mPFC Recruitment During Self-Administration of Quinine Adulterated Alcohol

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MEDICAL DISCOVERY TEAM ADDICTION

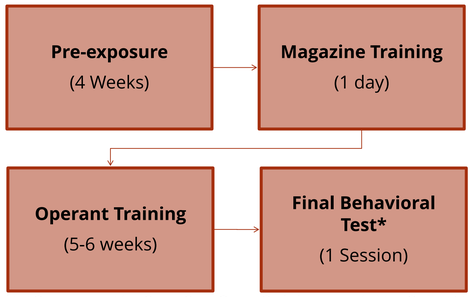
Introduction

•Alcohol Use Disorder (AUD) involves a variety of behaviors, including compulsive alcohol consumption
•Compulsive alcohol consumption = continued consumption of alcohol despite negative consequences
•Ignoring negative consequences activates regions of the brain involved in decision-making and conflict
•Medial prefrontal cortex (mPFC) = neural region involved in conflict navigation and decision making
- Theorized recruitment during compulsive alcohol consumption, but to what extent is unknown

•This study uses a self-administration alcohol consumption model in Long Evans rats (n = 9, M = 5, f = 4) focusing on aversion resistance via quinine adulterated ethanol to study mPFC activation during compulsive drinking
•Hypothesized increase of mPFC recruitment during aversion-resistant alcohol consumption

Materials & Methods

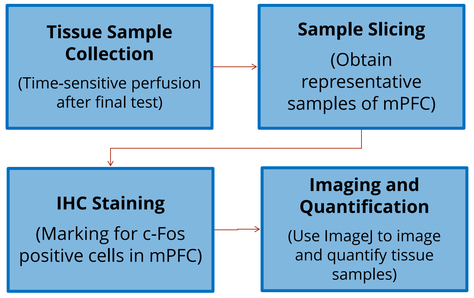
•Behavioral model: Self-administration aversion-resistance alcohol consumption model using quinine-adulterated ethanol as an aversive stimulus



*(EtOH: n = 5, M = 2 F = 3; EtOH + Quinine: n = 4, M = 3, F = 1)

•Tissue Processing: Neural samples of the mPFC are collected and quantified for c-Fos to compare levels of neural activation between treatment groups
- c-Fos = immediate early gene used as indicator for neural activation

•Images from fluorescent microscopy processed and quantified via ImageJ software



Results

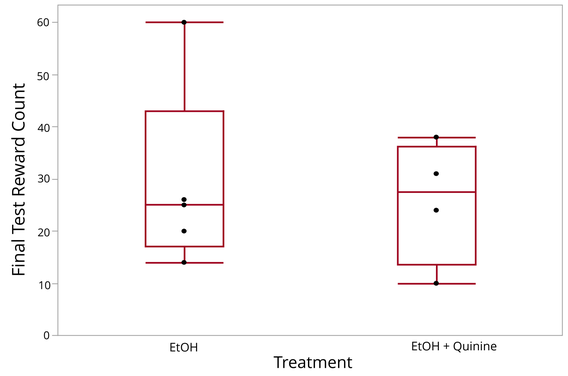


Figure 1: Final Test Reward Count Grouped by Ethanol Treatment. The EtOH group had a larger range of reward counts in comparison to the EtOH + Quinine group. However, the median reward count in the EtOH + Quinine group appears to be higher than that of the EtOH group. (EtOH: min = 14, max = 60, median = 25, mean = 29.00; EtOH + Quinine: min = 10, max = 38, median = 27.50, mean = 25.75; Main effect of treatment: F(1,7) = 0.095, p = 0.77)

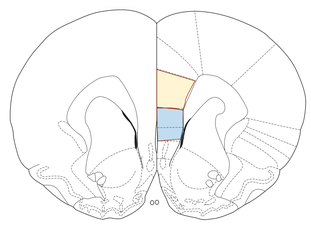


Figure 2: mPFC Highlighted Rat Brain Atlas Image, Prelimbic (Orange), Infralimbic (Blue). Highlighted portion in the medial region depicts the target area of the mPFC where prelimbic (orange) and infralimbic (blue) c-Fos quantification occurred. (Interaural 11.20 mm, Bregma 2.20 mm) (Paxinos and Watson, 1998)

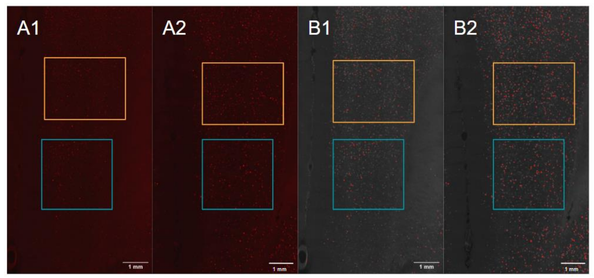


Figure 3: Sample Fluorescent Microscopy Images of the mPFC in EtOH and EtOH + Q rats. A) Sample images of the mPFC in ME-15 (A1, 15% EtOH treatment) and ME-9 (A2, 15% EtOH + 45 mg/L quinine) rats. Red signals within the images represent c-Fos positive cells. The orange rectangle represents the prelimbic mPFC with the blue rectangle representing the infralimbic mPFC. B) Sample images of the mPFC in ME-15 (B1, 15% EtOH treatment) and ME-9 (B2, 15% EtOH + 45 mg/L quinine) rats after being filtered through color splitting via ImageJ software.

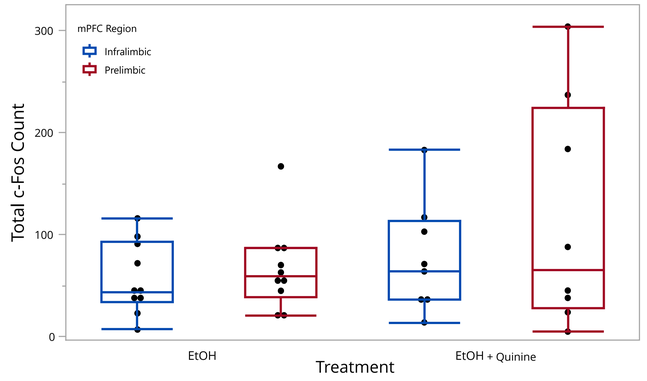


Figure 4: Total c-Fos Count in EtOH Compared to EtOH + Quinine Treatment Groups Within the Infralimbic and Prelimbic mPFC. In both subregions of the mPFC, there is an evident trend of higher variability of c-Fos counts in the EtOH + Quinine treatment group. Median and interquartile ranges remain similar across groupings except for the interquartile range for EtOH + Quinine treatment in the prelimbic mPFC. (EtOH [prelimbic, infralimbic]: min = 21, 7; max = 167, 116; median = 59.5, 44; mean = 66.90, 57.10) (EtOH + Quinine [prelimbic, infralimbic]: min = 5, 14; max = 304, 183; median = 65.5, 64.5;
mean = 115.38, 77.38) Linear Mixed Effect Model: (Main effect of mPFC region: F(1,26) = 2.05, p = 0.16; Main effect of treatment: F(1,7) = 0.91, p = 0.37)

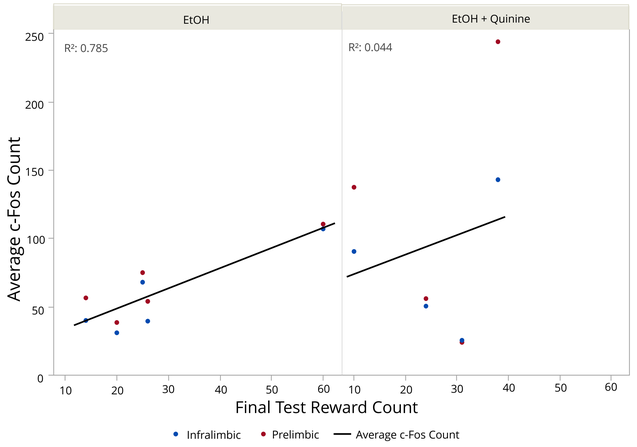


Figure 5: Final Test Reward Count Compared to Average c-Fos Counts in the infralimbic and prelimbic mPFC across treatment groups of EtOH (left) and EtOH + Quinine (right). Between treatment groupings, the EtOH + Quinine rats have a wider range of reward counts and c-Fos positive cells in comparison to the EtOH rats, suggesting the presence of quinine may influence activation of the mPFC. Upward trend lines for both treatment groups imply a positive correlation between final test reward count and average c-Fos count. Linear Regression: (EtOH: p = 0.0006, R^2 = 0.79; EtOH + Quinine: p = 0.62, R^2 = 0.044

Conclusions

•Greater variation in mPFC recruitment for the EtOH + Quinine group in comparison to the EtOH group (Fig 4)
•Though variation was greater, there were no statistically significant differences in mPFC activation
•Upward trends suggest a potential correlation of total reward count and c-Fos positive cells in EtOH group suggests a correlation between the two (Fig 5)
•Variation of relationship in EtOH + Quinine group suggest quinine may influence how the mPFC is recruited (Fig 5) during compulsive drinking through either:
- Recruitment of different input and output pathways
- Type of neural populations being recruited
- Recruitment of subregions within the mPFC
- Sex differences

•Hindrances of study/future improvements:
- Too small of sample size (imbalanced sex ratio)
- Use of an aversive stimulus paired with consumption versus latent punishment
- Limitations in abstract negative consequences
- Individual variability among rats in alcohol consumption and behavior in operant box

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