

# Acute and Chronic Molecular Signatures and Associated Symptoms of Blast Exposure in Military Breachers

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Icahn School  
of Medicine at  
**Mount  
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WALTER REED ARMY  
INSTITUTE OF RESEARCH

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# POLL QUESTION #1

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Has anyone in the audience has in the past or currently serving in the military?  
Choose what applies please!

Yes?

No?

If yes, are you a:

Veteran?

Service Member, Active Duty?



“It’s like being kicked by a horse, a horse with a foot that could cover your entire body,” said one survivor of an IED attack.”

<http://www.nationalgeographic.com/healing-soldiers/blast-force.html>

Peter van Agtmael,  
Magnum Photos

# The Unseen Wounds of TBI



**“Detonation happened and I was right there in the blast seat. I got blown up.”**

Marine Gunnery Sgt. Aaron Tam (Ret.)  
Iraq 2004-05, 2007-08





# POLL QUESTION #2

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Has anyone in the audience experienced a blast event (during training or combat)?

Yes?

No?

# The Effect of Blast

## Symptoms

Acute / Chronic

- Blast affects the whole body
- Exposure to blast often leads to polytrauma (multiple TBIs) and a multisystem response
- It is difficult to determine the neurologic effects of blast TBI is that these often occur concomitant with other organ injuries.

Headache

Tinnitus

Forgetfulness

Sleep Disturbance

Chronic Pain

Anxiety/ PTSD

Suicidal Behavior



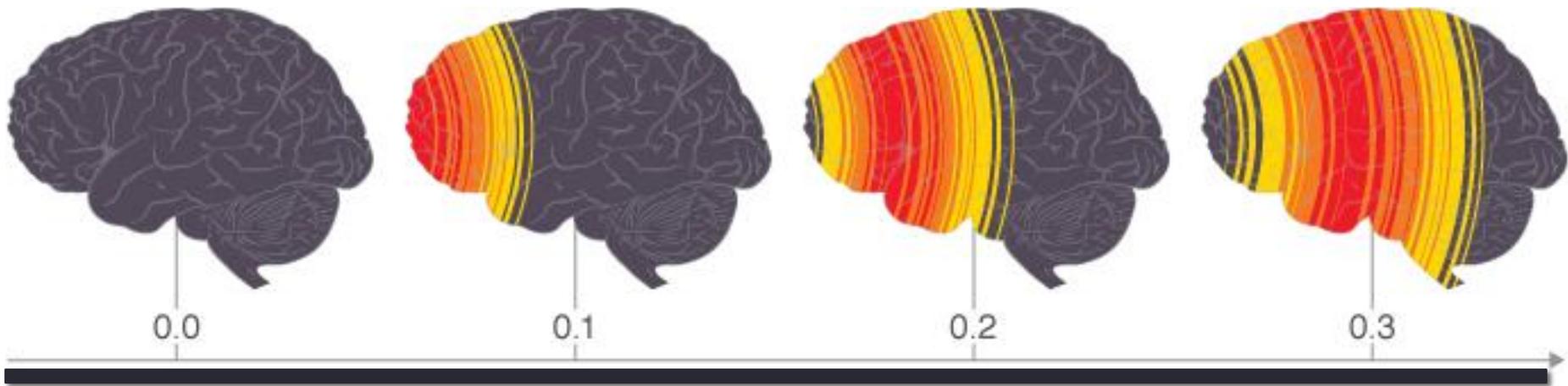
Repeated operational breaching exposure



Repeated exposure

# How Blast Affects the Brain

-  intracranial pressure: immediate effects upon impact. Pressure normalizes after a few ms.
- Brain's motion relative to skull: can occur for hundreds of ms post-impact.



## Blast Wave Transmission (milliseconds)

Jason Treat, National Geographic Staff. Source: Andrew Merkle, Johns Hopkins University Applied Physics Laboratory

# Traumatic Brain Injury (TBI)

- Iraq & Afghanistan wars led to highest number of military related TBIs since Vietnam
- ~20% of returning veterans suffered from TBI

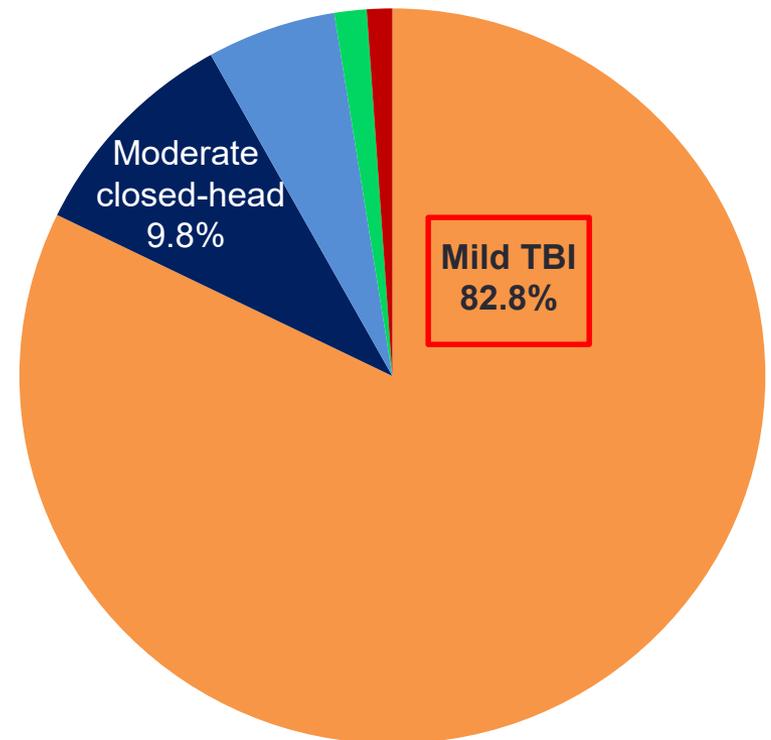
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2000 – 2019 3<sup>rd</sup> Quarter

	Penetrating	5,279
	Severe	4,110
	Moderate	40,378
	Mild	342,747
	Not Classifiable	21,344

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**TOTAL - All Severity 413,858**



# Sequelae of Blast Injury

## Neuropsychiatric Disorders:

- PTSD: Population based studies 30-40% (with >90% in clinical samples)
- Depression, & Increased Suicide Risk

## Spectrum of Blast Associated Symptoms

Difficulty with decisions, Mental slowness, Concentration problems, Headaches, Dizziness, Ringing in the Ear, Fatigue, Sadness, Sleep Disturbances



# POLL QUESTION #3

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Gauging the background of our group!

Clinical Research?

Basic Research?

Rehab Medicine?

Other Clinician?



# POLL QUESTION #4

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Are you familiar with the concept of epigenetics?

Yes?

No?

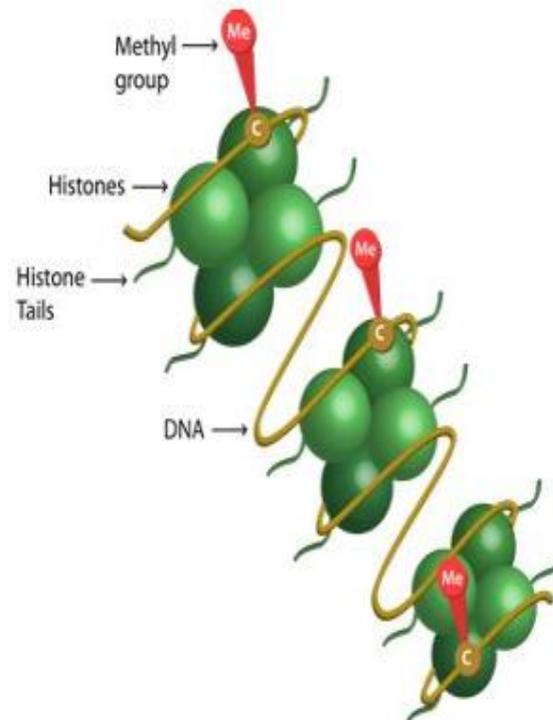
How about DNA Methylation?

Yes?

No?

# Introduction: DNA Methylation

- DNA methylation is a process in which methyl (Me,  $-\text{CH}_3$ ) groups are added to molecules of DNA at cytosine residues, typically resulting in changes in gene expression
- Represents highly stable, long-lasting marks that encapsulate a lifetime of environmental exposures (diet, stress, trauma, blast exposure, etc.)



# Objectives

1. Identify **chronic** DNA methylation changes associated with accumulative lifetime blast exposure
2. Identify **chronic** DNA methylation changes that track with blast-related symptoms
3. Does prior exposure history to TBI or blast effect responsiveness to blast acutely?



Operational Training; typical exposures ~4 psi.  
Maximum breacher exposure=12.9 psi (88.9 kPa)

Courtesy of the Walter Reed Breacher Consortium



# 1<sup>st</sup> Objective

Identify **chronic** DNA methylation changes associated with accumulative lifetime blast exposure

# Human Blast Exposure from Pilot Breacher Study



N=32, All male, average age  $35.8 \pm 9.5$  years

Day 1 - Baseline



Day 2



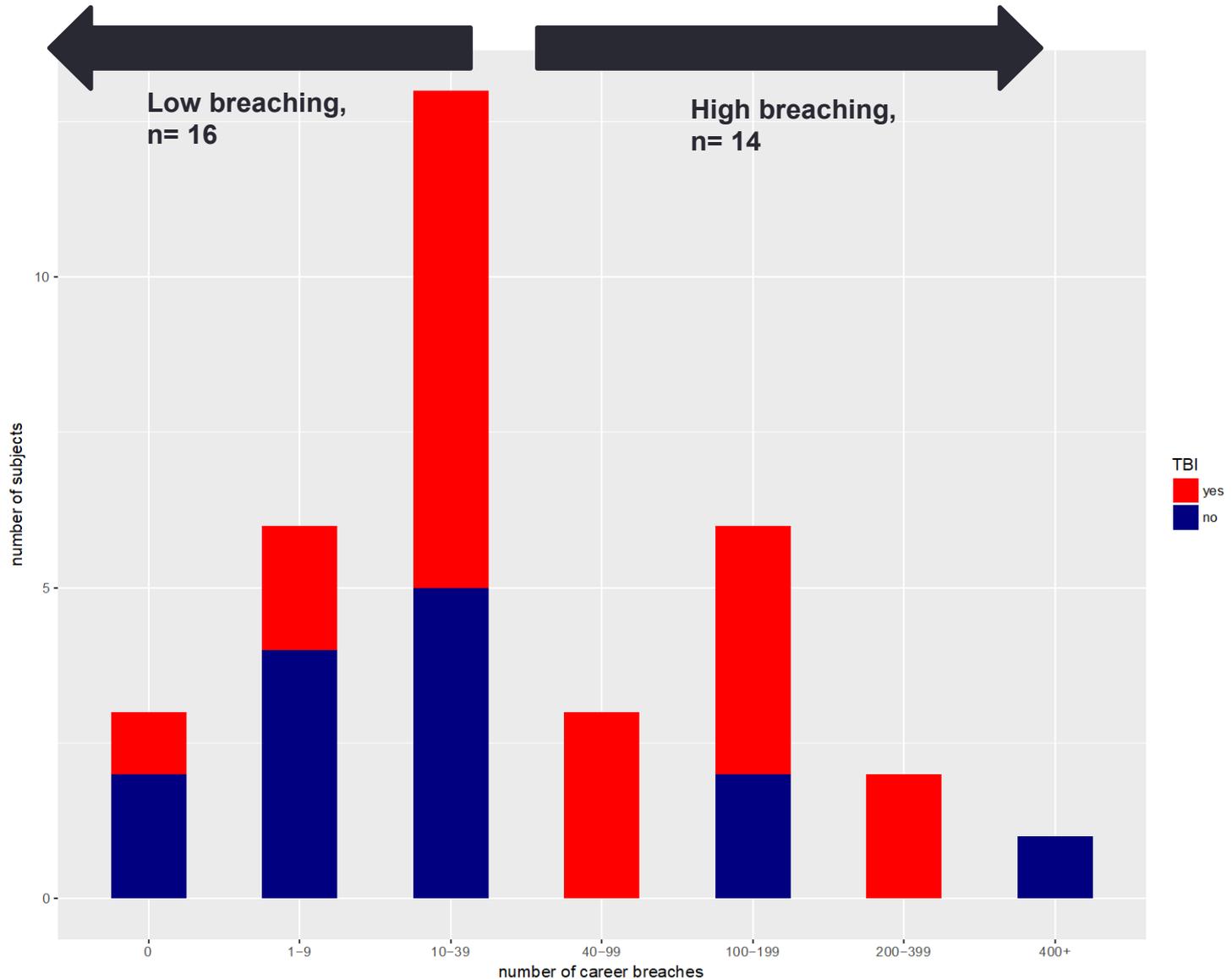
Day 7



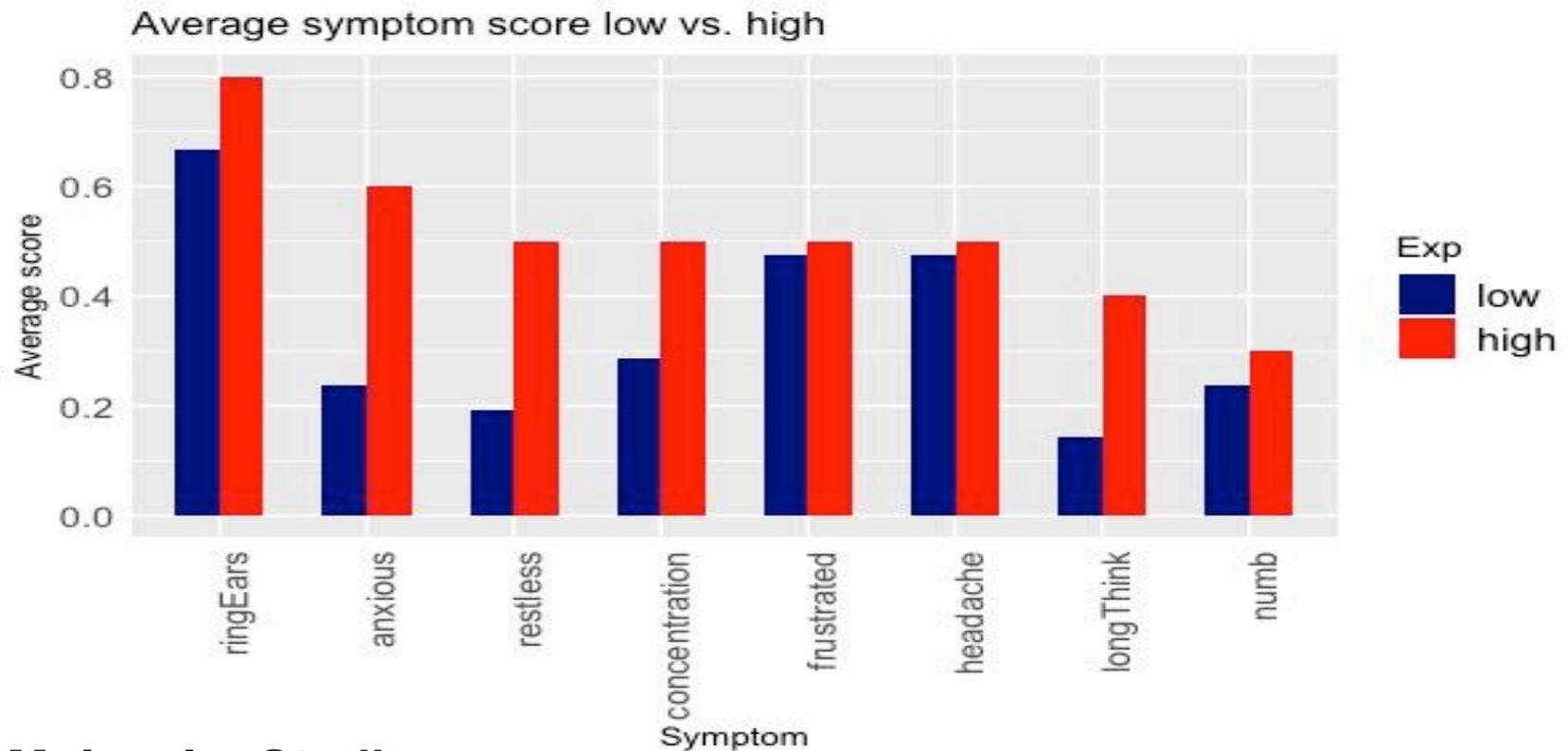
Day 9



Day 10 - End



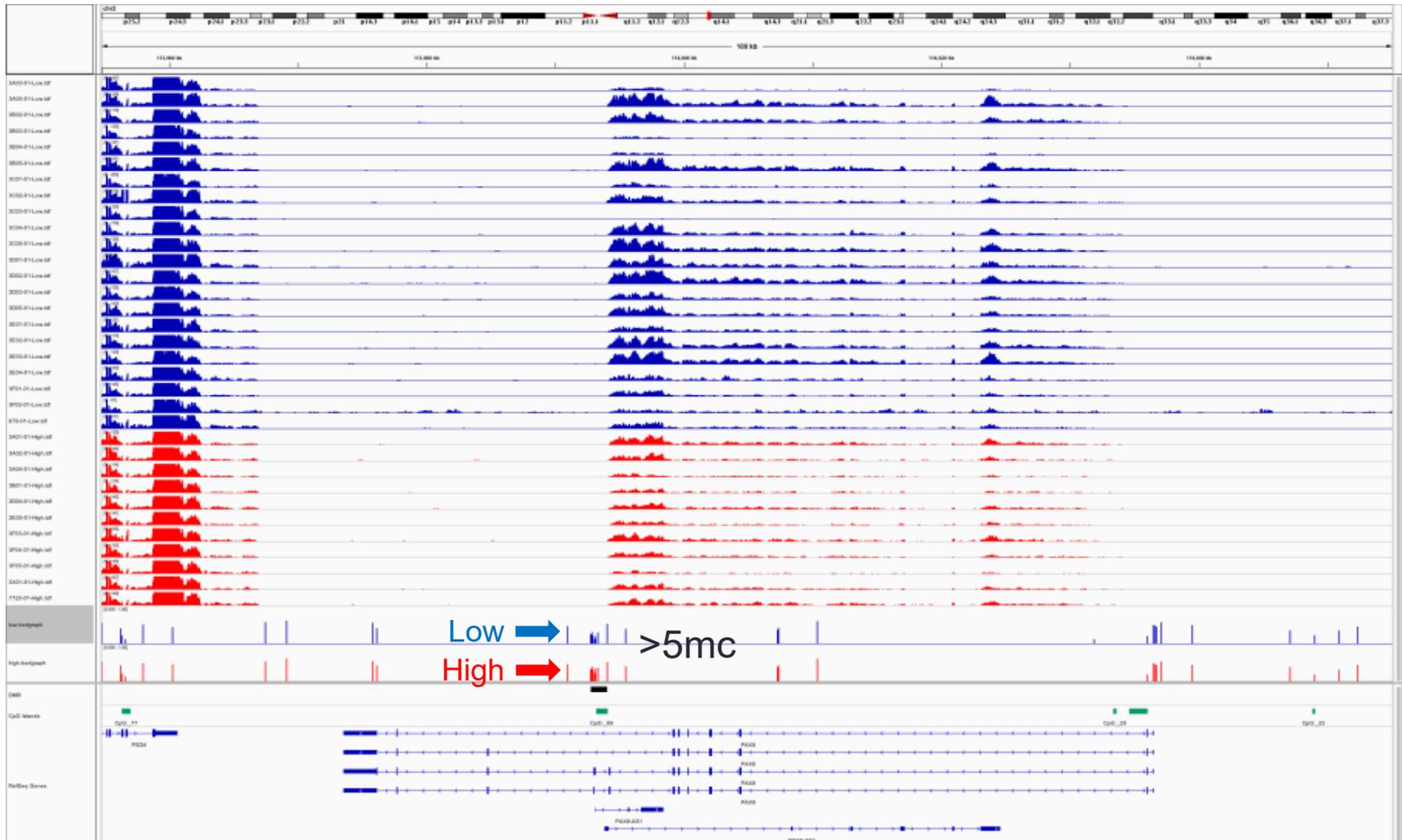
# Self-reported Symptoms at Baseline



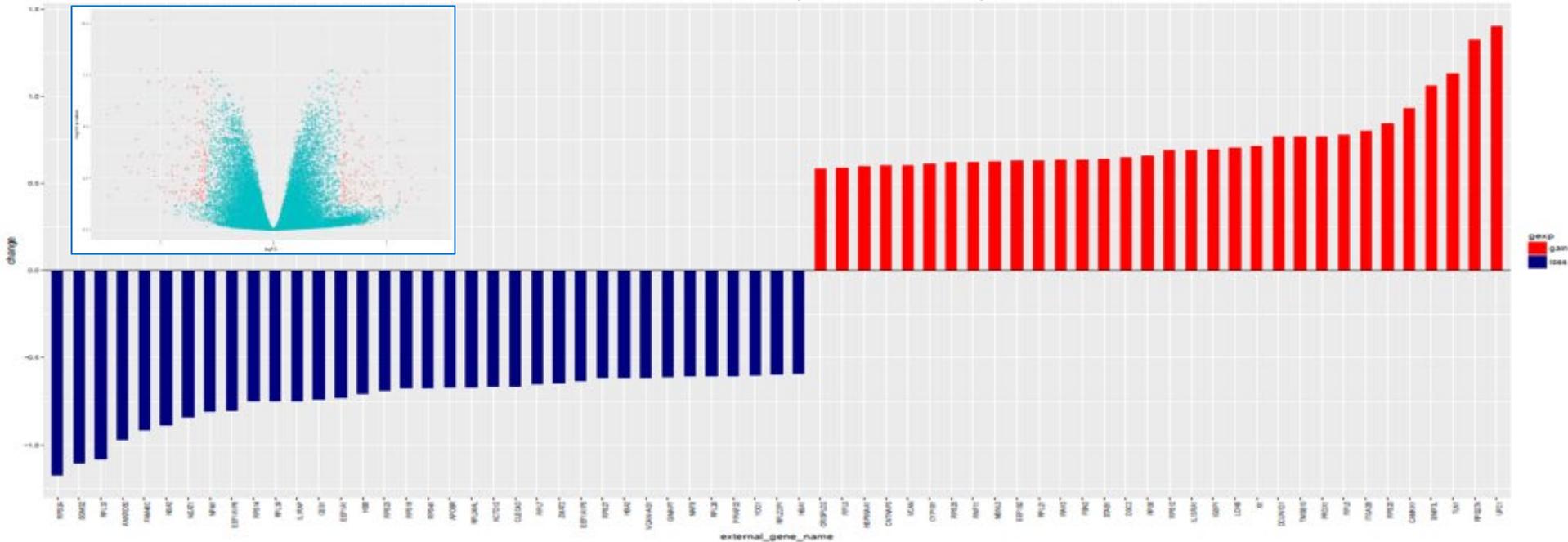
## Molecular Studies:

- Epigenetic analysis: Illumina Infinium HumanMethylation 450K or Next Generation EPIC BeadChip
- Whole genome transcriptome analysis via RNA-seq
- Published: Wang, Zhaoyu, et al. "Acute and chronic molecular signatures and associated symptoms of blast exposure in military breachers." *Journal of Neurotrauma* (2019).

# PAX8-AS1 Differentially Methylated Region (DMR) Low vs High Lifetime Blast Exposure



# Pre vs Post Blast Training



- Found significant gene expression and no DNA methylation changes
- Showing genes with robust gene expression fold changes ( $\geq 1.5$  FC)
- 30% are ribosomal proteins: heatshock & ubiquitin proteins
- These have been implicated in neurodegeneration and TBI

# Fort Leonard Wood: Replication in Independent Breacher Cohort

Day 1 – Baseline



Day 2 – Pre-blast



Day 2 – Post-blast

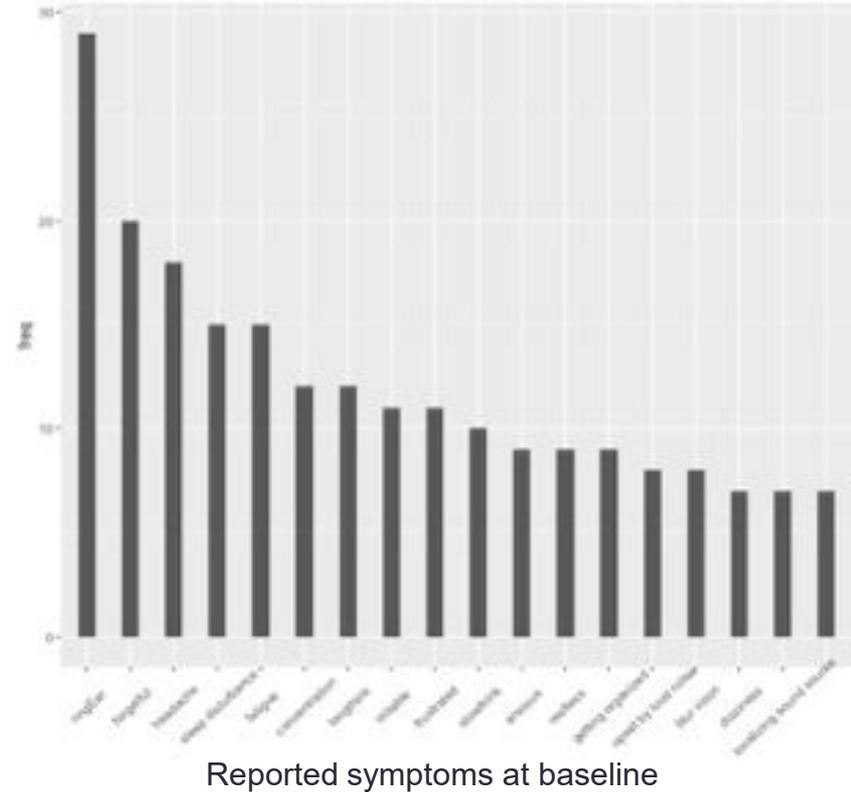
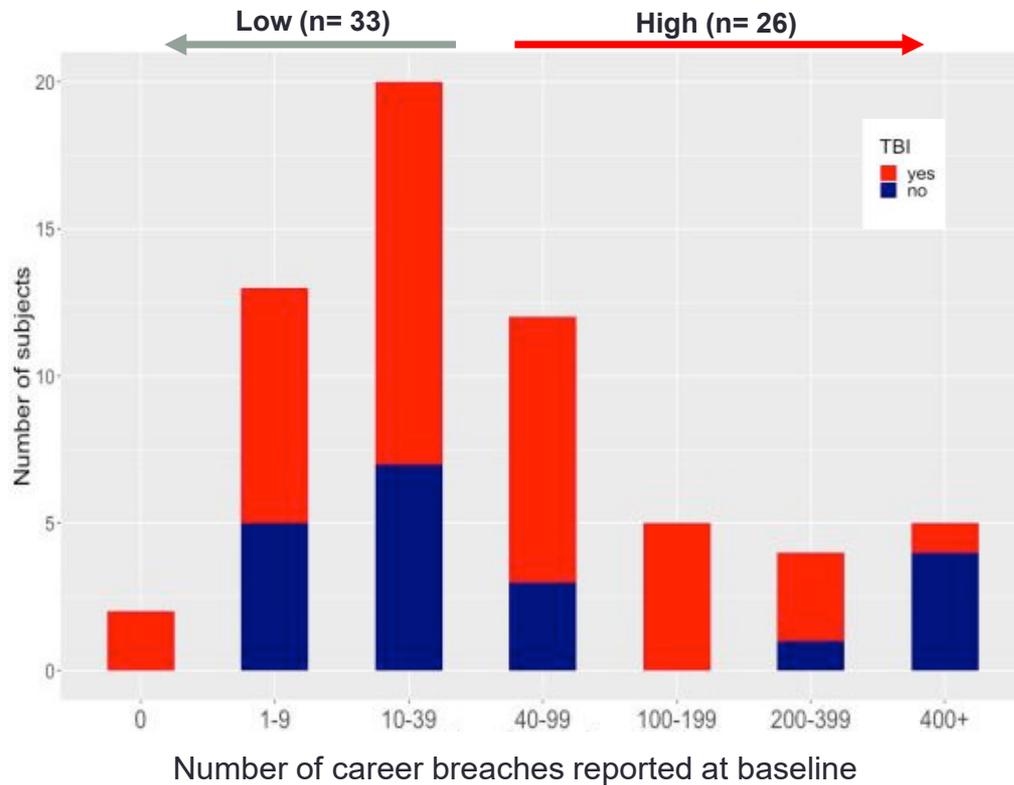


Day 3 - End



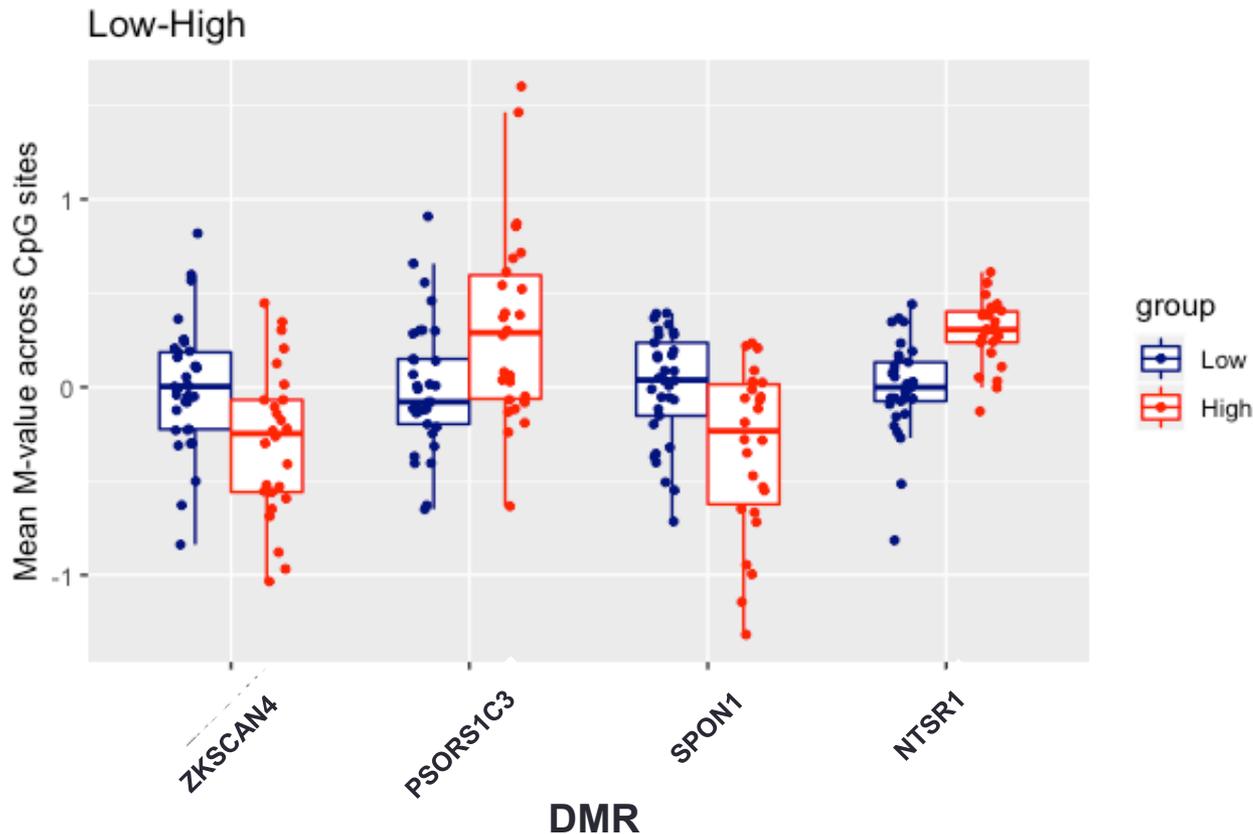
Courtesy of the Walter Reed Breacher Consortium

# Fort Leonard Wood: Breaching Operations Course Participants and Associated Symptoms



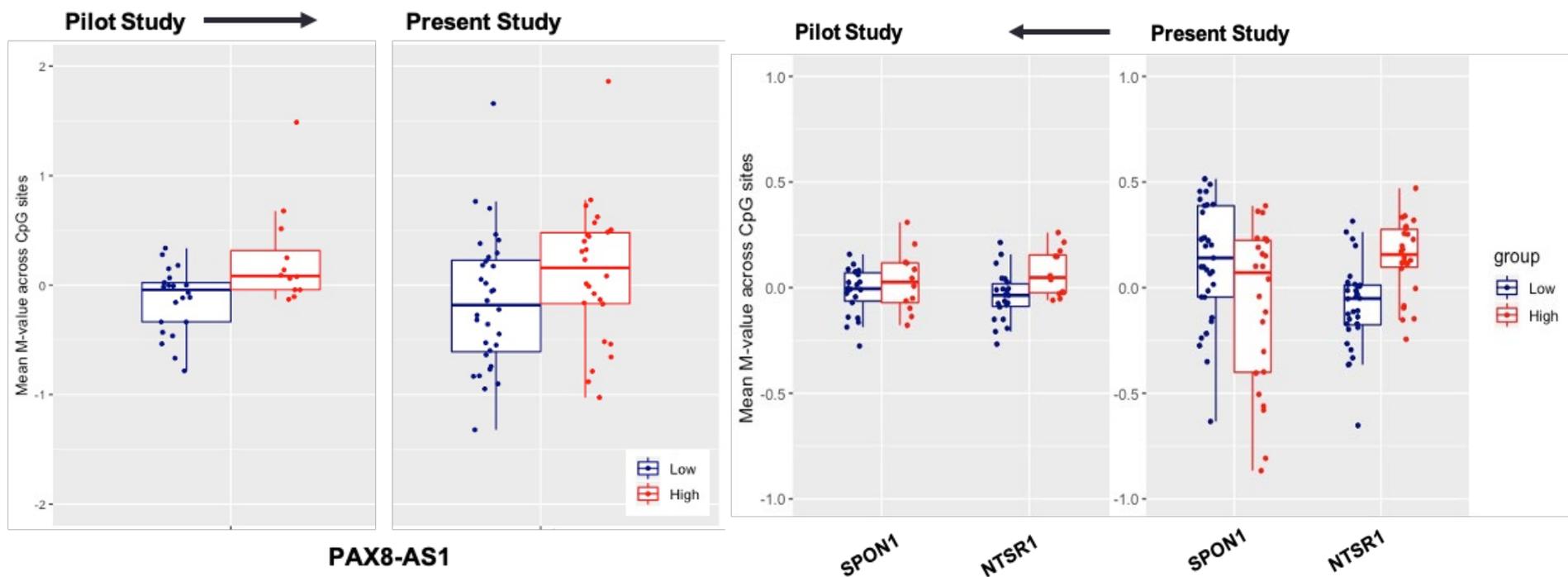
- 59 total subjects (from two training cohorts), with varying history of TBI, number of career breaches, and reported symptoms
- All male, age  $31.0 \pm 7.2$  years

## Results: Low vs high accumulative blast exposure causes changes in DNA methylation



- NTSR1 is associated with anxiety/depression and sleep dysregulation, and knockout mice exhibit decreased time spent in REM sleep relative to wildtype anxiety-related traits
- SPON1 is associated with sleep, with *Spon*<sup>-/-</sup> knockout mouse models showing disrupted circadian cycles

# Confirmation of replication with previous DMRs on low-high analyses of sleep-related genes



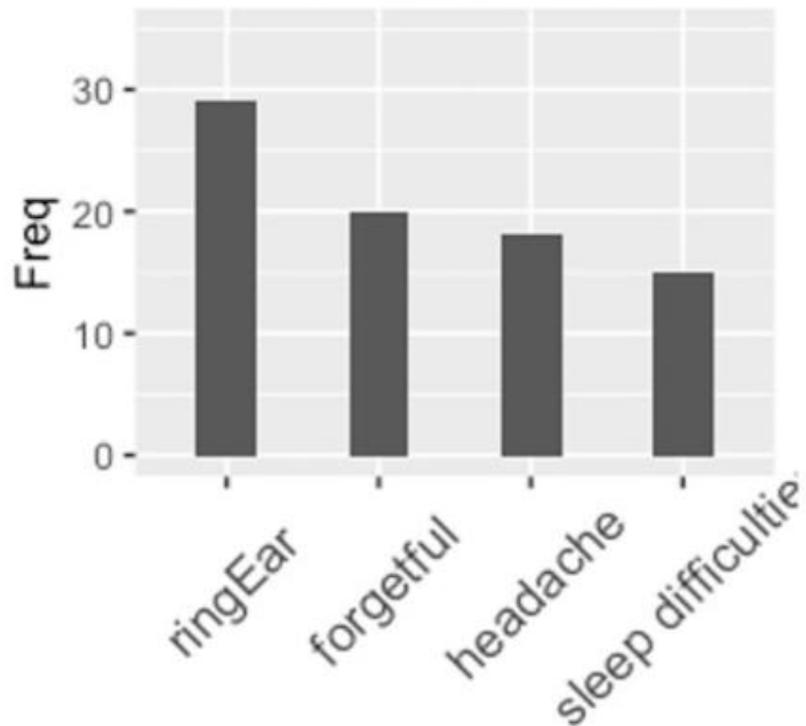
- Replication of previously reported genes for low-high blast exposure analyses of genes (left) *PAX-AS1* and (right) *SPON1* and *NTSR1*, with mean methylation M-values across CpG sites on the y-axis and the respective M-values for low-(blue) and high (red) lifetime blast exposed individuals.

## 2<sup>nd</sup> Objective

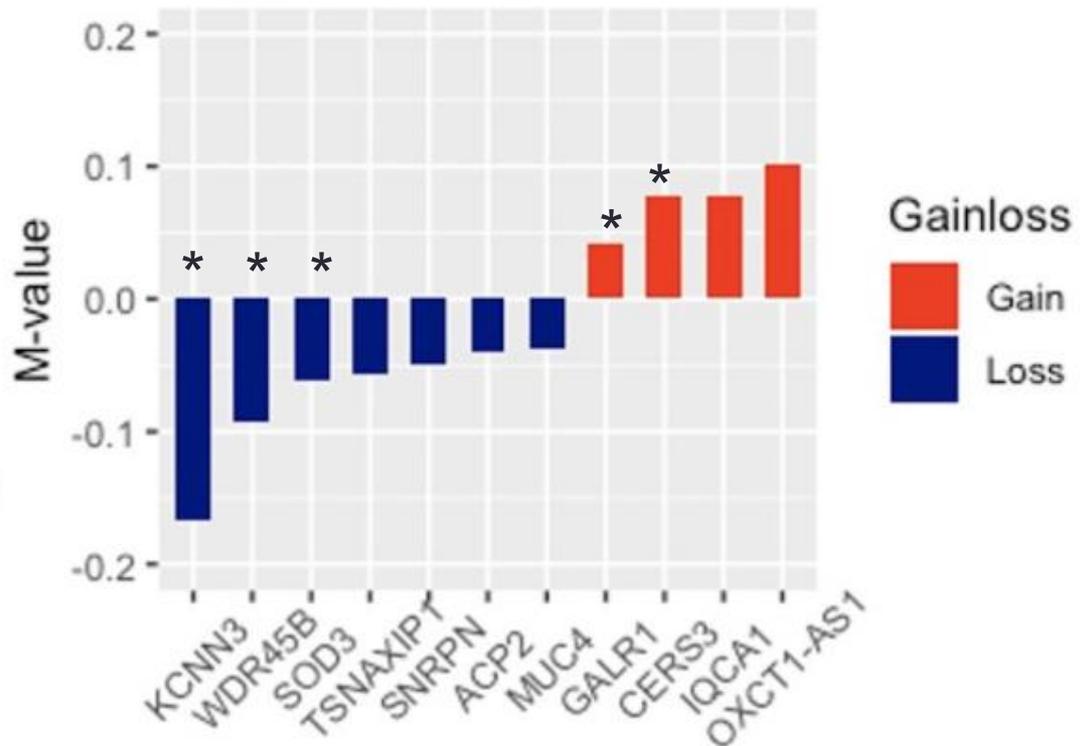
Identify **chronic** DNA methylation changes that track with blast-related symptoms

# DNA Methylation Tracks with Reported Symptoms of Tinnitus

Baseline symptoms



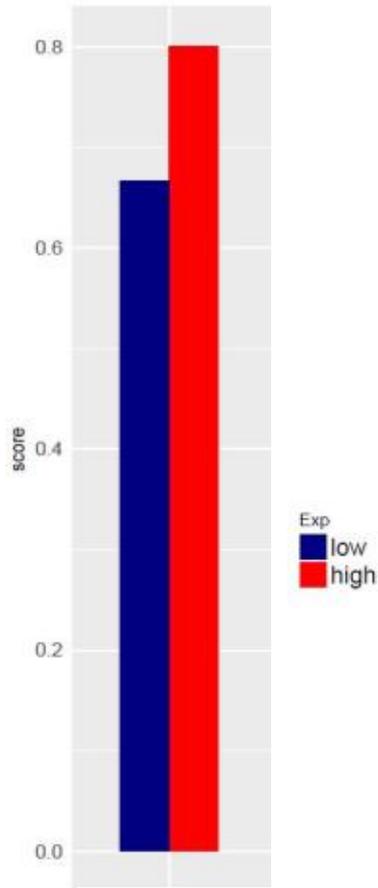
RingEar



- We found elevated symptoms of tinnitus/ringing in the ear at baseline (left).
- Differentially methylated genes associated that track with tinnitus denoted by (\*) (right).

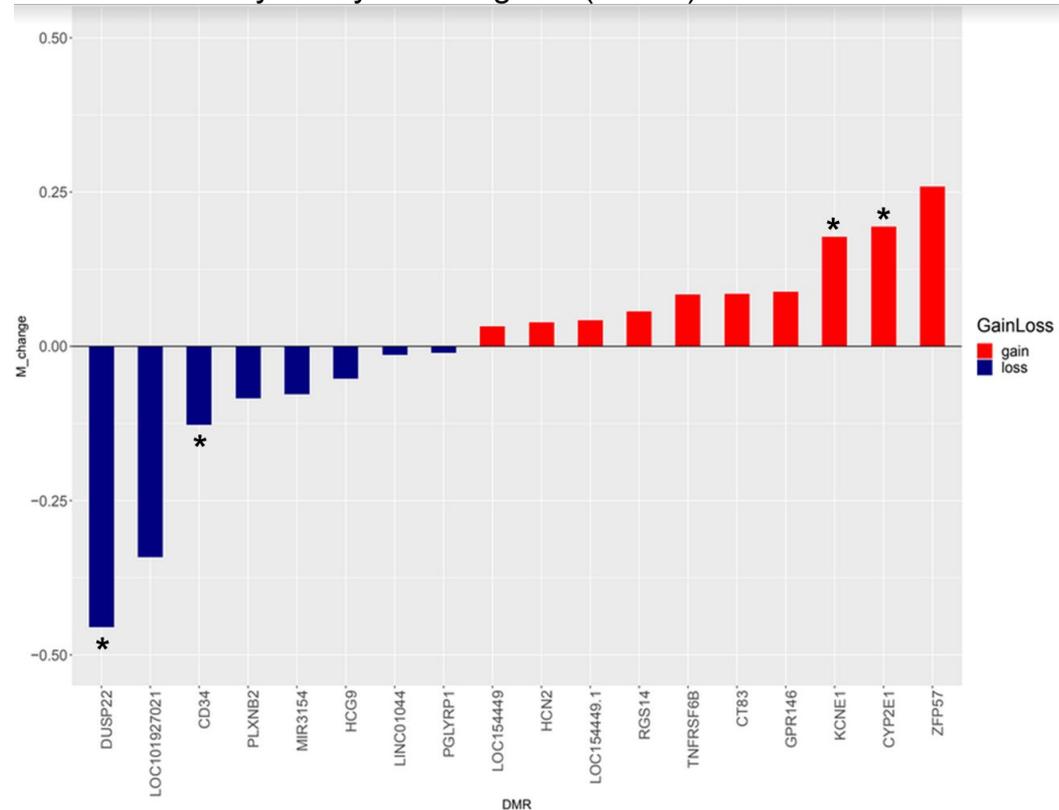
# DMRs & Proximal Genes Associated With Ringing in the Ear and Accumulative Blast

Avg Symptom Score Low vs High



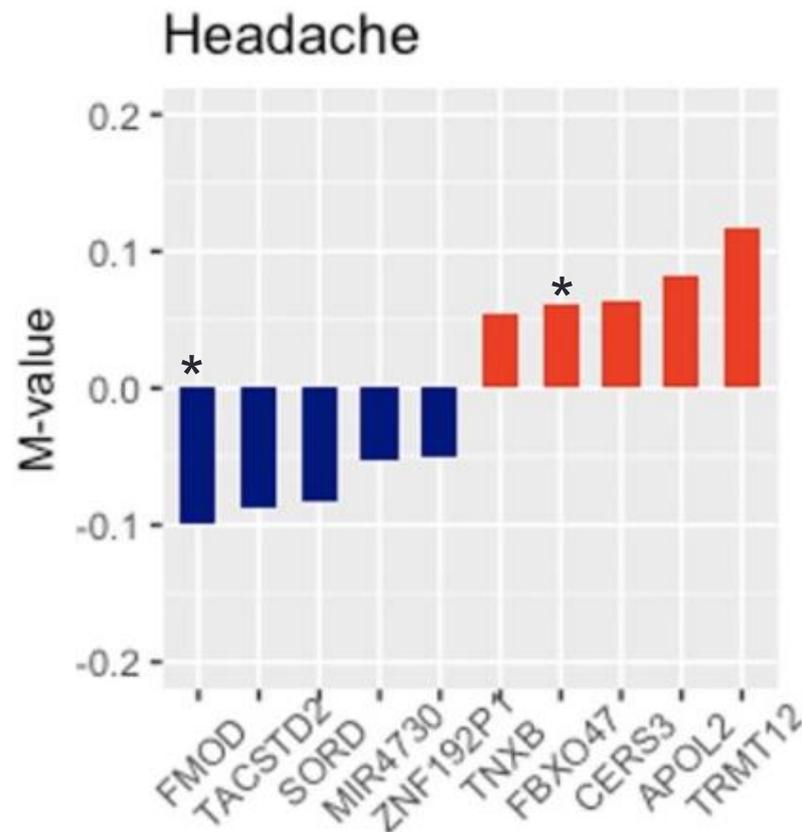
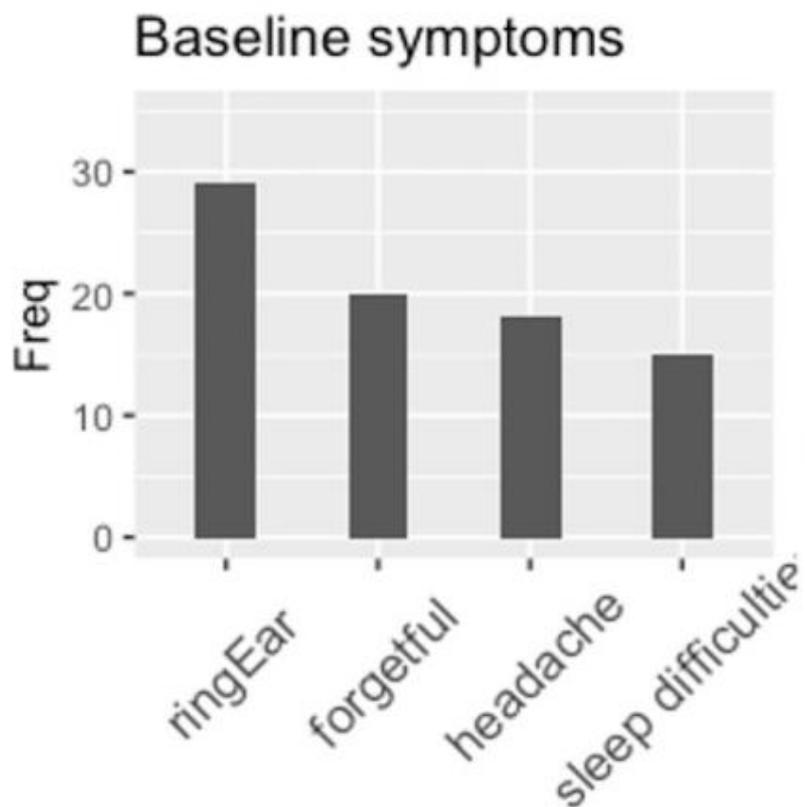
Ringing in the Ears

Differentially Methylated Regions (DMRs)



- We found elevated symptoms of tinnitus/ringing in the ear at baseline for those with low vs high career breaching (left).
- Differentially methylated genes associated with tinnitus with those implicated in auditory function denoted by (\*) (right).

# DNA Methylation Tracks with Reported Symptoms of Headache



- We found elevated symptoms of headache at baseline (left).
- Differentially methylated genes associated that track with symptom of headache denoted by (\*) (right).
- *FMOD* and *TNXB* are associated with temporomandibular joint disorder and chronic pain

## 3<sup>rd</sup> Objective

Does prior exposure history to TBI or blast affect responsiveness to blast acutely?

# Single Breacher Cohort at Fort Leonard Wood: In-depth Serial Sampling



Day 1 – Baseline



Day 2 – Pre-blast



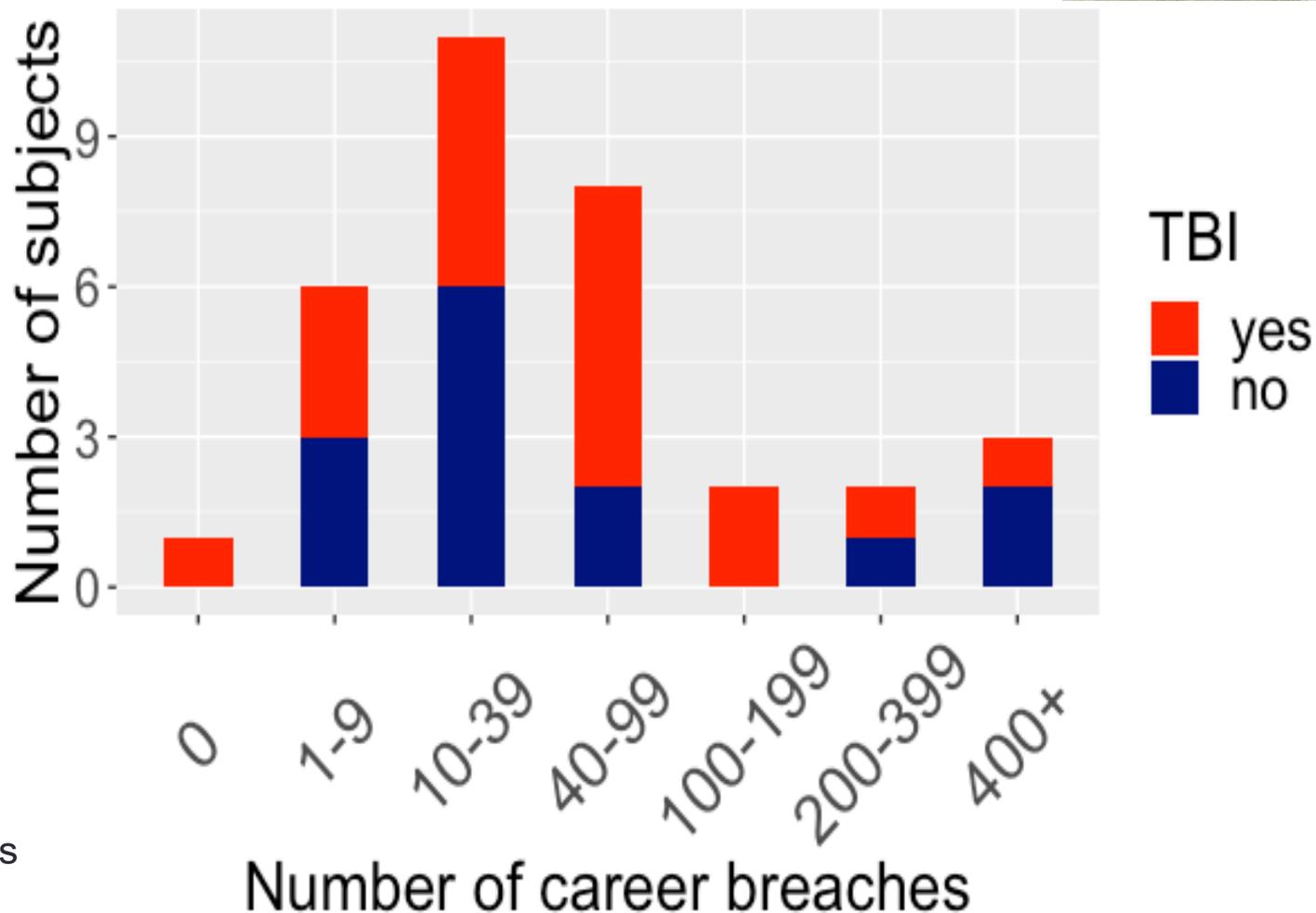
Day 2 – Post-blast



Day 3 - End

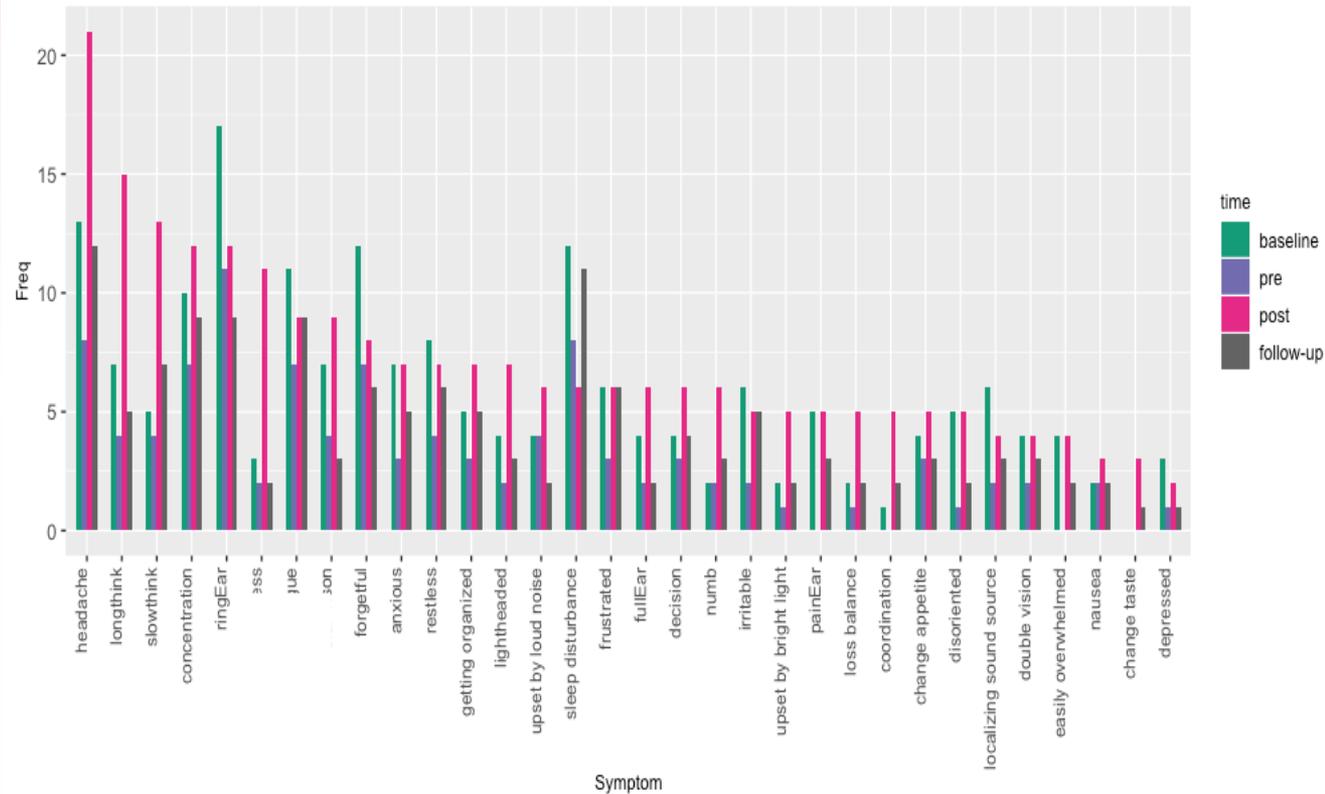
31 subjects, all male  
Age  $30.3 \pm 7.2$  years

## Site 8



# Serial Measurement of Symptoms During Operational Breaching Training

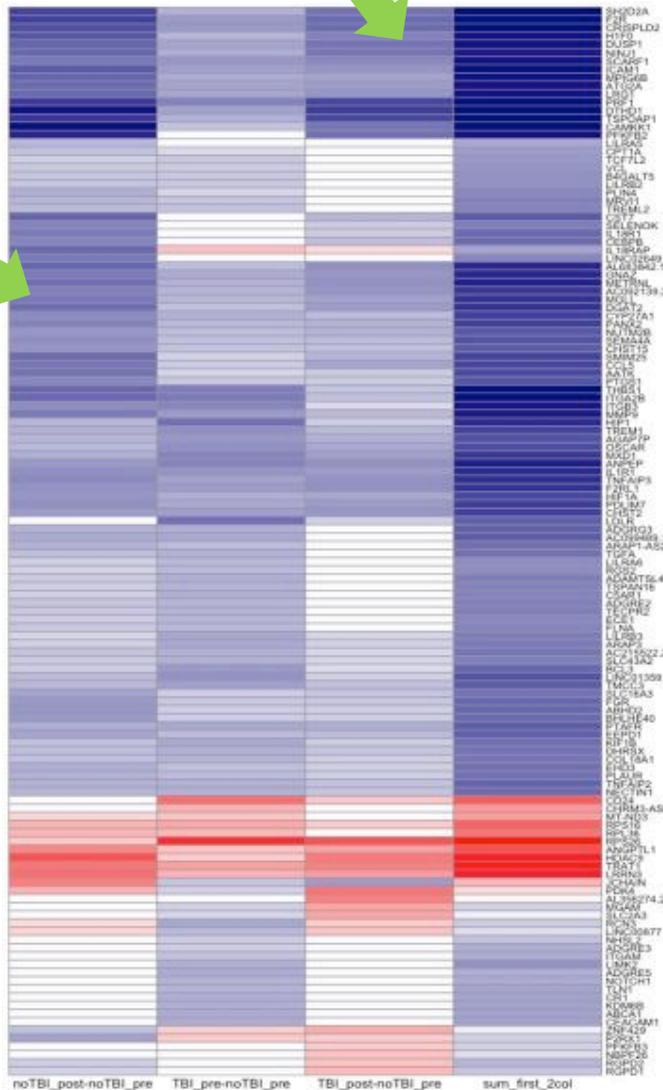
- Serial self-reported assessments show that acutely post blast headache and symptoms related to memory and cognitive functioning are frequently endorsed by breachers



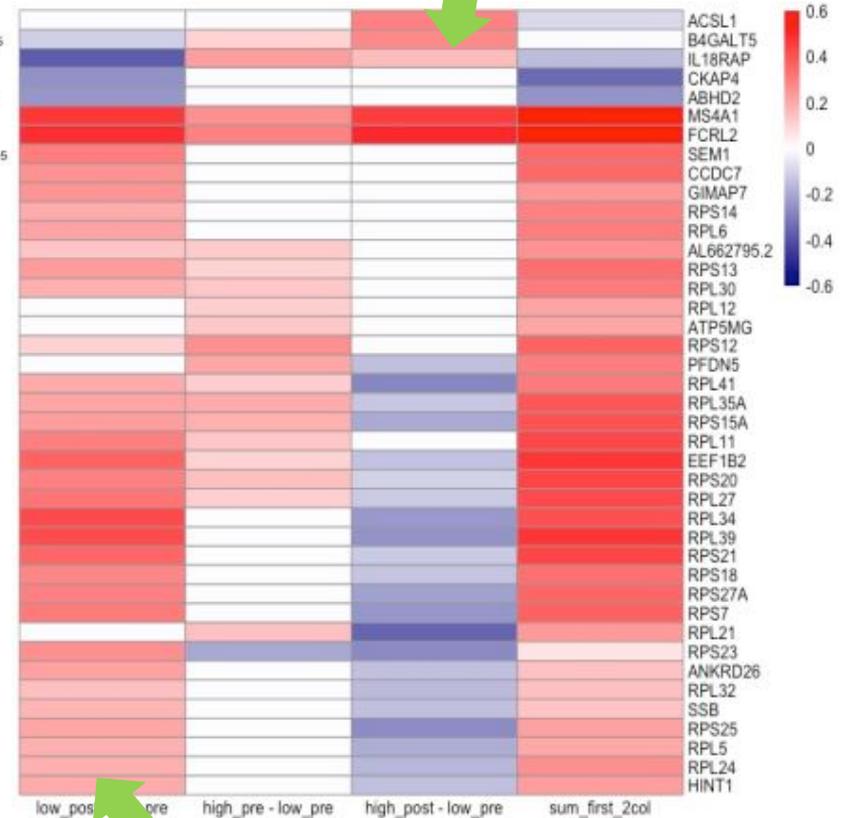
# Differential Responsivity to Blast Dependent on Prior Exposure Load

Blunted response; low gene expression responsivity in TBI vs non-TBI

Gene expression change in non-TBI "reference" group

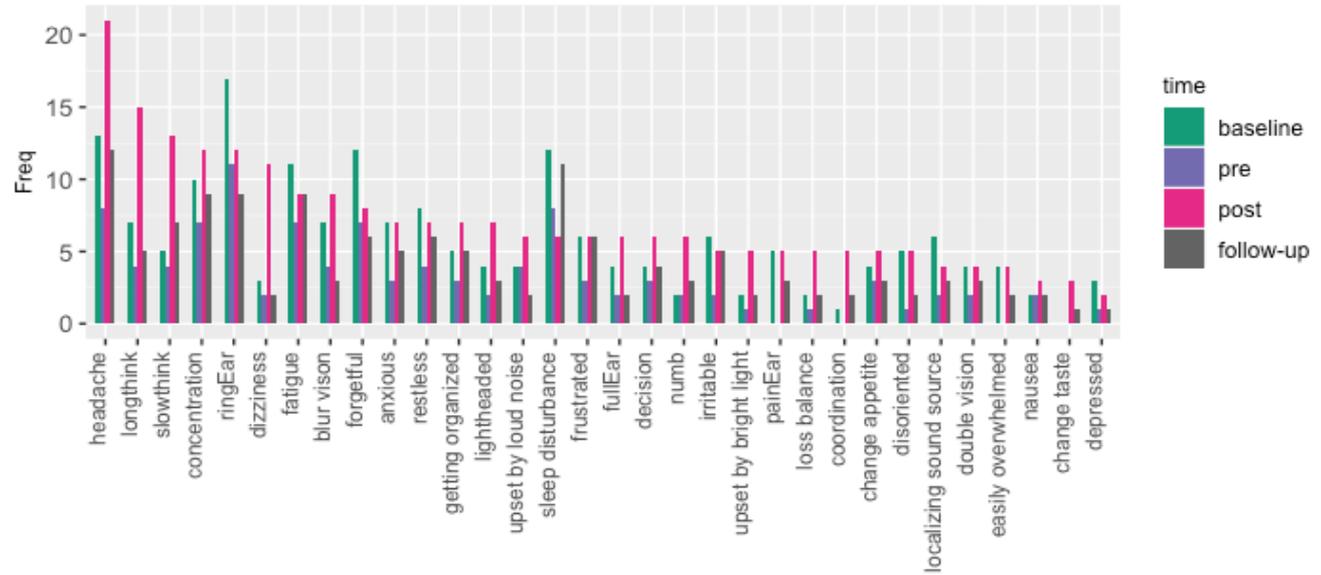


Blunted response; low gene expression responsivity in high cumulative exposed vs low cumulative exposed

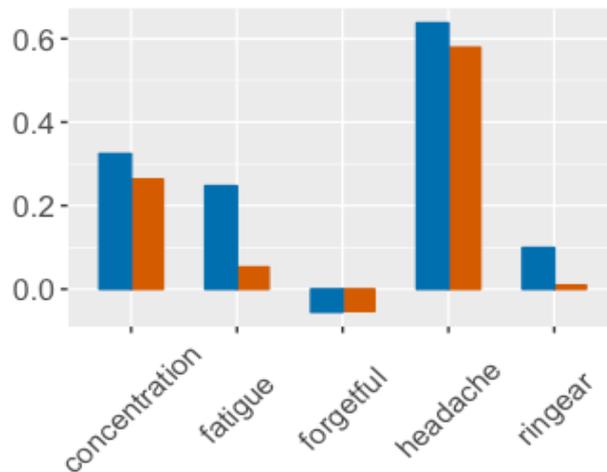


Gene expression change in low cumulative exposed "reference" group

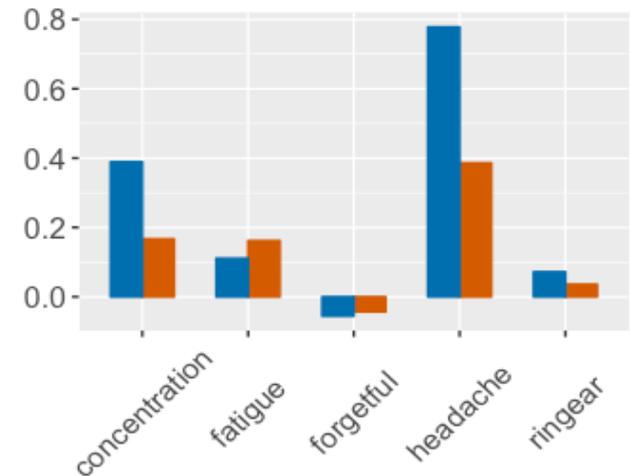
# How Does Blunted Molecular Response Track with Symptoms Acutely Following Blast?



- **Bottom left:** Acutely following blast, non-TBI group shows greater symptom severity
- **Bottom right:** Acutely following blast, low lifetime breaching exposed group shows greater symptom severity



group noTBI TBI



group low high

# Conclusions

- Exposure to blast overpressure is capable of altering DNA methylation chronically through high numbers of accumulative blast overpressure across a military career
- DNA methylation represents highly stable, long-lasting marks, and capture lifetime accumulative exposures to repeated blast events
- Tinnitus (ringing in the ear), sleep difficulties, and headache are the highest reported symptoms in military Breachers investigated
- Identified differentially methylated regions and associated genes that track with these reported symptoms
- Replicated findings of these differentially methylated genes in independent cohort of Breachers.

# POLL QUESTION #5

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After viewing this talk, how relevant do you think molecular studies are in development of novel rehabilitative treatment interventions for blast injury and TBI?

Very

Somewhat

Not really

Not at all

Other, please comment

# Acknowledgements

➤ Icahn School of Medicine/JJPVAMC:

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Firsthand assessment of blast exposure

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Questions?