ALTERATIONS IN GLIAL ACTIVATION FOLLOWING BINGE-LIKE ETHANOL CONSUMPTION

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Introduction

Binge-like Drinking and the Neuroimmune System

Consistent bouts of alcoholic binge drinking increases the likelihood of eliciting hyper reactive responses of the neuroimmune system (Marshall, Geil, & Nixon, 2016). As a result, brain damage may accumulate over time as continual hyper reactive neuroimmune responses are elicited. Many people who binge drink ultimately show no neurodegeneration due to the spaced out, episodic time period of the binge. However, non-dependent individuals can have neuronal maladaptations that become permanent with repeated exposure, most especially within glial cells. Previous research suggest that non-dependent binge-like consumption can promote a pro-inflammatory cytokine environment, but the impact on glial cells is still of interest.

Glial Activity in the Presence of Ethanol

Glial cells, including microglia and astrocytes, are key players in the neuroimmune response. Specifically, they play a prominent role in recovering from neuronal damage by promoting the removal of damaged cells and clearing debris. When exposed to binge-like amounts of ethanol, glial cell function is altered to a pro-inflammatory state (Mayfield, Ferguson, & Harris, 2013). Therefore, the brain's immune system begins to respond inappropriately with the potential to exacerbate damage, essentially harming rather than helping itself. Previous studies have examined the effects of ethanol on glial activation in dependent, damaging alcoholic binge models, but this study will examine the effects of ethanol on glial activation in a non-dependent, non-damaging alcoholic binge model. Our interest in glia also includes examining how ethanol alters the glial regulation of the control of behavior within neuronal brain circuits.

Methods

Drinking in the Dark Paradigm and Tissue Extraction

Binge drinking was modeled with male C57BL/6J mice in the Drinking in the Dark (DID) paradigm. This model effectively simulates nondependent binge-like consumption. Over a period of 4 consecutive days, mice are given access to 20% ethanol (v/v) or water (control) 3 hours into the 7AM – 7PM dark cycle. On the first three days, mice are given 2 hours of access, but on the last day animals have 4 hours to drink ethanol. During this period, the consumption levels induce blood ethanol concentrations greater than 100 mg/dL (Thiele & Navarro, 2014). Brains were harvested immediately following the DID (0-days post-ethanol), 1-day post-ethanol, or 10-days post-ethanol ingestion. Punches of the hippocampus were taken from snap frozen brains and homogenized for RNA extractions.

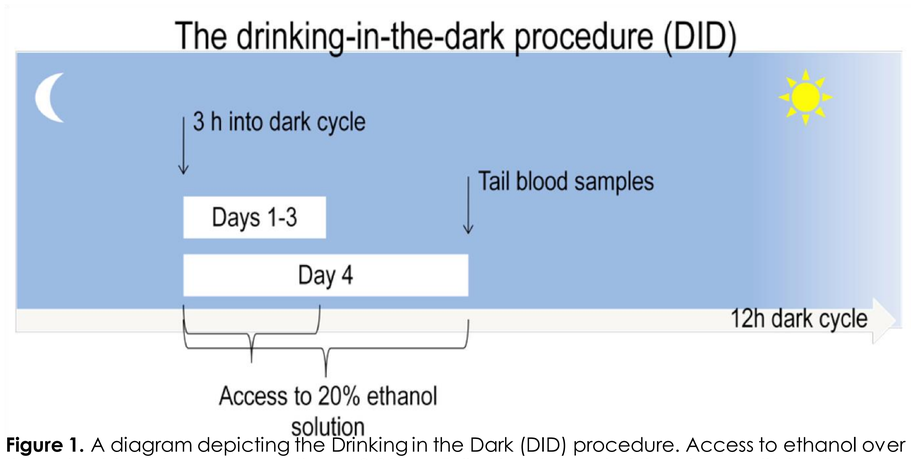

Figure 1. A diagram depicting the Drinking in the Dark (DID) procedure. Access to ethanol over the 4 day binge began promptly at 10:00AM. Following the end of the binge, tail blood samples were not collected because restraint stress has previously been shown to induce changes in glial mRNA expression.

qRT-PCR Analysis of Microglia and Astrocyte mRNA Expression

Samples were plated using a PCR (384-well) plate containing runs organized in duplicates for each glial marker. The microglia specific markers, integrin alpha M (Itgam), and allograft inflammatory factor 1 (Aif1) were used. Itgam is upregulated by microglia activation, whereas Aif1 is expressed in all microglia. An astrocyte specific marker, glial fibrillary acidic protein (GFAP), was used to determine if ethanol induced astrocyte activation. The PCR plate was normalized to peptidylprolyl isomerase A, PPIA, which is known not to be affected by ethanol. A one-way ANOVA analysis was completed in order to analyze our results.

Results

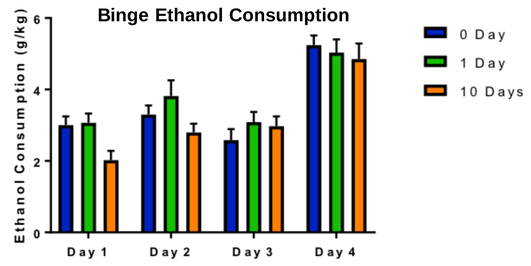

Figure 2. Average ethanol consumption of the experimental group spanning over 2 hours (Days 1-3) or 4 hours (Day 4) during the DID model. No significant differences in average ethanol consumption were observed over the 4-day DID model.

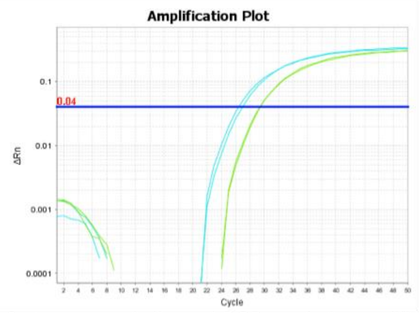

Figure 3. A representative qrt-PCR analysis amplification plot of Aif1 mRNA expression of an ethanol consuming group (blue) versus control group (green).

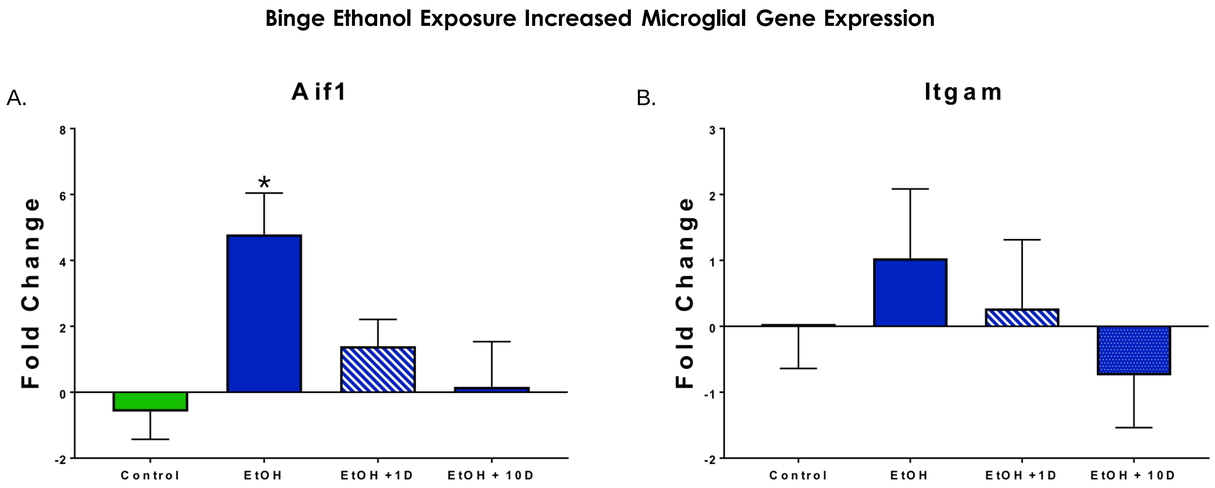

Figure 4. There was a significant 4 fold increase in changes of Aif1 on the fourth and final day of ethanol consumption (Aif1: $F(3,34)=4.10$, $p=0.014$) (A). This effect did not persist into abstinence, but a one-way ANOVA indicated no significant changes in Itgam, a gene upregulated in activated microglial cells (Itgam: $F(3,33)=067$, $p=0.57$). Both Aif1 and Itgam mRNA showed no significant changes between the control, 0-days post-ethanol, 1-day post-ethanol, or 10-days post-ethanol.

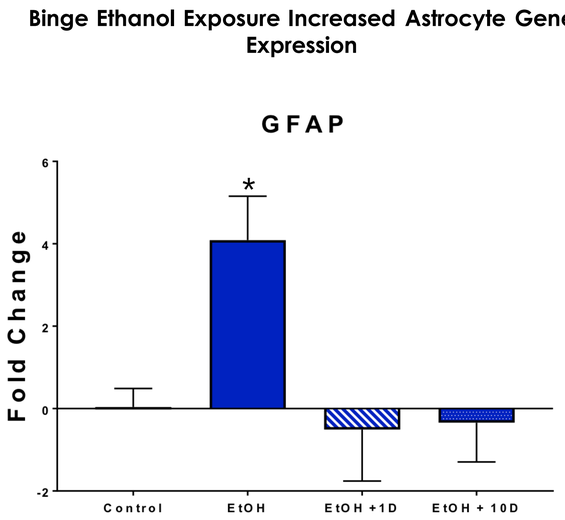

Figure 5. A One-way ANOVA indicates a significant 3 fold increase in changes of the hippocampal activated astrocytic cells on the fourth and final day of ethanol consumption when marked with GFAP mRNA (GFAP: $F(3,36)=4.67$, $p<0.01$).

Conclusions

- Changes in the mRNA are only prevalent during intoxication in non-dependent animals. There are no persisting alterations during abstinence.
- Acute binge drinking in nondependent individuals only produces short-term effects on the glial responses
- The effects of repeated binge exposures are likely to produce more long-term effects.

Future Work

Future studies will examine whether repeated cycles will evoke long-term effects on glial activation.

References

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