Adolescent Ethanol Impacts Brain Immediate Early Gene Expression, and Peripheral Neuroimmune, Neuroendocrine, and Behavioral Responsivity to an Adult Ethanol Challenge

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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, immune, and endocrine 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) and immune/endocrine reactivity an ethanol challenge in adulthood following AIE (**Figure 1**).

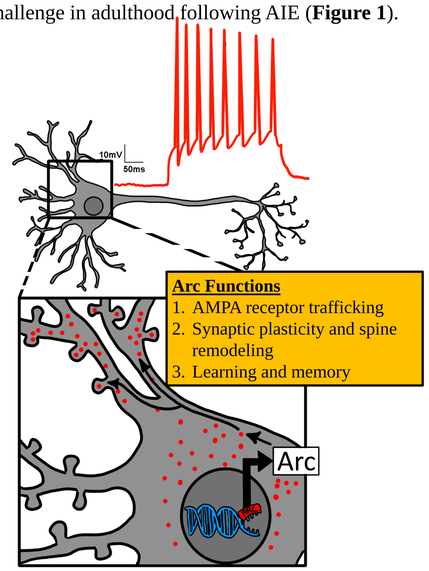


Figure 1. Arc is an immediate early gene which is upregulated in response to action potentials and intracellular calcium influx. Arc transcription and protein are tightly regulated, and upon upregulation, Arc protein is translocated to the cell body and dendrites where it regulates a variety of cellular learning and memory related processes. Disruption of Arc signaling results in severe long-term but not short-term memory deficits.

We hypothesized AIE will:

- Exacerbate peripheral endocrine and immune responsivity to an adult ethanol challenge after abstinence.
- Will attenuate acute adult ethanol-induced hippocampal immediate early gene expression (evidenced by Arc+immunoreactivity; IR) (**Figure 1**).
- Increase behavioral and physiological tolerance to an acute ethanol challenge in adulthood relative to controls.

Ethanol Response Battery

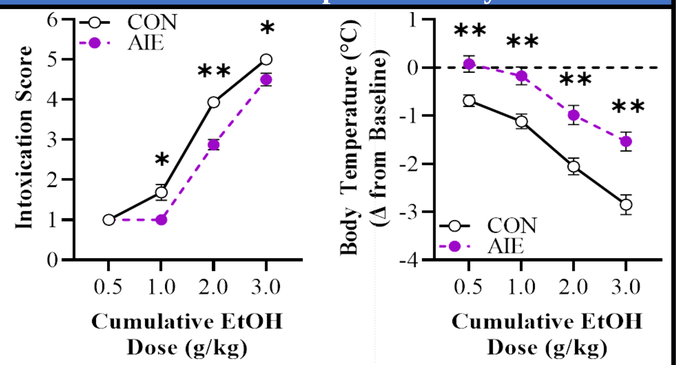


Figure 5. A prior history of AIE causes low responsivity to escalating EtOH exposure compared to that of control (CON) adult rats. A. Intoxication scores increased as ethanol dosage increased but AIE exhibited relatively lower intoxication indices than CON rats at each dose after 1 g/kg ethanol (p = 0.014).. **B.** AIE-treated rats exhibited attenuated hypothermia relative to CON adult rats in response to escalating ethanol exposure during the ERB ($p < 0.01$).

Methods

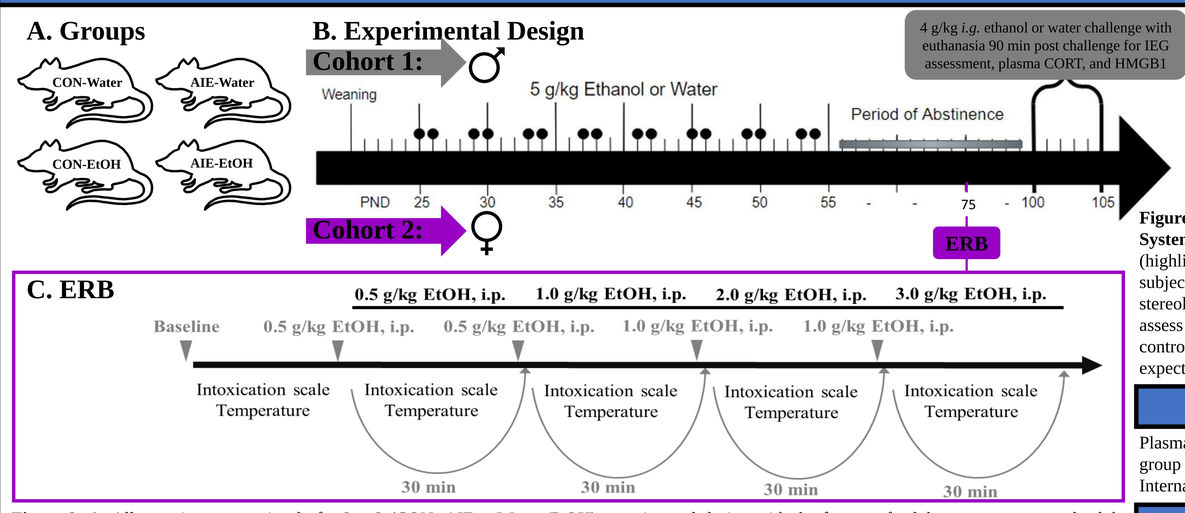


Figure 2. A. All experiments consisted of a 2 × 2 (CON, AIE × Water, EtOH) experimental design with the factors of *adolescent exposure* and *adult challenge*. For all studies, AIE rats were exposed to either 5g/kg/day ethanol or water, i.g., 2-days-on/2-days-off from postnatal days (PND) 25 – PND 54 followed by a period of abstinence. **B.** There were two separate cohorts of animals in this study which went through two different ethanol challenge paradigms in adulthood. **C.** In the ERB paradigm (**Cohort 2**), rats received escalating intraperitoneal injections of ethanol whereupon there were assessed for intoxication indices and body temperature.

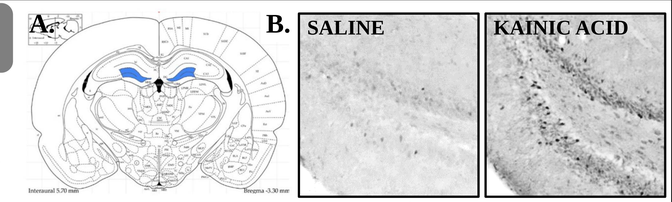


Figure 3. Cohort 1: Immunohistochemical assessment of hippocampal Arc+IR (Synaptic Systems, #156-003). A. The granule cell layer of dentate gyrus in the dorsal hippocampus (highlighted in blue) was the region histologically analyzed for Arc+IR. Four sections from each subject were quantified by a blind experimenter using a modified version of unbiased stereological quantification with Nikon NIS-Elements AR46 software. **B.** As a positive control to assess antibody validity, we assessed Arc+IR on hippocampal sections from (A) saline-treated control rat and (B) a kainic acid treated rat which causes hyperexcitability via seizures. As expected, there is an elevated Arc+ IR in the kainic acid-treated tissue compared to the control.

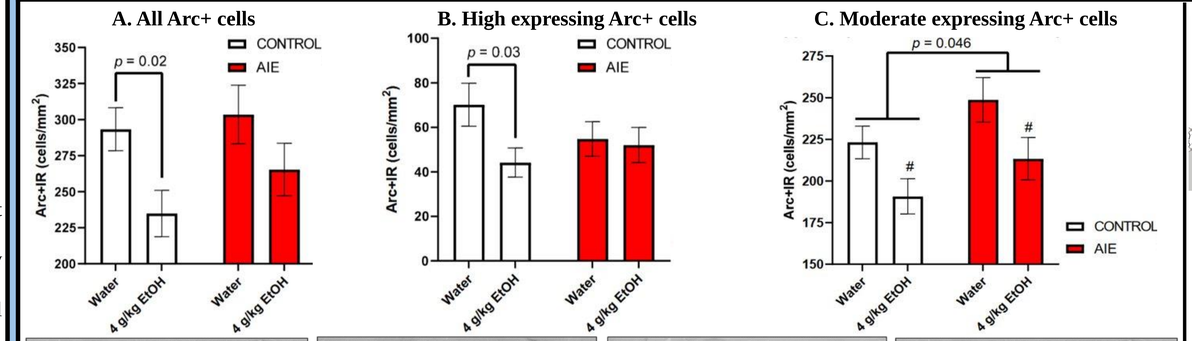
Plasma HMGB1 and CORT ELISAs

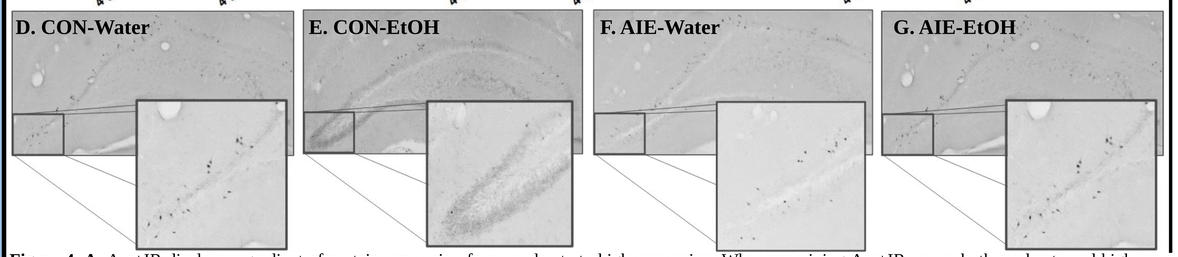
Plasma was collected to assess content of corticosterone and the immune marker high mobility group box 1 (HMGB1) using the rat CORT (Enzo, #ADI-900-097) and HMGB1 Express (IBL International, #30164033) ELISA kits as per manufacturing instructions.

Statistical Analyses

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; $\alpha = 0.05$.

Brain and Plasma Assessment Following Acute Ethanol Challenge in Adulthood





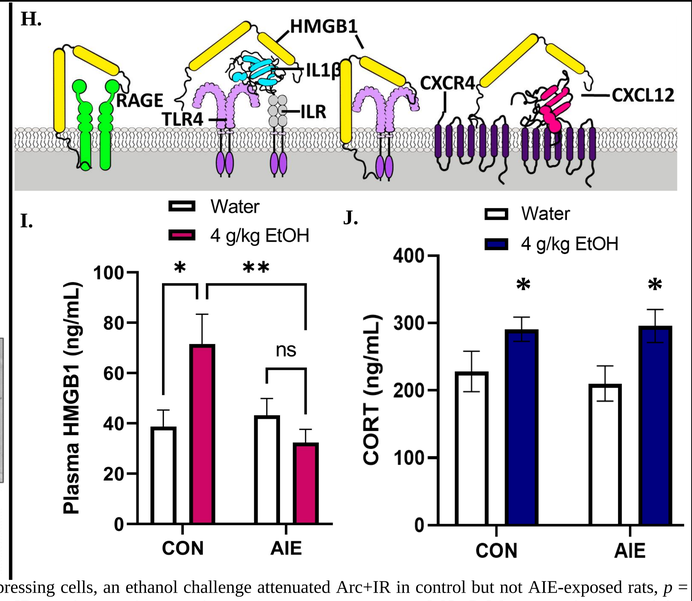


Figure 4. A. Arc+IR displays a gradient of protein expression from moderate to high expression. When examining Arc+IR across both moderate and high expressing cells, an ethanol challenge attenuated Arc+IR in control but not AIE-exposed rats, p = 0.02. **B.** When examining only cells which were densely expressing Arc+IR (i.e., high expressing Arc+ cells), an adult ethanol challenge similarly attenuated Arc+IR only in rats without a history of ethanol exposure, p = 0.03. **C.** When selectively examining cells with moderate Arc+IR, there was a main effect where AIE was significantly higher than CON, p = 0.046. However, the ethanol challenge attenuated Arc expression in both AIE and CON groups, p's < 0.05. Example photomicrographs were taken for each condition: **(D)** CON-Water **(E)** CON-EtOH **(F)** AIE-Water **(G)** AIE-EtOH. **H.** 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. **I.** Acute ethanol induction of plasma HMGB1 expression is attenuated in AIE rats, $p < 0.05$. **J.** Corticosterone (CORT) is a stress-related hormone which is produced by the adrenal glands in response to stimulation of the HPA axis. Ethanol acutely increases CORT. An adult ethanol challenge acutely increased plasma CORT in both CON and AIE groups, $p < 0.05$.

Conclusions & Future Directions

- **An adult ethanol challenge decreases Arc expression in the dorsal hippocampal dentate gyrus in control-treated but not AIE-treated male rats.**
- **The adult ethanol challenge increased plasma HMGB1 in control but not AIE-treated rats. These findings were paralleled by behavioral changes in ethanol tolerance, where AIE-treated rats exhibit lower intoxication scores coupled with blunted hypothermic responses relative to adult controls.**
- **A history of AIE did not impact corticosterone responsivity to an ethanol challenge in adulthood, suggesting diverging endocrine, immune, and behavioral tolerance mechanisms.**
- **AIE induces behavioral, physiological, peripheral immune, and hippocampal neuronal tolerance even after months of abstinence.**
- **Future directions: expand brain regional assessments and include both sexes. In addition, we will expand assessment of peripheral markers.**

Support

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