The Effects of Chronic Ethanol Consumption and Severe Stress on Dorsal Hippocampal IL-1β, TNF-α, and GFAP

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Introduction

- Alcohol use disorder (AUD) and post-traumatic stress disorder (PTSD) are highly comorbid. The susceptibility hypothesis explains this comorbidity by describing how prior alcohol dependence can influence the future development of PTSD (Gilpin and Weiner 2017; Blanco et al. 2013; Chilcoat and Breslau 1998).
- Prior ethanol consumption and withdrawal enhances stress enhanced fear learning, but the neural mechanisms underlying this behavior remain unknown (Barkell et al. 2022).
- The neuroimmune system, specifically astrocytes in the dorsal hippocampus, has been shown to be involved in both ethanol withdrawal and severe stress in rodents (Knapp et al. 2016; Jones et al. 2018).
- Cytokine signaling has been highlighted as a potential mechanism behind this behavior, specifically the cytokines interleukin-1β and tumor necrosis factor-α (Erickson et al. 2020; Lippai et al. 2013; Zhang et al. 2021).
- We hypothesize that animals that undergo ethanol withdrawal and severe stress will exhibit the highest level of IL-1β, TNF-α, and GFAP protein levels in the dentate gyrus of the dorsal hippocampus.

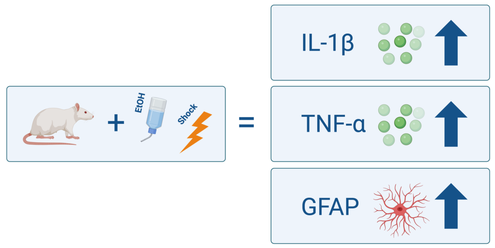


Figure 1. Chronic ethanol consumption and withdrawal and severe footshock stress were hypothesized to elevate IL-β, TNF-α, and GFAP immunoreactivity and cell count in the dentate gyrus of the dorsal hippocampus. Created with BioRender.com.

Methods

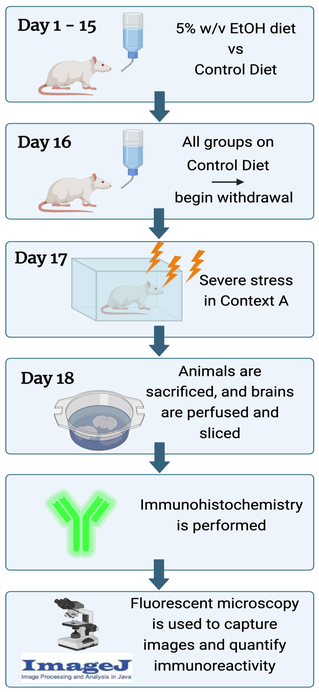
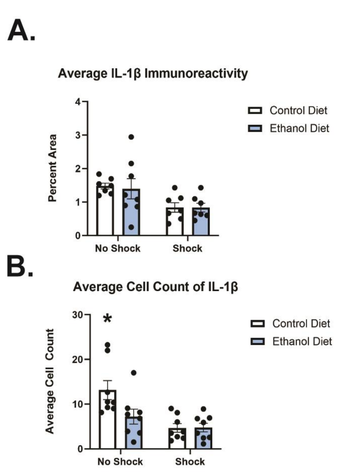


Figure 2. Schematic of the experimental timeline. Created with BioRender.com.

Results



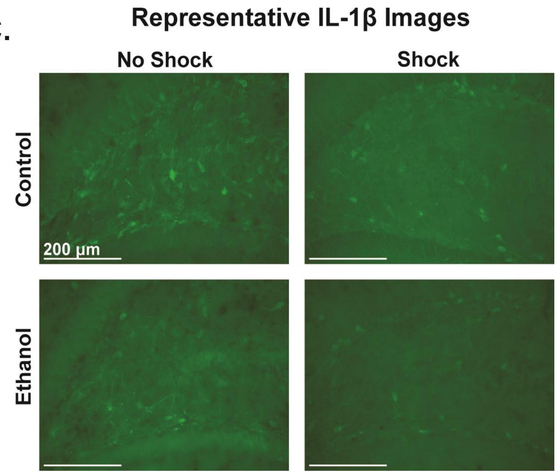
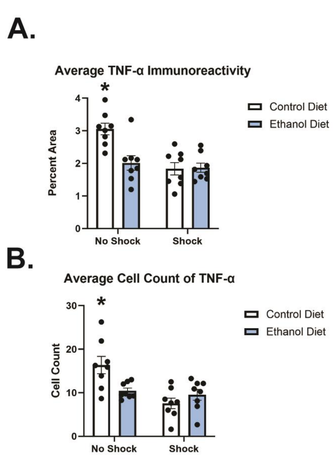


Figure 3. Ethanol has no effect on IL-1β immunoreactivity and positive cell count, but IL-1β positive cell count decreases following severe stress in the dentate gyrus of the dorsal hippocampus. Quantification of percent area of positive fluorescence of IL-1β (AlexaFluor 488; N = 28) (A). Quantification of IL-1β positive cells (N = 32) (B). Representative 20x images for all four experimental groups of the ethanol (control or ethanol) x context A treatment (shock or no shock) taken within the dentate gyrus of the dorsal hippocampus (C). * represents statistically significant differences relative to respective control. Error bars indicate SEM.



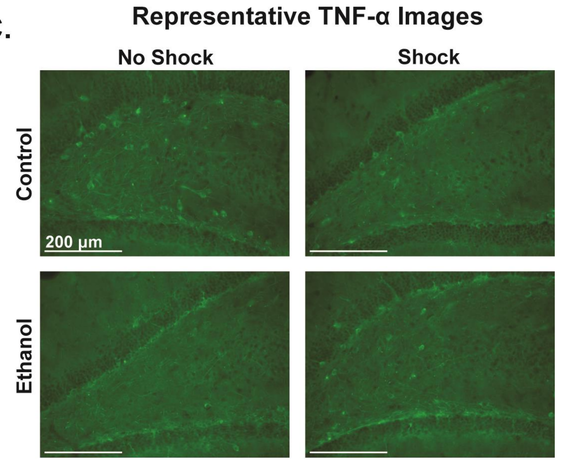
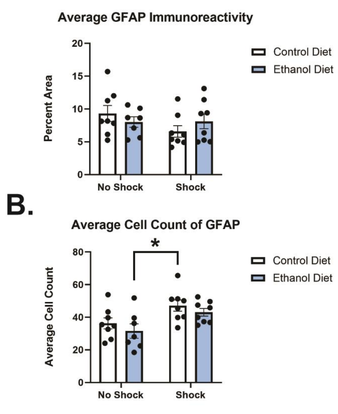


Figure 4. Ethanol decreases TNF-α immunoreactivity and severe stress decreases both TNF-α immunoreactivity and positive cell count in the dentate gyrus of the dorsal hippocampus following severe stress. Quantification of the percent area of positive fluorescence of TNF-α (AlexaFluor 488; N = 32) (A). Quantification of TNF-α positive cells (B). Representative 20x images for all four experimental groups of the ethanol (control or ethanol) x context A treatment (shock or no shock) taken within the dentate gyrus of the dorsal hippocampus (C). * represents statistically significant differences relative to respective control. Error bars indicate SEM.



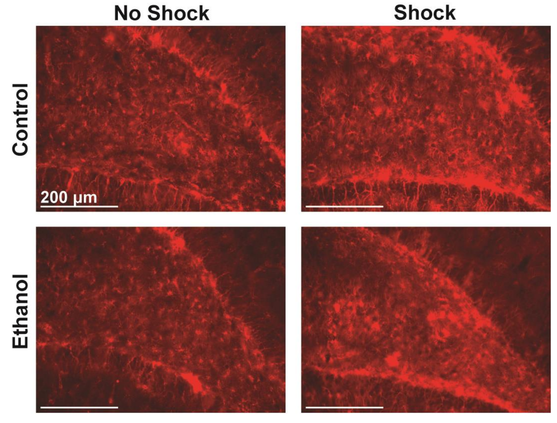


Figure 5. Ethanol has no effect on GFAP immunoreactivity and positive cell count, and GFAP positive cell count increases following severe stress in the dentate gyrus of the dorsal hippocampus following severe stress. Quantification of the percent area of positive fluorescence of GFAP (AlexaFluor 594; N = 31) (A). Quantification of GFAP positive cells (B). Representative 20x images for all four experimental groups of the ethanol (control or ethanol) x context A treatment (shock or no shock) taken within the dentate gyrus of the dorsal hippocampus (C). * represents statistically significant differences relative to respective control. Error bars indicate SEM.

Discussion

- IL-1β positive cell count and TNF-α immunoreactivity and positive cell count in the DG of the DH were decreased following severe stress.
- TNF-α immunoreactivity in the DG of the DH was decreased following ethanol withdrawal.
- GFAP positive cell count in the DG of the DH was increased following severe stress.
- These results suggest that decreased expression of proinflammatory cytokines in the DG of the DH could be a mechanism underlying the comorbidity of AUD and PTSD.
- Ongoing studies are working to replicate these results using tissue from a new group of rats that underwent the same paradigms and are utilizing confocal microscopy to analyze colocalization between GFAP and IL-1β and TNF-α.

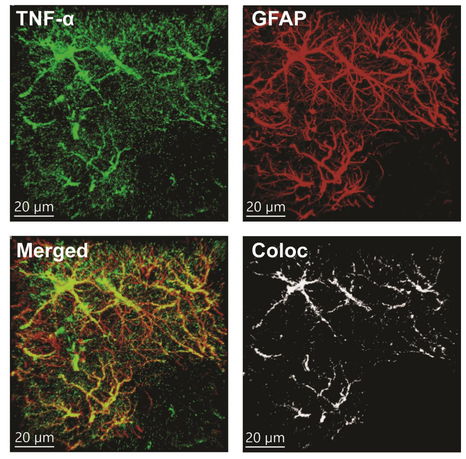


Figure 6. Representative (63x) z-stack images of colocalization of TNF-α (upper left) and GFAP (upper right) using confocal microscopy. Bottom left panel shows the merged channels of TNF-α and GFAP. Bottom right panel shows the colocalization between TNF-α and GFAP in white.

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Acknowledgements

I would like to thank Gillian Barkell, Dr. Shveta Parekh, and Dr. Todd Thiele for being on my Honors Committee.

I would also like to thank Dr. Donald Lysle and all the Lysle Lab members, as well as the NSCI694H class and Dr. Kelly Giovanello.

This work was funded by NIH grants R21AA027463, T32DA007244, R01DA034721, and R21DA048241.