Alcohol and Repeated Mild Traumatic Brain Injury Synergistically Exacerbate Hippocampal Neuroinflammation During Adolescence

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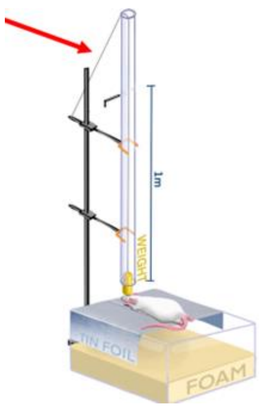
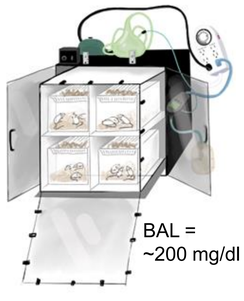
Background

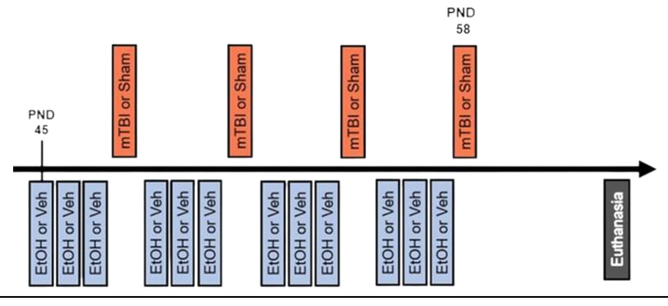
- Adolescent athletes are more likely to experience traumatic brain injuries (TBIs) and engage in underage drinking than non-athletic peers.
- Compared to adults, adolescents have increased severity and persistence of subsequent symptoms following TBI.
- TBI and alcohol are known to lead to neuroinflammation independently.
- The hippocampus has increased vulnerability to the effects of alcohol and TBIs.
- Previously, our lab showed alcohol exposure following a single mild-to-moderate TBI increased inflammation in adult male rats compared to TBI-only.
- While most TBIs are classified as mild (mTBI), the damage caused by repeated mTBI (rmTBI) can accumulate.
- Here, we used female and male adolescent Wistar rats to model a frequently observed pattern of rmTBI and binge alcohol consumption in the adolescent athlete population, testing the hypothesis that rmTBI and alcohol exposure may produce neuroinflammation both independently and synergistically.

Methods: TBI

- Adolescent female and male Wistar rats were anesthetized with isoflurane and received an injection of bupivacaine, a local anesthetic, into the scalp.
- Rats were placed upon a perforated foil sheet taped over top of a chamber containing a collection sponge.
- A 300g weight was dropped from a height of 1m, impacting the dorsal surface of the head at a force of ~2.94 Joules.
- The rat then fell through the foil sheet, landing on the sponge, and was immediately transferred to a recovery chamber and monitored until recovery.
- At 7 days post injury, animals were euthanized, and their brains harvested.
- Brains were cryosectioned at 40µm.







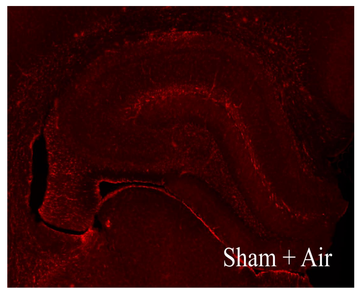
Methods: GFAP IHC

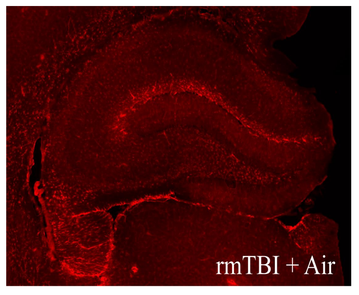
Methods

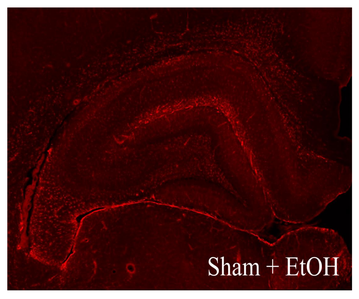
- Brain sections of animals (n=3-5/group) were incubated overnight with mouse-hosted anti-glial fibrillary acidic protein (GFAP; 0.133 µg/ml, MA5-12023, Invitrogen) and visualized using a goat anti-mouse Alexa Fluor 555 (4 µg/ml, A-21127, Invitrogen)
- We imaged the whole hippocampus at 10x on the Keyence BZ-X810
- The CA1, CA2/3 and dentate gyrus were traced and analyzed using ImageJ
- Density was normalized to %Sham + Air

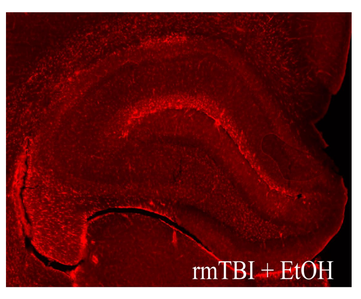
The hippocampus is associated with learning and memory. We focused on the following subregions:

A. CA1
B. CA2/3
C. Dentate Gyrus

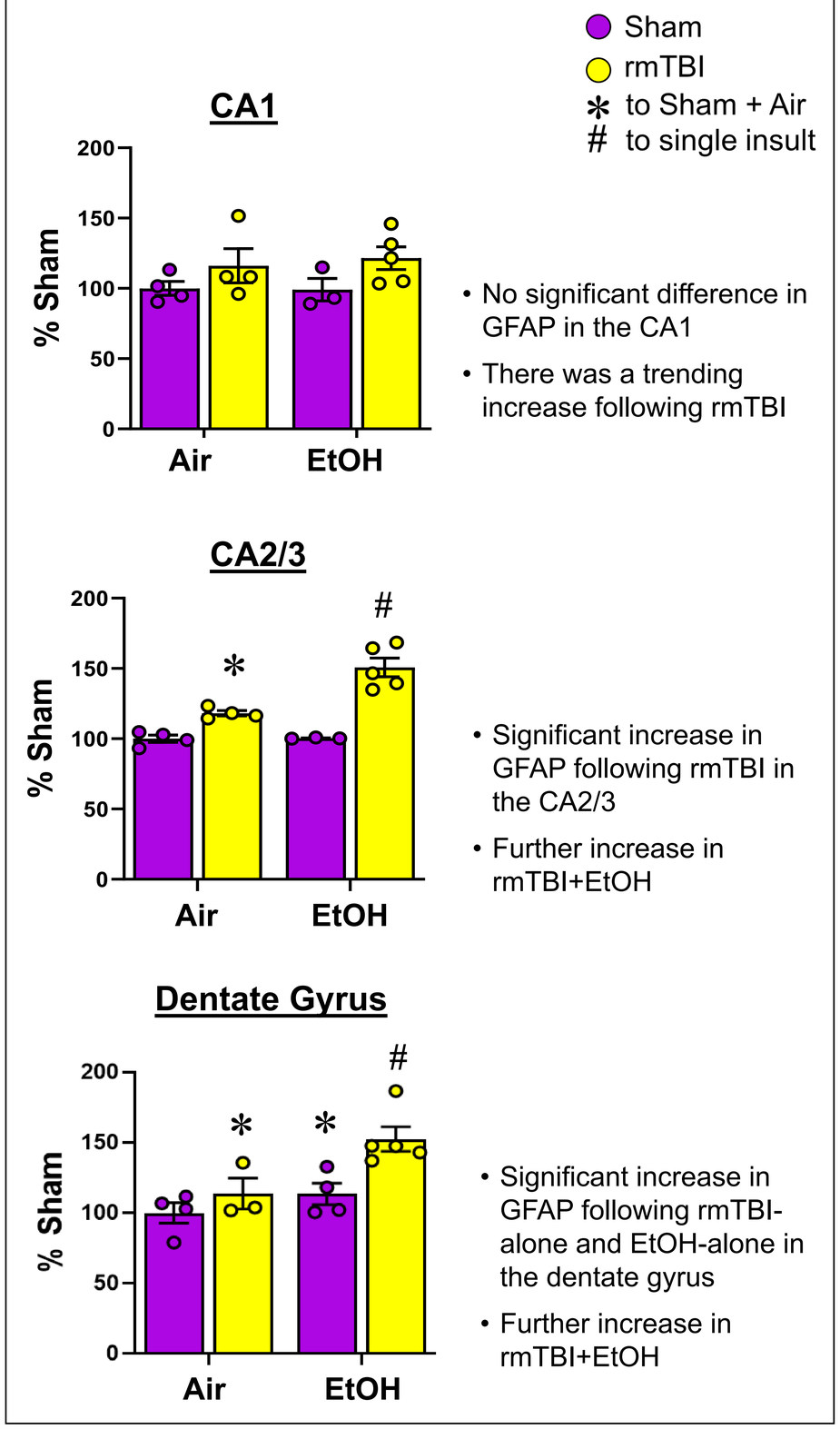








Results: GFAP



- No significant difference in GFAP in the CA1
- There was a trending increase following rmTBI
- Significant increase in GFAP following rmTBI in the CA2/3
- Further increase in rmTBI+EtOH
- Significant increase in GFAP following rmTBI-alone and EtOH-alone in the dentate gyrus
- Further increase in rmTBI+EtOH

Conclusions

- These results suggest that the combination of rmTBI and alcohol synergistically exacerbates neuroinflammation within the hippocampus of adolescent rats.
- Future directions will be to increase the N and analyze for sex differences.
- Future directions will be to measure additional markers of inflammation, such as Iba-1, vimentin, and CD68.

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