AN ANIMAL MODEL FOR ADHD WITH RESISTANCE TO ALCOHOL-INDUCED CHANGES IN FOREBRAIN RECEPTORS AND BEHAVIOR

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BACKGROUND & HYPOTHESIS

- Attention Deficit Hyperactivity Disorder (ADHD) is typically treated with psychostimulants such as the amphetamine-based Adderall©.
- Off prescription use of these stimulants is a growing concern especially when combined with adolescent binge consumption of alcohol (Egan et al., 2013)
- Previous work in our lab (Popkin et al., 2018) modeled repeated ethanol-amphetamine co-use in adolescent Long-Evans (LE) rats and provided evidence that amphetamine can attenuate alcohol withdrawal symptoms in a manner that may lessen an individual's awareness of impending alcohol dependence.
- This led to interest in the Spontaneously Hypertensive Rat (SHR), an experimental model for ADHD (Sagvolden and Johansen, 2012). How would a brain for which amphetamine is therapeutic respond to combined use of alcohol and amphetamine?
- However, SHR showed no anxiety-like behavior nor any of the major alcohol withdrawal symptoms used to assess alcohol dependency in rodents (see Figure 2).
- Other work from our lab has shown that severity of adolescent alcohol withdrawal symptoms is associated with the upregulation of two excitatory glutamate receptors (AMPA and NMDA) combined with downregulation of the modulatory adenosine A1 receptor (Bolewska et al., 2019).

Hypothesis

Following consumption of an ethanol-containing diet, SHR brain will not show upregulation of excitatory glutamate receptors (NMDA and AMPA) and/or downregulation of modulatory adenosine receptors (A1)

METHODS

Figure 1. Administering Ethanol-containing Liquid Diet to Adolescent SHR

Animals and Drug Administration.

- Juvenile male rats were obtained from Charles River Breeding Laboratories (Raleigh NC) at P26: Long-Evans (LE), Wistar-Kyoto (WKY) and Spontaneous Hypertensive Rat (SHR).
- At P33, rats were randomly assigned to one of four treatment groups receiving isocaloric liquid diets: control, alcohol (3.5%), amphetamine (20 mg/L), and alcohol with amphetamine (Popkin et al., 2018).

Behavioral Testing

- For behavioral testing of overall withdrawal severity, rats were withdrawn from the liquid diet on day 26. Alcohol withdrawal symptoms and severity were scored using a 4-point scale (Penland et al., 2001), 6-8 hours after withdrawal (Popkin et al., 2018).

Biochemical Analyses

- For biochemical studies, rats were sacrificed on day 26 and brains were dissected and frozen at -80 degrees C.
- Forebrain hemispheres were homogenized and mixed with equal parts Laemmli Sample Preparation Buffer (Bio-Rad) before polyacrylamide gradient (4-20%) gel electrophoresis and Western blotting with receptor specific antibody as described previously (Bolewska et al., 2019).
- Protein determination (Bradford, Bio-Rad) was used to adjust protein loading to 40ug per lane. Antibody against glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal loading control.

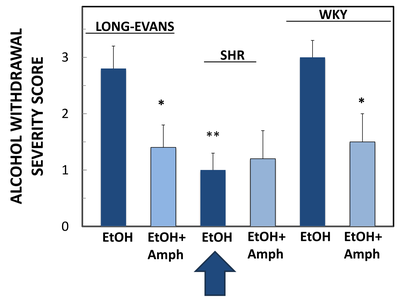
Statistical Analysis.

- Differences among treatment groups were analyzed by ANOVA with Tukey's post-test analysis where a significant main effect was detected. Significance was set at $p < 0.05$.

All protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Monmouth University (ASp1801).

RESULTS

Figure 2. Withdrawal Behavior of Ethanol-fed Rats



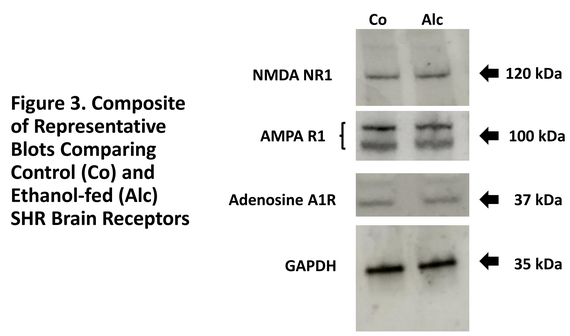
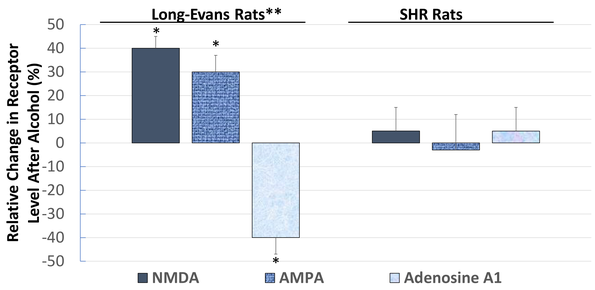


Figure 3. Composite of Representative Blots Comparing Control (Co) and Ethanol-fed (Alc) SHR Brain Receptors

Figure 4. Relative Changes in Receptor Levels in Ethanol-Fed Rats



**Results from Bolewska et al., 2019

CONCLUSIONS

- SHR adolescents are resistant to development of alcohol withdrawal symptoms following prolonged alcohol consumption.
- In comparing control and alcohol fed SHR adolescents, there were no statistically significant differences in NMDA, AMPA or Adenosine A1 receptor expression.
- Thus, the absence of alcohol withdrawal symptoms in SHR adolescents was correlated with the absence of alcohol-induced receptor dysregulation.
- This is in sharp contrast to the results with Long-Evans and WKY adolescents which show pronounced alcohol withdrawal symptoms and significant receptor dysregulation under identical conditions

Significance

- Childhood ADHD has been correlated with later substance use disorders including alcohol use disorder (Groenman et al., 2019).
- If these results translate to individuals with an ADHD diagnosis, are these individuals similarly resistant to development of alcohol withdrawal symptoms?
- The appearance of alcohol withdraw symptoms (including increased anxiety) may serve as cues that alcohol dependency is developing and be important in diagnosing an alcohol use disorder.

Future Directions

- Conduct literature review of alcohol studies involving individuals with an ADHD diagnosis. Is there evidence for attenuated alcohol withdrawal symptoms?
- Investigate the subunit structure of the SHR NMDA receptor. SHR have been reported to have impaired glutamatergic transmission and an altered subunit composition (Jensen et. al., 2009). Does an altered subunit structure impart resistance of the SHR receptor to adaptive changes as are expected during chronic alcohol consumption?

REFERENCES

Bolewska et al. 2019. Sequential changes in brain glutamate and adenosine A1 receptors may explain severity of adolescent alcohol withdrawal after consumption of high levels of alcohol. Neuroscience Journal, vol. 2019, Article ID 5950818, 7 pages. https://doi.org/10.1155/2019/5950818

Egan et al. 2013. Simultaneous use of non-medical ADHD prescription stimulants and alcohol among undergraduate students. Drug and Alcohol Dependence, 131:71-77.

Groenman et al., 2019. Stimulant treatment profiles predicting co-occurring substance use disorders in individuals with attention-deficit/hyperactivity disorder. European Child & Adolescent Psychiatry 28:1213–1222

Jensen et. al., 2009 N-methyl-D-aspartate receptor subunit dysfunction at hippocampal glutamatergic synapses in an animal model of attention-deficit/hyperactivity disorder. Neuroscience 158 (2009) 353-64.

Penland et al. 2001. Effects of nicotine on ethanol dependence and brain damage. Alcohol 24:45–54.

Popkin et al. 2018. Co-Administration of Amphetamine with Alcohol Results in Decreased Alcohol Withdrawal Severity in Adolescent Rats. Behavioural Pharmacology 29:547–550.

Sagvolden & Johansen 2012. Rat models of ADHD. Current Topics in Behavioral Neurosciences. 9:301-15.

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