Neuroimmune Effect of MDMA on Dorsal Hippocampus in Heroin Withdrawal

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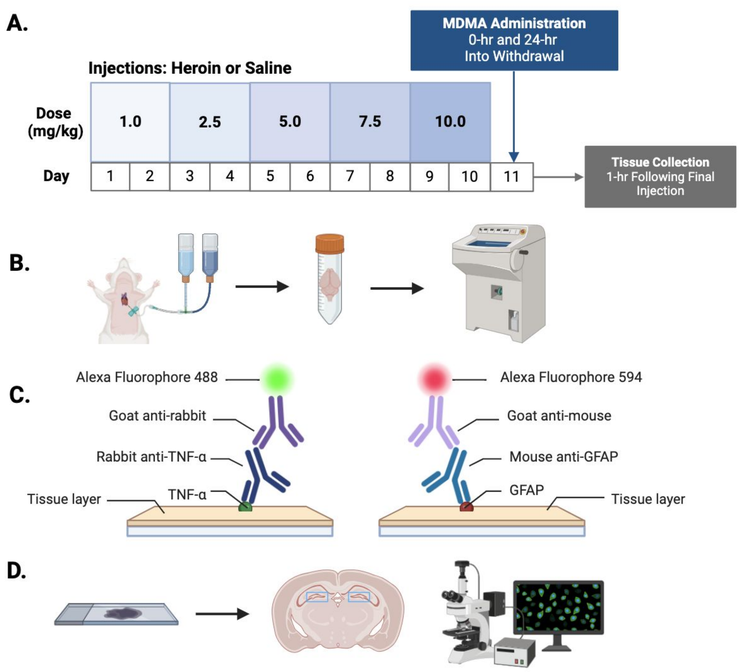
INTRODUCTION

- 33% of people with opioid use disorder (OUD) have post-traumatic stress disorder (PTSD), compared to 5% of the population.[1,2]
- Heroin withdrawal increases the dropout rate for PTSD therapy.[2] Additionally, only 6.8% of comorbid individuals receive treatment for both disorders.[1]
- Heroin withdrawal can lead to an inflammatory phenotype, increasing levels of TNF-α, and has been shown to produce a PTSD phenotype in rats.[3]
- MDMA is a promising new treatment for PTSD, and has shown anti-inflammatory effects in the dorsal hippocampus, a region implicated in hyperarousal and fear learning.[4]
- Our study explores the neuroimmune mechanism of MDMA and how it may be useful in the treatment of OUD in addition to PTSD.
- Our results aim to establish the neuroimmune effect of MDMA on TNF-α and GFAP in the dentate gyrus (DG) of the dorsal hippocampus (DH).

HYPOTHESIS

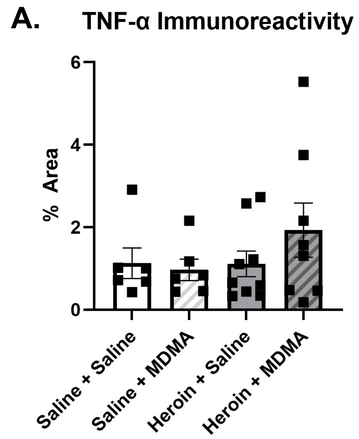
I. **Heroin withdrawal will increase TNF-α and GFAP expression in the DH.**
II. **MDMA administration during withdrawal will decrease TNF-α and GFAP expression in the DH.**

EXPERIMENTAL DESIGN



A. **Experimental timeline.** Chronic escalating heroin and withdrawal paradigm. Rats given MDMA or saline 0-hr and 24-hr into withdrawal.
B. **Tissue preparation.** Rats were sacrificed by transcardial perfusion 1-hr following final injection. Brains were post-fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, and 30% sucrose with 0.1% sodium azide were used for cryoprotection. 40 µm coronal sections were cut on a cryostat.
C. **Immunohistochemistry.** Tissues were stained with rabbit anti-TNF-α (Ab66579), goat anti-rabbit 488 (A11008), mouse anti-GFAP (Ab-6-1376P), and goat anti-mouse 594 (MS1376P).
D. **Mounting and imaging.** Tissues were mounted onto slides with DAPI and coverslipped. Fluorescent widefield microscopy was used to image the DG of the DH.

RESULTS



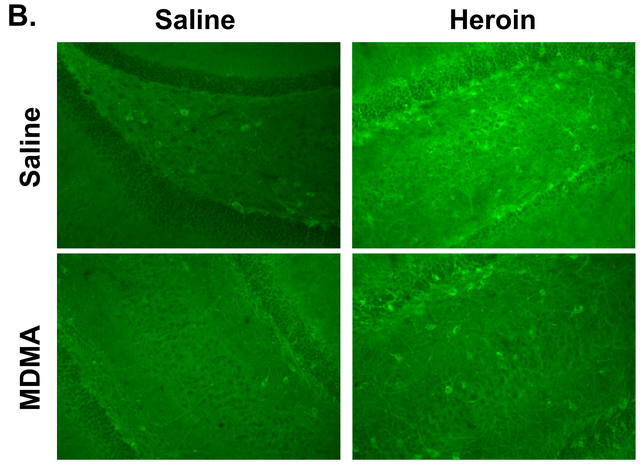
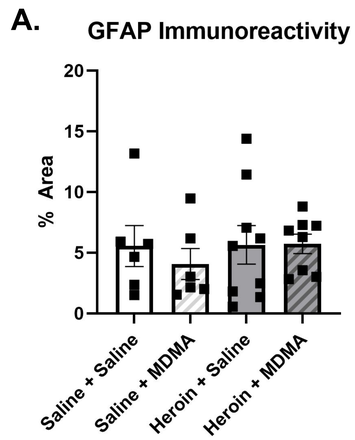


Figure 1. TNF-α immunoreactivity does not significantly differ among saline-saline (n=6), saline-MDMA (n=6), heroin-saline (n=10), or heroin-MDMA (n=8) treated rats. A) Percent (%) area TNF-α immunoreactivity did not significantly differ between treatment groups. **B)** Representative 20X widefield fluorescent images of TNF-α IHC staining in the DG.



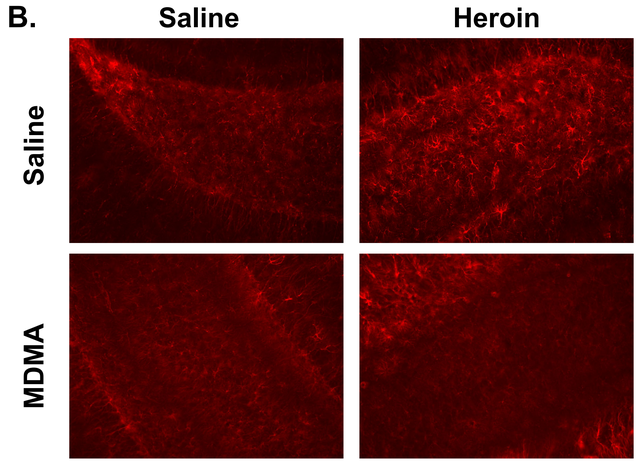


Figure 2. GFAP immunoreactivity does not significantly differ among saline-saline (n=6), saline-MDMA (n=6), heroin-saline (n=10), or heroin-MDMA (n=8) treated rats. A) Percent (%) area GFAP immunoreactivity did not differ significantly between treatment groups. **B)** Representative 20X widefield fluorescent images of GFAP IHC staining in the DG.

DISCUSSION

- No significant difference in TNF-α or GFAP immunoreactivity was found between heroin-withdrawn and control rats.
  - Additionally, no significant difference in TNF-α or GFAP immunoreactivity was found when MDMA was administered.
- These results conflict with previous research that has demonstrated that heroin withdrawal increases TNF-α immunoreactivity in the DG. One explanation for these results may be that the rats given heroin did not experience withdrawal.[3]
  - We can not confirm if withdrawal was present, which presents a major limitation.
  - This study acquired antibodies from a new lot source which could have contributed to the insignificant data.
- A previous study found that MDMA significantly reduced IL-1β expression, a pro-inflammatory cytokine, in the DG in a PTSD animal model.[4] However, this effect was not seen for TNF-α or GFAP. [4]
  - Other evidence has shown that MDMA reduces TNF-α expression in animals exposed to a lipopolysaccharide (LPS) immune challenge.[5]
- Future studies may:
  - Repeat the experimental procedure, collecting weight measurements to determine if withdrawal is present.
  - Examine the influence of MDMA on other pro-inflammatory cytokines like IL-1β and on the microglial marker Iba-1.

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