Impact of Blast-Induced Hearing Loss in a Murine Model of Sporadic Alzheimer's Disease

Megan Barber[1,2,5], Shinelle Williams[2], Rachele Sangaletti[2], Federica Raciti[2], Laura Rodriquez[2], Kayla Minesinger[2,3], Curtis S. King[4], Nadine A. Kerr[1,5,6], and Suhrud M. Rajguru[1,2,3,4,5,6]

[1]Dept. of Neuroscience University of Miami, FL ,[2]Dept. of Otolaryngology, Miami FL, [3]Dept. of Biomedical Engineering University of Miami, FL , [4]Restor-Ear Devices LLC, [5]Dept. of Neurological surgery, University of Miami, FL, [6] The Miami Project to Cure Paralysis, University of Miami

INTRODUCTION

Hazardous impulse noise exposures, including blasts, can cause significant inner ear dysfunction and pose a long-term risk to neurological health. A single blast event can induce two of the strongest known risk factors for Alzheimer's Disease (AD): traumatic brain injury and hearing loss. The primary shock wave injures the brain by reducing cerebral blood flow and compromising the blood-brain barrier, while simultaneously damaging the inner ear. Hearing loss is the most prevalent service-connected disability in the United States. Both brain injury and hearing loss can accelerate cognitive decline. This study aims to reveal how blast-induced hearing loss accelerates cognitive decline and AD progression, specifically through pro-inflammatory mechanisms. Using a blast model in both wild-type and genetically susceptible APOE4 mice, we will longitudinally measure functional outcomes while assessing central and peripheral pro-inflammatory expression post-trauma. These findings have the potential to reveal novel therapeutic targets to mitigate blast-related auditory dysfunction and ultimately reduce the long-term risk of AD progression.

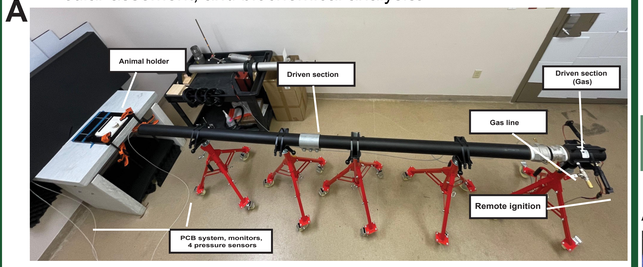
METHODS

1

Model: B6.129P2-Apoetm3(APOE*4)MaeN8 and C57BL/6NTac mice (19-20 weeks old) .

Blast Trauma: Exposed to single blast with oxy-acetylene gas tube under anesthesia.

Testing: Functional assesment, behavioral assesment, cebrovascular assement, and biochemical analysis.



RESULTS

2 Blast induces permanent auditory and vestibular threshold shifts across a range of frequencies in both wild type and AD mice

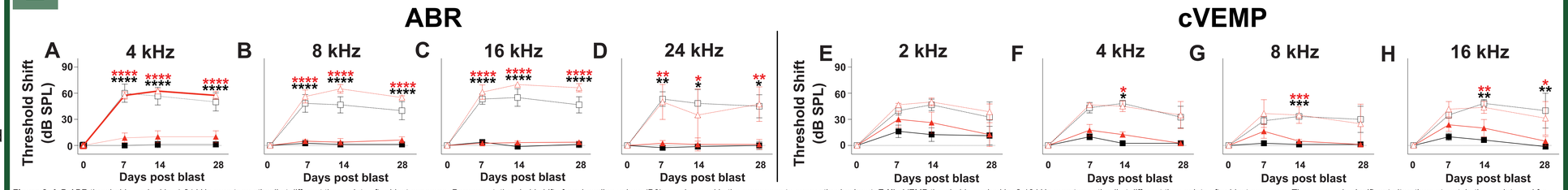


Figure 2. A-D ABR thresholds evoked by 4-24 kHz pure tone stimuli at different time points after blast exposure. Permanent threshold shifts from baseline values (D0) are observed in the responses to every stimulus input. **E-H)** cVEMP thresholds evoked by 2-16 kHz pure tone stimuli at different time points after blast exposure. There are only significant alterations at certain time points and frequencies, but overall, it does appear blast does cause vestibular dysfunction. Black asterisks compares WT Sham to WT Blast. Red asterisks compare AD Sham to AD Blast. The only significant differences in threshold shifts between WT Blast and AD Blast occurs on D28 at 16 kHz. Data analyzed on Prism 10.3.1. Performed Holm-Sidak's multiple comparison test *p<0.05, **p<0.01, ***p<0.001, ****p<0.00010. Data analysis conducted across time points.

3 WT Mice exhibit more exploratory behavior in open field

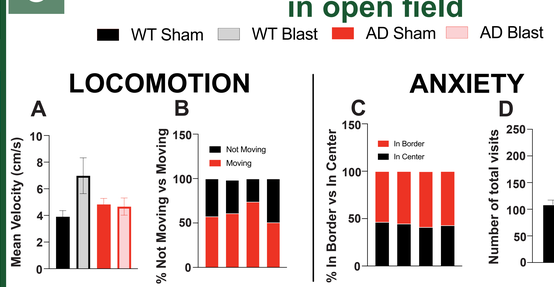
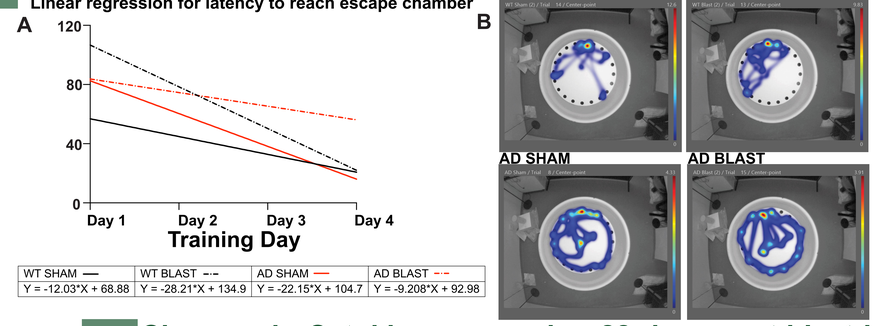


Figure 3. A). WT Blast (gray) moves with a greater velocity compared to WT Sham (black), AD Sham (dark red), and AD Blast (light red). **B)** Overall, each group has comparable locomotion with similar percentages of not moving and moving. **C)** With respect to anxiety levels, all groups spent the same amount of time in the border and in the center. **D)** WT Blast mice appear to move more frequently between the border and center compared to the other groups, which is supported by their higher velocity. Following a blast, which can cause a traumatic brain injury (TBI), the amygdala can become structurally altered. This includes adverse neurological effects such as increased dendritic branching and increased activity. These changes are linked to the development of post-traumatic stress disorder (PTSD). Future behavior studies will look into this. Data analyzed on Prism 10.3.1.Performed Two-way Anova (N=4-6) *p<0.05, **p<0.0 ***p<0.001. Data analysis conducted at 3-days post blast.

4 AD mice show spatial learning deficits 84 days post blast



WT SHAM ——	WT BLAST -·-	AD SHAM ——	AD BLAST -·-
Y = -12.03*X + 68.88	Y = -28.21*X + 134.9	Y = -22.15*X + 104.7	Y = -9.208*X + 92.98

Figure 4. A) Lines represent simple linear regression fits for each group. The WT Sham group (Y = −12.03X + 68.88) demonstrated a moderate rate of learning with the lowest initial escape latency, serving as the healthy control baseline. The WT Blast group (Y = −28.21X + 134.9) exhibited the highest initial escape latency, suggesting acute spatial memory impairment following blast injury; however, the steep negative slope indicates a robust learning rate and strong improvement across training days. The AD Sham group (Y = −22.15X + 104.7) showed an elevated initial latency consistent with AD-associated spatial memory deficits, yet retained the ability to improve performance across days at a moderate rate. The AD Blast group (Y = −9.21X + 92.98) displayed the shallowest slope among all groups, indicating the most impaired learning rate across training days despite a moderate starting latency, suggesting that the combination of AD pathology and blast injury may have a compounding effect on spatial learning acquisition. **B)** Representative heat map images of the path taken throughout the trial for WT Sham, WT Blast, AD Sham, and AD Blast animals in the Barnes Maze setup. Data analyzed on Prism 10.3.1. Performed Two-way ANOVA (N=4-8) *p<0.05, **p<0.0 ***p<0.001. Data analysis conducted at 84-day time point.

5 Blast reduces superficial cerebral and cochlear bloodflow in AD mice

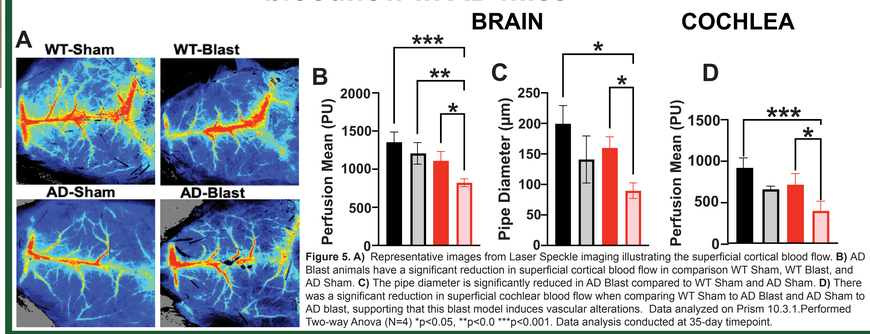


Figure 5. A) Representative images from Laser Speckle imaging illustrating the superficial cortical blood flow. **B)** AD Blast animals have a significant reduction in superficial cortical blood flow in comparison WT Sham, WT Blast, and AD Sham. **C)** The pipe diameter is significantly reduced in AD Blast compared to WT Sham and AD Sham. **D)** There was a significant reduction in superficial cochlear blood flow when comparing WT Sham to AD Blast and AD Sham to AD blast, supporting that this blast model induces vascular alterations. Data analyzed on Prism 10.3.1.Performed Two-way Anova (N=4) *p<0.05, **p<0.0 ***p<0.001. Data analysis conducted at 35-day timepoint.

6 Changes in Cytokine expression 28 days post-blast in the cochlea and brain

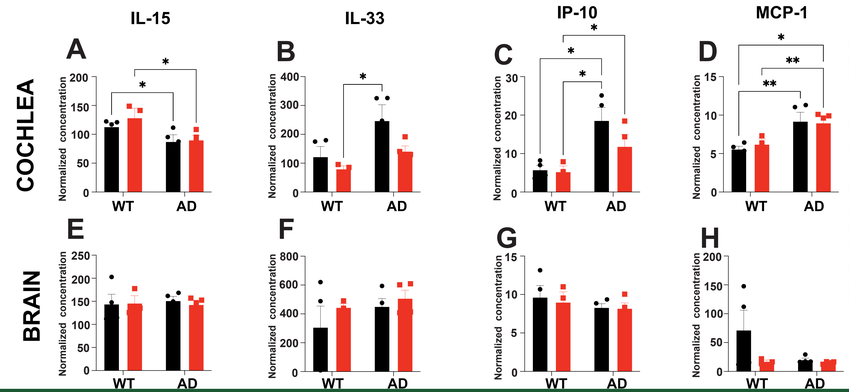


Figure 6. A) AD Sham and AD Blast have signficant levels of IL-15 reduction in the cochlea. **B)** AD Sham mice cochlea have a higher level of IL-33 compared to all groups. **C)** Both AD Sham and AD Blast cochlea have high levels of IP-10 when compared to WT Sham and WT Blast. **D)** MCP-1 levels in the cochlea are significantly elevated in AD Sham and AD Blast when compared to WT Sham and WT Blast. **E)** In the brain, there are no differences in IL-15, **F)** IL-33, **G)** IP-10 **H)** and MCP-1. It is evident at this time point that there is a robust peripheral inflammatory response that may have an effect on the central system; however, further studies need to explore this more in depth and look into acute time points. Data analyzed on Prism 10.3.1.Performed Two-way ANOVA with Tukey correction. (N=4). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

CONCLUSIONS & FUTURE DIRECTIONS

Our preclinical model effectively induces both auditory and vestibular dysfunction. While blast exposure triggered no significant locomotive alterations and anxiety-like behaviors, the wild-type response indicates a compensatory effect which are absent in the AD cohort. This discrepancy may be attributed to the baseline fragility of AD mice, a factor we intend to investigate further using nesting behavior and grip strength assays. Post-blast, we observed global superficial vascular dysfunction in the brain and cochlea, which are significantly reduced in the AD blast group. This provides insight that our blast model is inherently capable to cause vascular disruption and that genotype may increase susceptibility to vascular dysfunction. Furthermore, although AD mice exhibited spatial learning deficits, an increased sample size is necessary to confirm these trends. Notably, we identified distinct inflammatory activation within the cochlea but not the brain; this suggests that peripheral inflammation may drive long-term central impacts, a hypothesis that requires longitudinal studies to substantiate.

SPECIAL ACKNOWLEDGMENTS: VA I01RX003532, NIDCD R01DC019158, and DOD HT9425-23-1-0709