Differential Amygdala and Hippocampal Responsivity to an Ethanol Challenge in Adulthood After Adolescent Ethanol Exposure in Male Rats

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Introduction

While binge drinking (4-5+ drinks/2 hours) is prevalent in adolescence, preclinical models of human adolescent binge drinking (adolescent intermittent ethanol exposure; AIE) have revealed that there are long-term consequences on neural and innate immune signaling systems which persist into adulthood despite abstinence. Moreover, individuals who drink during adolescence are at a heightened risk for alcohol use disorder later in life, yet the impact of AIE on the physiological responses to ethanol exposure later in adulthood remain poorly characterized. This study will therefore test the neuronal responsivity (evidenced by activity regulated cytoskeletal associated protein (Arc)+immunoreactivity; IR), HMGB1 immunoreactivity (HMGB1+IR), and plasma corticosterone, immune (HMGB1, CRP) and liver enzymatic (ALT) reactivity with a later acute ethanol challenge in adulthood following AIE.

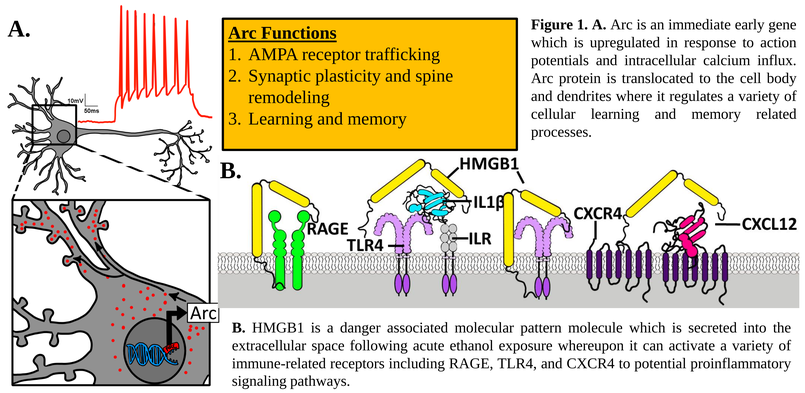


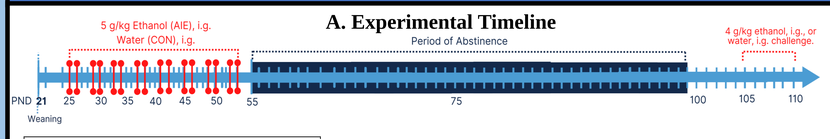
Figure 1. A. Arc is an immediate early gene which is upregulated in response to action potentials and intracellular calcium influx. Arc protein is translocated to the cell body and dendrites where it regulates a variety of cellular learning and memory related processes.

B. HMGB1 is a danger associated molecular pattern molecule which is secreted into the extracellular space following acute ethanol exposure whereupon it can activate a variety of immune-related receptors including RAGE, TLR4, and CXCR4 to potential proinflammatory signaling pathways.

The overarching hypothesis of this study is that AIE will:

- Exacerbate peripheral endocrine and immune responsivity to an adult ethanol challenge after abstinence.
- Attenuate acute adult ethanol-induced hippocampal and amygdalar immediate early gene expression (evidenced by Arc+immunoreactivity; IR) (**Figure 1**).
- Sensitize acute adult ethanol-induced brain immune response, evidenced by reductions in nuclear HMGB1.

Methods



B. Experimental Groups	
CON-Water (n = 12)	AIE-Water (n = 12)
CON-EtOH (n = 12)	AIE-EtOH (n = 12)

Figure 2. A. All experiments consisted of a 2 × 2 (CON, AIE × Water, EtOH) experimental design with the factors of *adolescent exposure* and *adult challenge*. **B.** AIE rats were exposed to either 5g/kg/day ethanol or water, i.g., 2-days-on/2-days-off from postnatal days (PND) 25 – PND 54 followed by a multi-month period of abstinence. This was followed by a 4 g/kg *i.g.* ethanol or water challenge (PND 105-110) with euthanasia 90 min post challenge to assess brain IEG response to ethanol as well as CORT, HMGB1, and ALT levels.

Immunohistochemistry for Arc and HMGB1

Immunohistochemical assessment of hippocampal and amygdalae Arc+IR (Synaptic Systems, #156-003, 1:500) and HMGB1+IR (Abcam, #ab18256, 1:1000). The granule cell layer of dentate gyrus (DG) in the dorsal hippocampus, basolateral and central amygdala, were the regions histologically analyzed for Arc+IR and HMGB1 **(Figure 3).** Four sections from each subject were quantified by a blind experimenter using a modified version of unbiased stereological quantification with Nikon NIS-Elements AR46 software.

Plasma HMGB1 and CORT ELISAs

Plasma was collected to assess content of corticosterone and the immune marker high mobility group box 1 (HMGB1) using the ra CORT (Enzo, #ADI-900-097) and HMGB1 Express (IBI International, #30164033) ELISA kits as per manufacturing instructions.

Statistical Analysis

All results were analyzed as a 2 × 2 analysis of variance (ANOVA). *A priori* analyses to compare the effects of the ethanol challenge on Arc+IR were performed. For all other measures, Bonferroni corrected *post hoc* analyses were performed as appropriate; $\alpha = 0.05$.

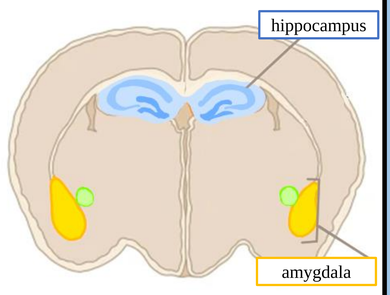


Figure 3. The dentate gyrus of the hippocampus as well as the central and basolateral amygdala were quantified in all histochemical assessments.

BLA: Effects of acute ethanol challenge on Arc and HMGB1+IR

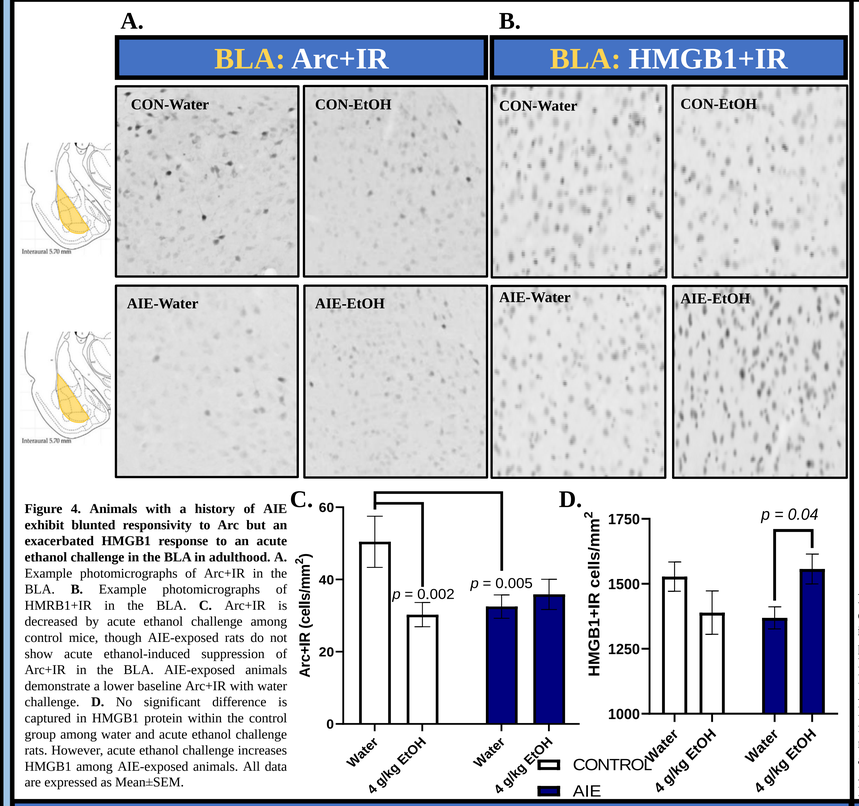


Figure 4. Animals with a history of AIE exhibit blunted responsivity to Arc but an exacerbated HMGB1 response to an acute ethanol challenge in the BLA in adulthood. A. Example photomicrographs of Arc+IR in the BLA. **B.** Example photomicrographs of HMRB1+IR in the BLA. **C.** Arc+IR is decreased by acute ethanol challenge among control mice, though AIE-exposed rats do not show acute ethanol-induced suppression of Arc+IR in the BLA. AIE-exposed animals demonstrate a lower baseline Arc+IR with water challenge. **D.** No significant difference is captured in HMGB1 protein within the control group among water and acute ethanol challenge rats. However, acute ethanol challenge increases HMGB1 among AIE-exposed animals. All data are expressed as Mean±SEM.

DG: Effects of acute ethanol challenge on Arc and HMGB1+IR

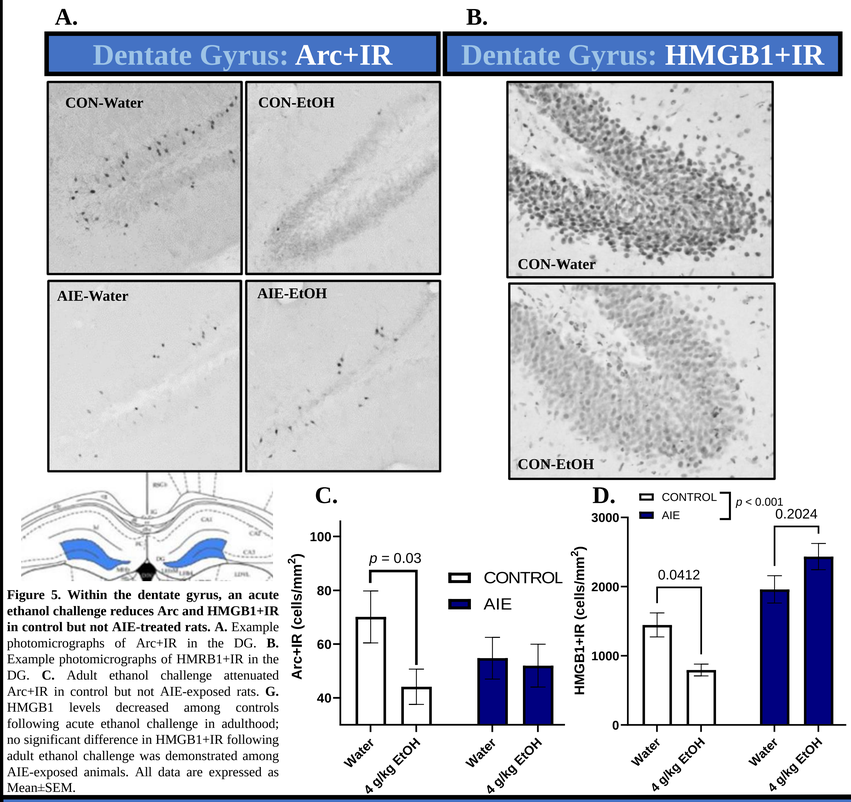


Figure 5. Within the dentate gyrus, an acute ethanol challenge reduces Arc and HMGB1+IR in control but not AIE-treated rats. A. Example photomicrographs of Arc+IR in the DG. **B.** Example photomicrographs of HMRB1+IR in the DG. **C.** Adult ethanol challenge attenuated Arc+IR in control but not AIE-exposed rats. **G.** HMGB1 levels decreased among controls following acute ethanol challenge in adulthood; no significant difference in HMGB1+IR following adult ethanol challenge was demonstrated among AIE-exposed animals. All data are expressed as Mean±SEM.

CeA: Effects of acute ethanol challenge on Arc and HMGB1+IR

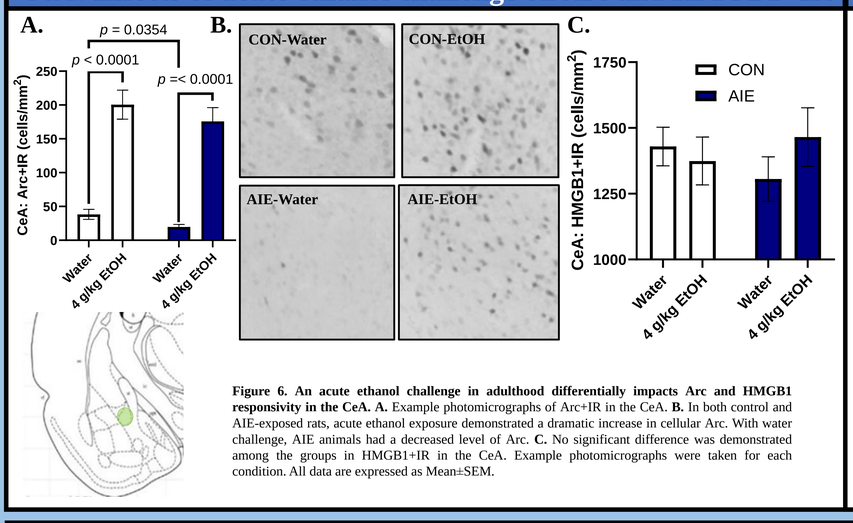


Figure 6. An acute ethanol challenge in adulthood differentially impacts Arc and HMGB1 responsivity in the CeA. A. Example photomicrographs of Arc+IR in the CeA. **B.** In both control and AIE-exposed rats, acute ethanol exposure demonstrated a dramatic increase in cellular Arc. With water challenge, AIE animals had a decreased level of Arc. **C.** No significant difference was demonstrated among the groups in HMGB1+IR in the CeA. Example photomicrographs were taken for each condition. All data are expressed as Mean±SEM.

Plasma: Peripheral Endocrine and Innate Immune Effects

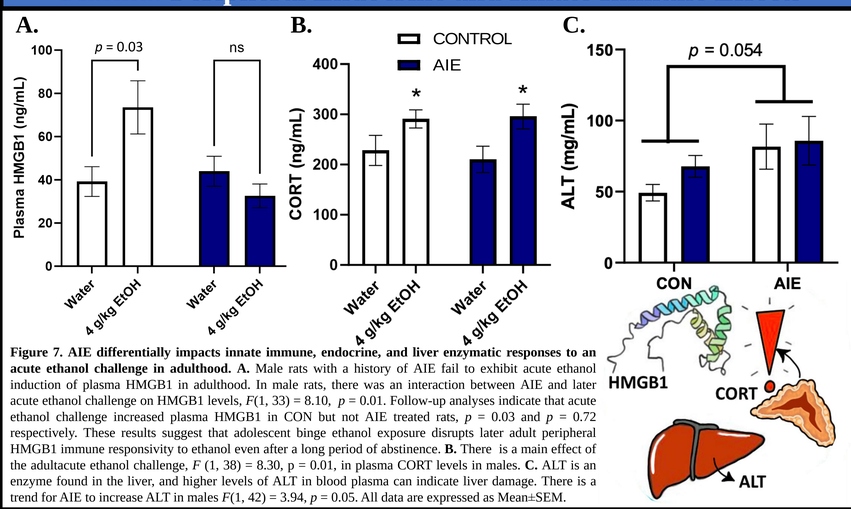


Figure 7. AIE differentially impacts innate immune, endocrine, and liver enzymatic responses to an acute ethanol challenge in adulthood. A. Male rats with a history of AIE fail to exhibit acute ethanol induction of plasma HMGB1 in adulthood. In male rats, there was an interaction between AIE and later acute ethanol challenge on HMGB1 levels, $F(1, 33) = 8.10$, $p = 0.01$. Follow-up analyses indicate that acute ethanol challenge increased plasma HMGB1 in CON but not AIE treated rats, $p = 0.03$ and $p = 0.72$ respectively. These results suggest that adolescent binge ethanol exposure disrupts later adult peripheral HMGB1 immune responsivity to ethanol even after a long period of abstinence. **B.** There is a main effect of the adultacute ethanol challenge, $F(1, 38) = 8.30$, $p = 0.01$, in plasma CORT levels in males. **C.** ALT is an enzyme found in the liver, and higher levels of ALT in blood plasma can indicate liver damage. There is a trend for AIE to increase ALT in males $F(1, 42) = 3.94$, $p = 0.05$. All data are expressed as Mean±SEM.

Conclusions & Future Directions

- An adult ethanol challenge in ethanol-naïve animals produces significant Arc and HMGB1 responsivity across hippocampal and amygdalar regions. Prior history of AIE produces persistent reductions in responsivity to an adult ethanol challenge, despite a long period of abstinence, suggesting persistent shifts in brain ethanol sensitivity after AIE.
- The adult ethanol challenge increased plasma HMGB1 in control but not AIE-treated rats. Corticosterone was not differentially effected by an acute ethanol challenge indicated divergence between endocrine and immune responses.
- **Future directions: We are investigating other immune markers; currently quantifying cyclooxengenase-2. Additionally, we are examining the role of HMGB1 in neuronal cell death.**

Support

Supported by the Neurobiology of Adolescent Binge Drinking in Adulthood (NADIA) consortium of the NIAAA (U24 AA020024, U01 AA020023) and the Bowles Center for Alcohol Studies (P60 AA011605), K01 AA025713 (RPV), and K99 AA030089 (VMP)