrier is a sign of curiosity overpowering the fear.

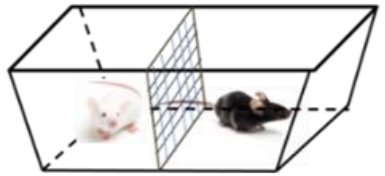


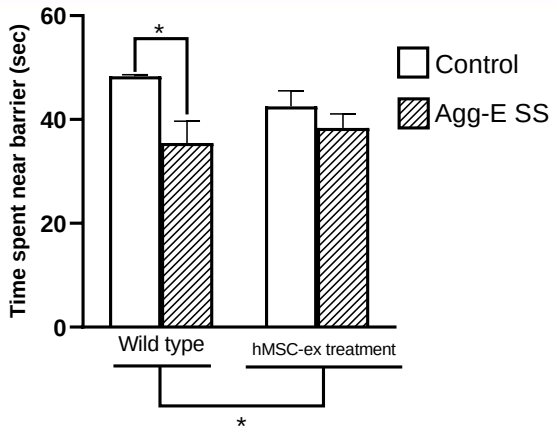
Increased frequency to freeze is a sign of fear.

Overall, hMSC-ex mice showed high curiosity and *high fear*, potentially a mixed sign of a high activity and anxiety.

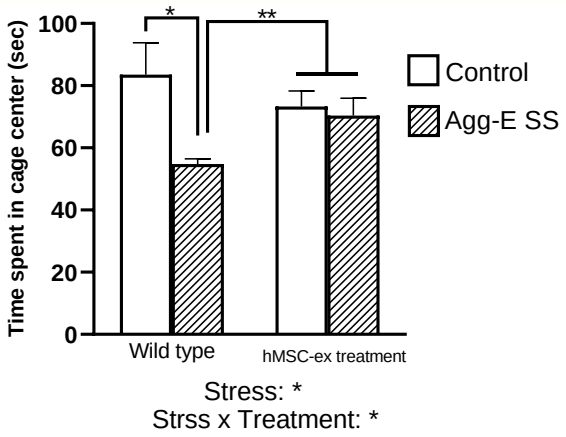
Behavioral profile : Distribution of time spent during barrier test



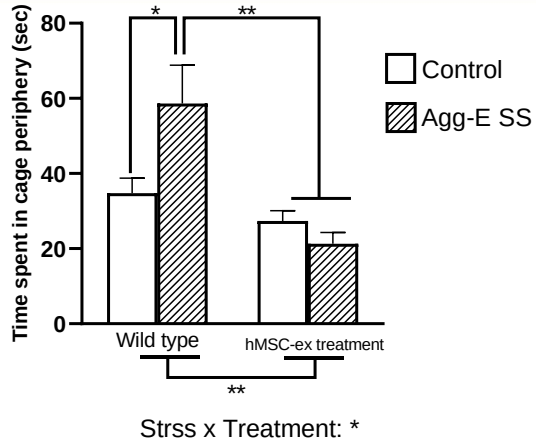




Longer time spent near barrier is a sign of high curiosity (natural trait of rodents) overpowering the fear.



Time spent inside the cage center, rather an open space is a sign of less fear.

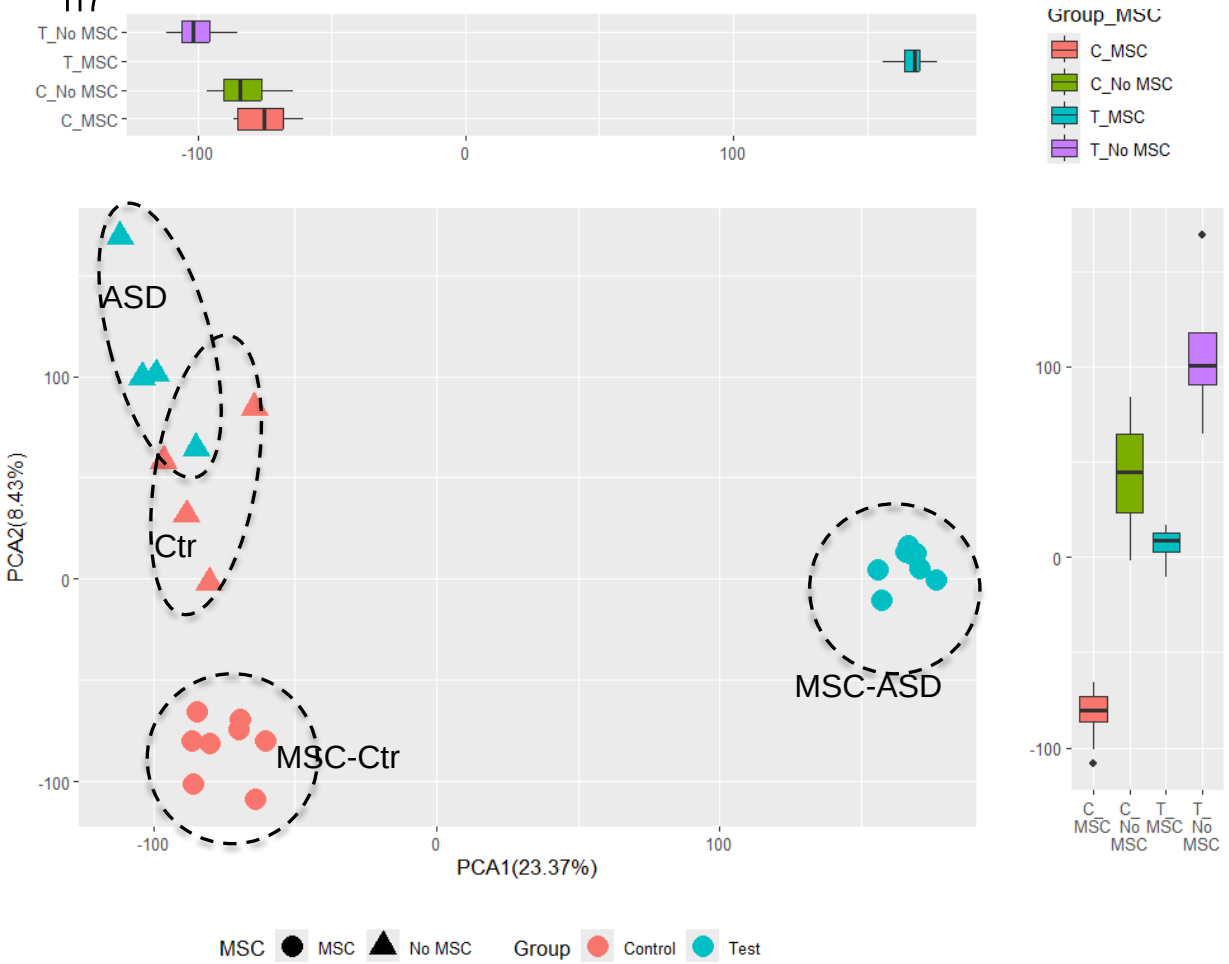


Time spent near periphery is a sign of anxiety and a trait underlining the withdrawal behavior from traumatic experience.

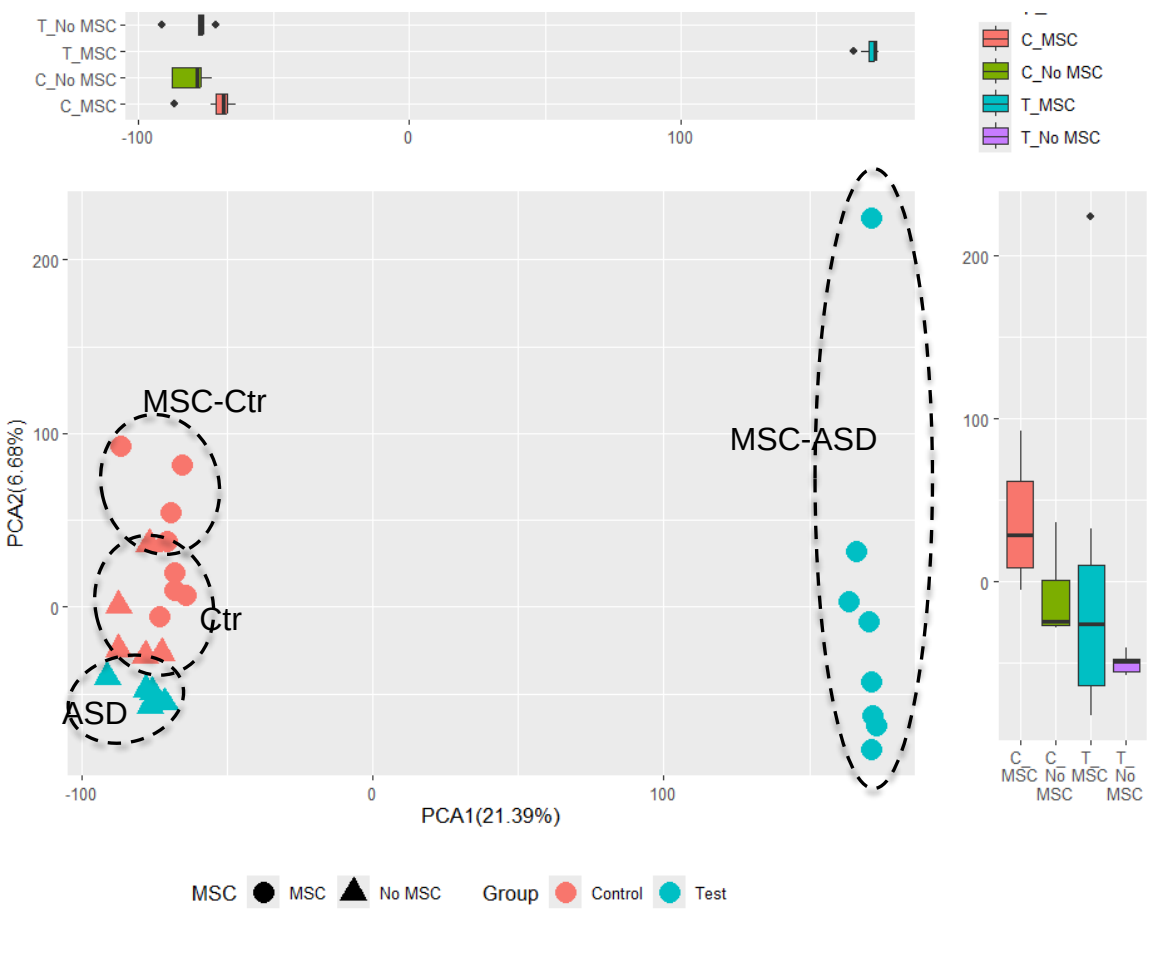
Overall hMSC-ex treatment showed a sign of mixed effects immediately after the removal of Agg-E SS

Whole genome analysis: Principal Component Analysis (PCA)

Brain



Heart



MSC-ASD mice showed very different overall genomic profile.

Functional networks perturbed by hMSC-ex

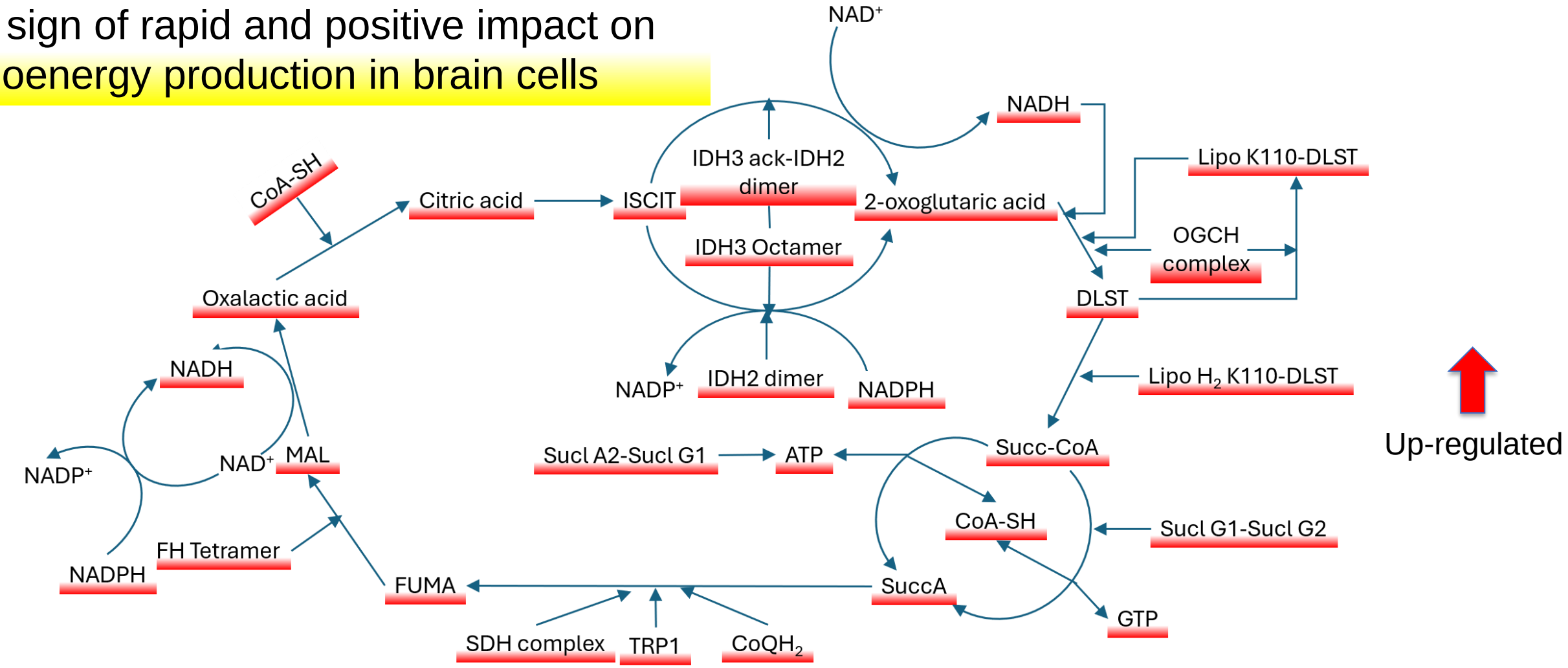
	Brain Pathways	Z score
	Mitochondrial protein degradation	5.6
	Oxidative Phosphorylation	5.2
	Mitochondrial translation	5
	Complex I biogenesis	4.6
Pain	Neurotransmitter release cycle	-4.6
Pain	Neuropathic Pain Signaling in Dorsal Horn Neurons	-4.9
Pain	Glutaminergic Receptor Signaling Pathway	-5.6
Pain	Synaptogenesis Signaling Pathway	-5.7

Cardiac Pathways	Z score
Glutaminergic Receptor Signaling Pathway	5.5
Synaptic Long-Term Potentiation	5.4
Neurotransmitter release cycle	4.2
Calcium Signaling	4.2
Collagen degradation	-3.2
Mitochondrial Fatty Acid Beta-Oxidation	-3.9
Mitochondrial protein degradation	-4.3
Oxidative Phosphorylation	-5.6

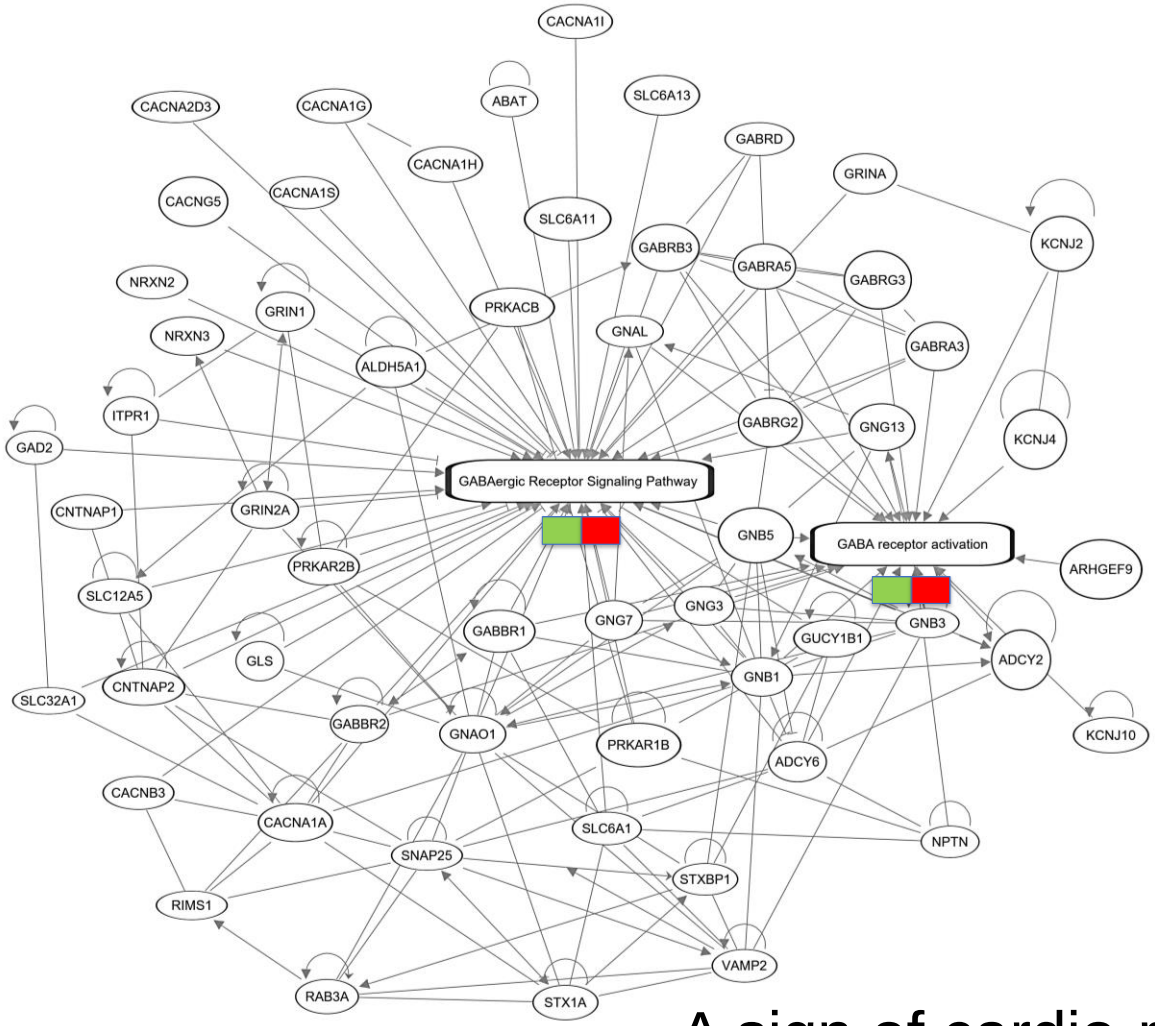
- Diverging activities by hMSC-ex were suggested by functional analysis.
- hMSC-ex enhanced bioenergy and inhibited pain networks in brain.
- hMSC-ex inhibited bioenergy related networks in heart, which could be due to the time sensitive measurement.

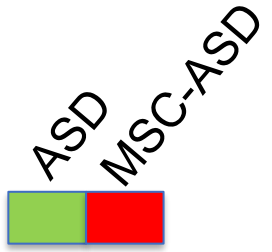
hMSC-ex activated TCA cycle in brain tissues of ASD mice

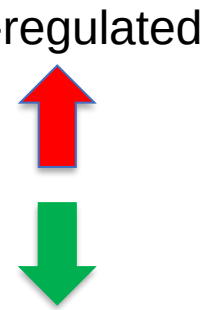
A sign of rapid and positive impact on bioenergy production in brain cells



hMSC-ex activated GABAergic cycle in heart tissues of ASD mice







A sign of cardio-protective impact on heart

Conclusions

- The Agg-E SS model mimics acute stress response (ASR) by inducing phenome-to-genome perturbations.
- Dose-response profiling and literature review suggested a hMSC-ex administration protocol that would cause minimum inflammation and maximum bioavailability to the tissues.

- In the barrier test:
  - Agg-E SS mice avoided traumatic cues and exhibited freezing behavior, indicating heightened anxiety and trauma.
  - MSC mice approached traumatic cues but still showed freeze motion, reflecting partial resilience.
  - hMSC-ex treatment increased activity but did not fully alleviate anxiety.
  - hMSC-ex administration made Agg-E SS mice less traumatized and more cautious when facing the aggressor.

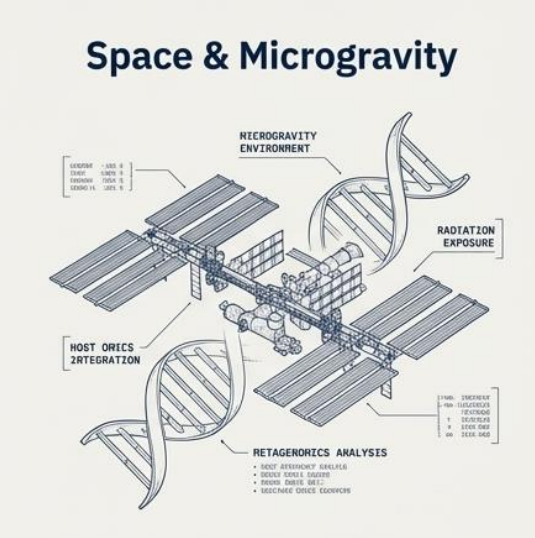
- Overall, hMSC-ex provided partial behavioral improvement, reducing trauma but not completely resolving anxiety-related behaviors in the Agg-E SS model.

Conclusions (Continued)

- Omics analysis revealed that hMSC-ex treatment following Agg-E SS is the most dominant factor causing biological perturbations, surpassing all other study variables.
    - Dose adjustment of hMSC-ex may be necessary to optimize therapeutic outcomes.
    - Future studies should investigate the long-term effects of hMSC-ex treatment.
- Major and positive changes were observed in the brain tissues of Agg-E SS mice after hMSC-ex administration.
- The impact of hMSC-ex on heart tissue was not adequate, possibly due to timing issues in the assay; histopathology data may provide more definitive insights.
- Overall, hMSC-ex treatment shows promise in ameliorating trauma-related changes, particularly in the brain, but further optimization and investigation are needed.

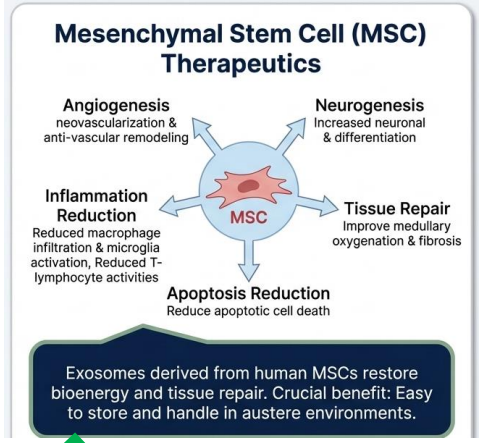
- ❖ hMSC-ex may serve as an early stabilizer to counter multiple military-relevant disorders.
- ❖ The capability of hMSC-ex to ameliorate cold stress has been demonstrated.
- ❖ Disorders associated with acute bioenergy depletion could potentially be mitigated by hMSC-ex.

Thank You



Review of our five Space studies

Session: Current Challenges in DoW Aerospace Medicine
Time: Monday, 1300-1500



hMSC-ex in Cold stress

Session: Human Performance Optimization in Cold-Weather & High-Altitude Environments
Time: Monday, 1530-1730

hMSC-ex in ASD

Session: Cellular Therapeutics for Trauma
Time: Thursday, 1115-1315

TBI Biomarker

Session: Managing the Golden Hour & Beyond: Innovative TBI Care in Contested Combat Environments
Time: Wednesday, 0830-1000

Radiation Therapeutics

Session: Ops in a Nuclear Environment: Ensuring Medical Readiness & Preparing for Complex Injuries
Time: Wednesday, 1530:1730

Nabarun.Chakraborty2.civ@health.mil
(301)-319-3398