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he Effect of Binge-Like Drinking on the Activation f Cells Within the Ventral Hippocampus

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Introduction

Binge Drinking brings the blood alcohol concentration ≥ 0.08% within a short amount of time

Binge Drinking ↑ risk of alcohol use disorder

- Regions of Interest Ventral Hippocampus (vHipp) and Nucleus Accumbens (NAc)
- vHipp linked with anxiety and stress, NAc linked with addiction and reward-seeking
- Excitatory projections from the vHipp to NAc

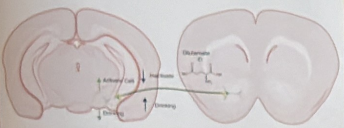

Figure 1. Ventral hippocampus to nucleus accumbens projection overview

Research Goal

- The goal of this research is to investigate the activation of cells in the ventral hippocampus during binge drinking.
- **Hypothesis: The number of inhibitory cells expressing *C-Fos* will be greater in mice that drank alcohol compared to those that did not drink alcohol.**

Methods

- The **Drinking-in-the-Dark paradigm (DID)** gives rodents limited access to alcohol early in the dark cycle for a limited amount of time

Figure 2. Mouse environment example during DID procedure

Days 1-3 2 hrs EtOH	Days 1-3 2 hrs EtOH	Days 1-3 2 hrs EtOH
Days 4 4 hrs EtOH	Days 4 4 hrs EtOH	Days 4 4 hrs EtOH
Days 5-7 Abstinence	Days 5-7 Abstinence	Days 5-7 Abstinence
Week 1	Week 2	Week 3

Figure 3. Timeline of DID procedure is depicted for 3 weeks

- **Perfusions:** Within 90 minutes following DID
- **Slicing:** 30 to 50-micron coronal slicing through vHipp and NAc

Figure 4. The machine used for slicing is the Cryostat (Left) and well plate where we place tissue to be transferred to stain (Right)

- **Immunohistochemistry:** A technique which uses antibodies to detect expression of proteins in tissue. Identified the cell populations that were expressing *C-Fos*
- ***C-Fos*** = immediate early gene marker for activation of cells

- **Fluorescent Microscopy:** Allowed for the viewing of fluorescent cells within our tissue
- **Image J:** Software that allowed for adjustment of images to improve clarity

Figure 5. Left image depicts the microscope used to view tissue samples. Right image is the software used to analyze data

Results

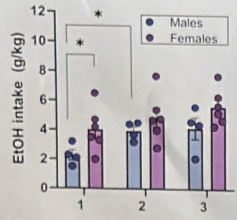

Figure 6. Graph of 4th day sessions of each week in C57BL/6J males and females in groups of 4-6 mice and the * means p<0.05

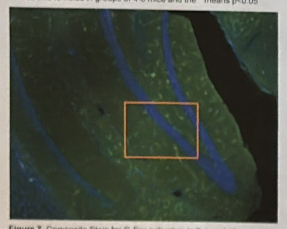

Figure 7. Composite Stain for *C-Fos* activation in the ventral hippocampus

Figure 8. Alexa Fluor stain for visualization of *C-Fos* activation. Arrow pointing to blood vessel

Figure 9. DAPI stain for nucleus of cell bodies

Figure 10. Composite stain of positive control (NAc). No significant expression was found.

C-Fos Activation

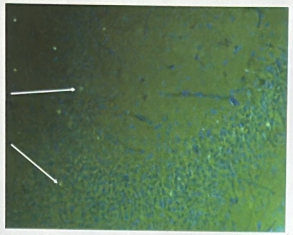

Figure 11. Example composite staining of *C-Fos* activation within cell bodies in the vHipp from a different experiment.

Conclusions

- Drinking in the dark procedure is working as expected
- Immunohistochemistry protocol requires optimization
- Our concentration of antibody needs refinement
- Perfusions also need to be optimized, autofluorescence was present
- Obtained cellular stains of *C-Fos* in the vHipp that indicate insufficient activation of *C-Fos*

Future Ou

- Run more trials of i address potential i
- Complete slicing compare agains
- Additional ampl needed to accu
- Identification o populations

Ac

I would like to Lab for showi with learning this project. the National and LSAMP UTRGV for opportunity.

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