Exploring the Effects of Alcohol Abstinence on H3K9me2 Levels and Colocalization with IBA1 in Key Brain Regions

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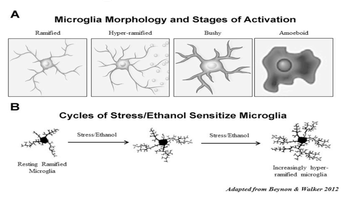
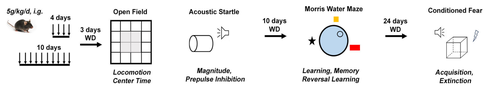
Introduction and Background

- Alcohol use disorder (AUD) is a medical condition characterized by an impaired ability to stop or control alcohol use despite adverse social, occupational, or health consequences.
- Alcohol consumption is known to increase activity of known innate immune signaling molecules, otherwise known as microglia.
- H3K9me2, catalyzed by G9a, is an epigenetic modification to the DNA packaging protein Histone H3 in which a demethylation the Histone.
- The presence of the H3K9me2 mark, which is typically associated with gene silencing, suggests that alterations in histone methylation states may influence the gene expression patterns/activity in microglia during alcohol exposure.[5]
- The regulation of H3K9me2 through G9a can possibly serve as a therapeutic target for AUD.
- **Hypothesis: Chronic Binge Drinking for ten days induces the levels of H3K9me2, IBA1 and colocalization of lH3K9me2 in IBA1 even after a period of abstinence through activation and inflammation.**



Methods

Chronic Binge Alcohol 10-Day



Mice Treatment

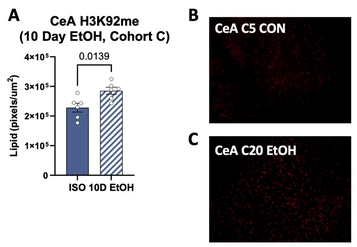
- The mice used in the experiment were all adult males and part of Cohort C. The mice in Cohort C were treated with 5 g/kg of EtOH for 10 days straight to ensure the mice received adequate exposure to alcohol and observed its effect on key brain regions.
- Mice were then abstained from Alcohol for 28 days. The 28-day longevity allowed us to see how much of H3K9me2 was being expressed, total IBA1 activation and inflammation as well as how much H3K9me2 in IBA1 colocalized.

Immunofluorescence

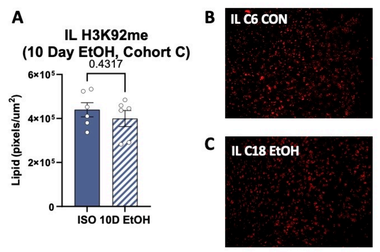
- Preparation and Washing: Mice brain sections were stored in a 12-well plate submerged in PBS within a walk-in freezer for 24 hours. Upon removal, the sections were washed three times with 0.1 M PBS for 10 minutes each 3 times at room temperature with gentle shaking on the shaker
- Blocking and Antibody Staining: The sections underwent a blocking step in a mixture of PBS, Goat serum (H3K9me2 at 1/500 and IBA1 at 1/1000), with shaking
- Secondary Antibody Incubation and Mounting: Following another PBS wash, sections were incubated in secondary antibody (Goat Anti-Rabbit) for 60 minutes, washed again, and then mounted on glass slides for subsequent analysis

Binge Drinking and H3K9me2 Expression in Key Brain Regions

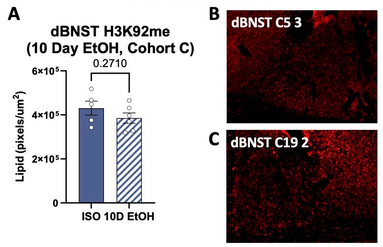
H3K9me2 is significantly induced in the CeA (Central Nucleus of the Amygdala after Abstinence



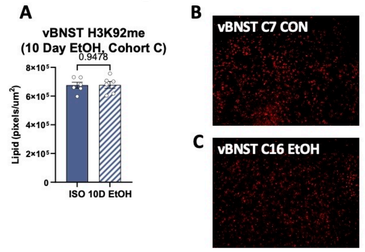
H3K9me2 is insignificantly reduced in the IL (Infralimbic Region)



H3K9me2 is insignificantly decreased in the dBNST (Bed Nucleus of the Stria Terminalis) after Abstinence

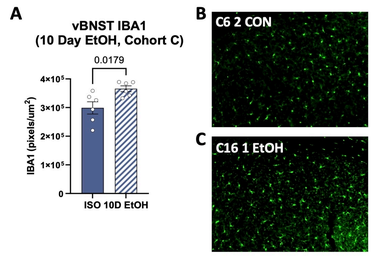


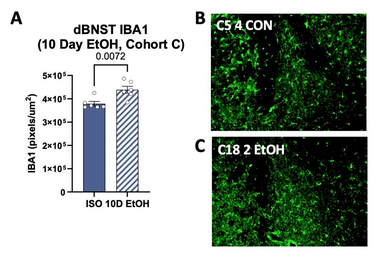
H3K9me2 does not show signs of change in vBNST

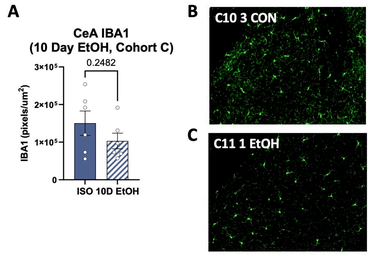


Binge Drinking and IBA1 Expression

IBA1 shows greater inflammation and activity in 10-day EtOH male mice (vBNST and dBNST) but decreased in CeA after Abstinence

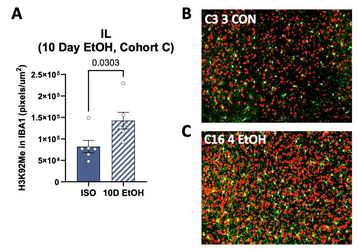


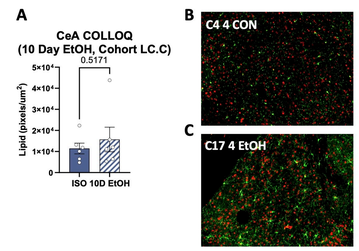


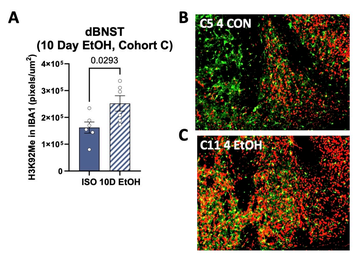


Binge Drinking and Colocalization of H3K9me2 and IBA1

10-Day Binge Drinking promotes colocalization in key brain regions even after Abstinence







Summary and Future Directions

- **Key Findings on H3K9me2:**
  - Significant increase in H3K9me2 in the Central Nucleus of the Amygdala (CeA) of male mice after 10-day ethanol (EtOH) intake and 28-day abstinence ($p = 0.0139$), as seen in Figure 1a.
  - No significant changes in H3K9me2 levels in the infralimbic (IL), dorsal bed nucleus of the stria terminalis (dBNST), and ventral BNST (vBNST) after 28 days of abstinence.
- **Key Findings on IBA1:**
  - Increased expression of IBA1 in all selected brain regions except for CeA
  - Significant increases in IBA1 expression observed in dBNST ($p = 0.0072$) and vBNST ($p = 0.0179$), in IL.
  - Indications of more defined and active microglia processes in EtOH-induced mice, suggesting persistent inflammation even after abstinence (Figures 3b, 4b, 5b).
- **Colocalization of H3K9me2 and IBA1:**
  - Increases in colocalization noted between control and EtOH-induced male mice after 10 days of treatment and 28 days of abstinence.
  - Significant increases in the IL ($p = 0.0303$) and dBNST ($p = 0.0293$), with insignificant increases in CeA and vBNST.
  - Greater density of H3K9me2 surrounding IBA1 molecules in EtOH-induced mice (Figures 6b, 7b, 8b).
- **Inverse Relationship and Implications:**
  - Inverse relationship observed between H3K9me2 and IBA1 in the CeA; higher H3K9me2 associated with lower IBA1 expression in EtOH mice.
  - This relationship is also observed in dBNST and IL but is not significant
  - This pattern suggests H3K9me2 may mitigate inflammation effects, linked to reduced IBA1-mediated inflammation in the CeA (Figure 5c).
  - Amygdala's role emphasized in the stress and arousal systems relevant to alcohol withdrawal symptoms.
- **Research Directions:**
  - Need for further studies to validate the findings and explore the exact mechanisms between H3K9me2 and IBA1 in alcohol-related behaviors and inflammation.
  - Further research required to confirm the relationships and findings in IL, vBNST, and dBNST regarding changes and significance with H3K9me2 and IBA1.

References

- U.S. Department of Health and Human Services. (n.d.-b). *Understanding alcohol use disorder*. National Institute on Alcohol Abuse and Alcoholism. https://www.niaaa.nih.gov/publications/brochures-and-fact-sheets/understanding-alcohol-use-disorder
- Crews, F. T., Lawrimore, C. J., Walter, T. J., & Coleman, L. G. (2017). The role of neuroimmune signaling in alcoholism. *Neuropharmacology*, *122*, 56–73. https://doi.org/10.1016/j.neuropharm.2017.01.031
- Shinkai, Y., & Tachibana, M. (2011). H3K9 methyltransferase G9a and the related molecule GLP. *Genes & development*, *25*(8), 781–788. https://doi.org/10.1101/gad.2027411
- Robison, A. J., & Nestler, E. J. (2011). Transcriptional and epigenetic mechanisms of addiction. *Nature reviews. Neuroscience*, *12*(11), 623–637. https://doi.org/10.1038/nrn3111