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 suggests that non-dependent binge-like consumption can promote a pro-inflammatory cytokine environment, but the impact on glial cells is still of interest.

Gliaal Activity in the Presence of Ethanol

Gliaal 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 to promote 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-day post-ethanol), or 0, 5, and 10-days post-ethanol ingestion. Punches of the hippocampus were taken from snap frozen brains and homogenized for RNA extractions.

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. 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 days (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).

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 is 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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