The Role of Central Amygdala Corticotropin-Releasing Factor Receptor 1 (CRF1) Neurons in Anxiety-Like Behavior and Alcohol Consumption

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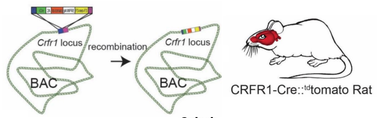
Background

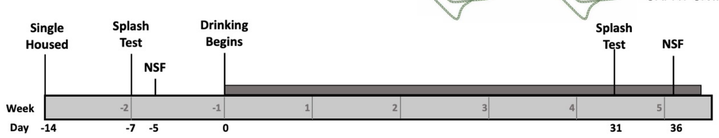
Alcohol use disorder (AUD) is a severe and complex condition that has negative impacts on human health and society. Despite its negative effects, alcohol is extensively used recreationally, and consumption rates continue to rise.[1] Anxiety disorders are highly prevalent and often co-occur alongside AUD. However, it is difficult to isolate the interactions between alcohol and anxiety from outside variables such as sex and stress. Sex differences are apparent in both AUD, which is more prevalent among men, and anxiety disorders, which are more prevalent among women.[1,2] However, preclinical research has shown female rodents to consume more ethanol.[3] Additionally, the CRF1 system is broadly implicated in both alcohol use and anxiety, however our understanding of these complex interactions remains incomplete. The present study utilizes a CRF1 transgenic rat line to investigate the dynamic interactions between alcohol, anxiety, and stress in a sex-specific manner.

Methods

Experimental Timeline

- We utilized male (n = 16) and female (n =16) CRF1:cretdTomato rats



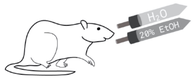


Behavioral Testing

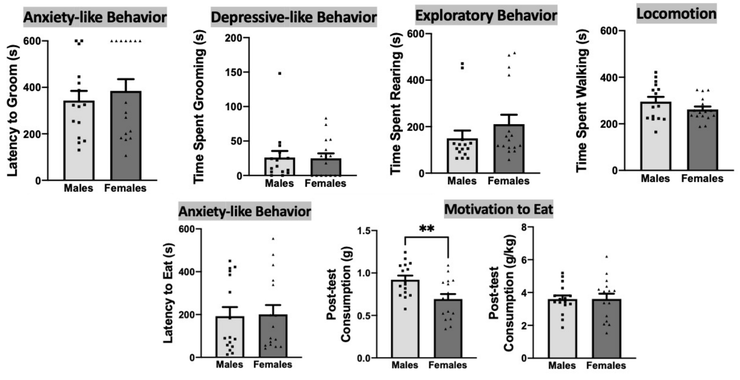
- Two behavioral tests were employed to measure anxiety-like behavior before and after ethanol exposure.
- Splash Test: Rats were removed from their home cage and sprayed with 10% w/v sucrose solution. Animals were placed into a behavioral testing chamber and recorded via a video camera for 10 minutes. Videos were later analyzed for latency to groom, time spent grooming, and time spent rearing.
- NSF: After undergoing a 24-hour food deprivation, rats were placed into a behavioral testing chamber with one Froot Loop at the center. Latency to eat and post test consumption were recorded.

Intermittent Access to Ethanol Two Bottle Choice

- Subjects were single housed and given 24h access to 20% ethanol on alternating days. Each 24h drinking session was followed by 24 hours of access to water only.



Baseline Anxiety-like Behavior



- Before ethanol exposure, no significant sex differences were found in the measures of anxiety-like or depressive-like behavior.

References:
1. Grant BF, Chou SP, Saha TD, Pickering RP, Kerridge BT, Ruan WJ, Huang B, Jung J, Zhang H, Fan A, Hasin DS (2017) Prevalence of 12-Month Alcohol Use, High-Risk Drinking, and DSM-IV Alcohol Use Disorder in the United States, 2001-2002 to 2012-2013: Results From the National Epidemiologic Survey on Alcohol and Related Conditions. JAMA Psychiatry 74:911–923.
2. Walker BM, Walker JL, Ehlers CL (2008) Dissociable effects of ethanol consumption during the light and dark phase in adolescent and adult Wistar rats. Alcohol 42:83–89.
3. Donner NC, Lowry CA (2013) Sex differences in anxiety and emotional behavior. Pflugers Arch 465:601–626.

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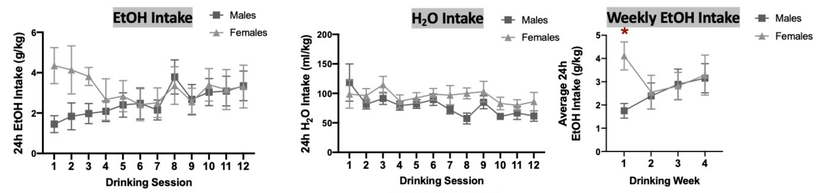
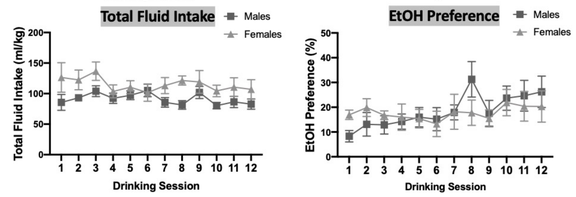
Ethanol Intake

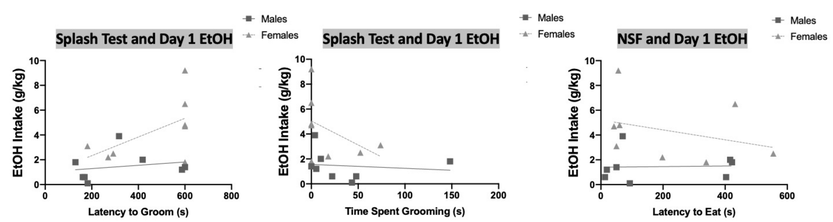




Voluntary Drinking

- Females drank significantly more ethanol during the first week.
- Overall, males and females drank similar amounts of EtOH.

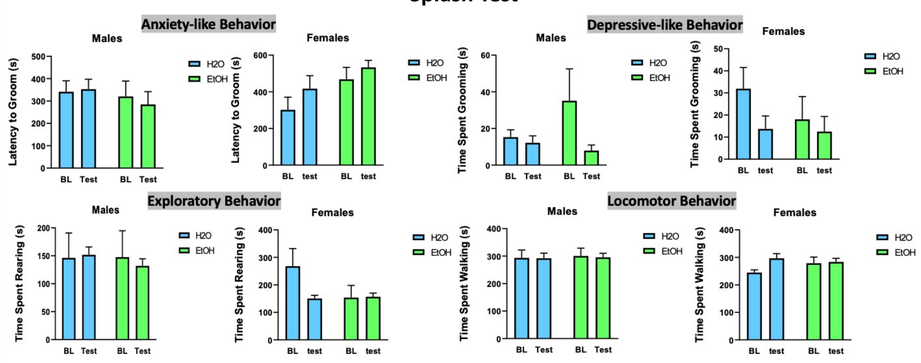
Baseline Anxiety-Like Behavior and Subsequent Drinking

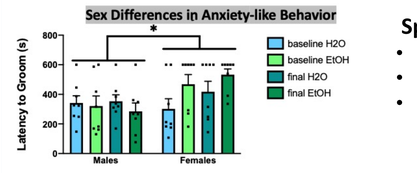


- Anxiety-like behavior did not predict ethanol intake for the first drinking session.

Behavioral Testing After Alcohol

Splash Test

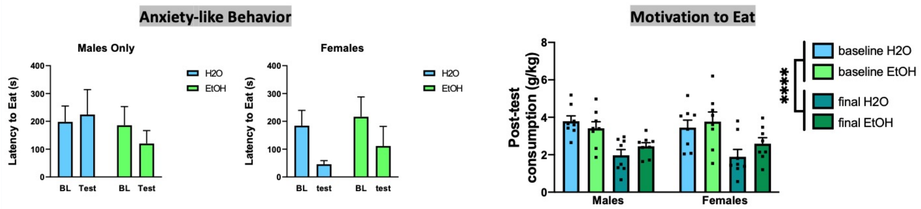




Splash Test

- Alcohol was not found to effect anxiety-like behavior.
- No differences in exploratory or locomotion behavior.
- Overall, females displayed more anxiety-like behavior than males as measured by latency to groom.

NSF Test

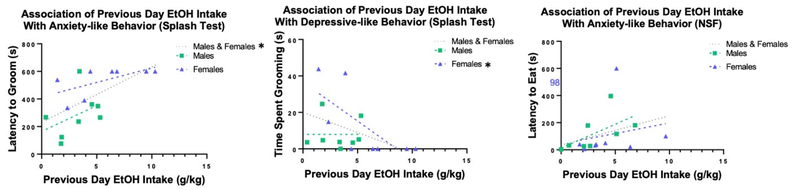


NSF Test

- Alcohol was not found to effect anxiety-like behavior.
- Motivation to eat was decreased for both the water and ethanol groups of males and females

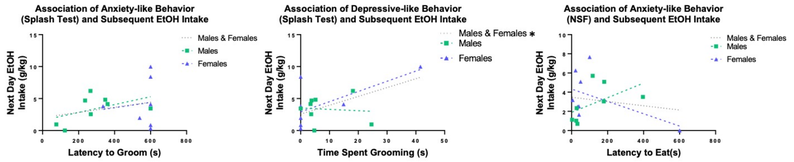
Drinking and Anxiety Interactions

Associations Between Previous Day Drinking and Subsequent Anxiety-like Behavior



- Previous day ethanol consumption was correlated to increased anxiety-like behavior in males and females combined.
- We found a positive correlation between ethanol intake and depressive-like behavior in females only.

Associations Between Anxiety-like Behavior and Subsequent Drinking



- No associations were identified between next day ethanol intake and anxiety-like behavior.
- Increased depressive-like behavior corresponded with decreased alcohol intake in males and females combined.

Summary

- **We found no baseline sex differences in basal anxiety-like behavior as measured by the splash test and NSF test**
- **Females consumed significantly more ethanol in the first week, however, no sex differences in overall EtOH drinking were found.**
- **Exposure to alcohol did not impact anxiety-like behavior in male or female rats.**
- **Increased ethanol consumption on the day prior to testing was correlated to increased anxiety-like behavior in males and females combined.**
- **In females, ethanol intake was positive correlated to depressive-like behavior.**

Conclusion

The present study illustrates the dynamic relationship between alcohol and anxiety and stresses the importance of considering sex as a biological variable. While we observed no overall effects of alcohol on anxiety-like behavior, when analyzing correlations on an individual level significant relationships began to emerge. These individual differences highlight the complex interactions between alcohol and anxiety and the need for more research to understand how alcohol use afflicts males and females differently.

While the relationship between alcohol and anxiety remains complex, our study expands our understanding of these interactions and aids in the development of more personalized therapeutic treatments for both complex disorders.

Future Directions:

- Employ different behavioral tests to measure anxiety-like behavior
- Explore alternate models of ethanol intake or investigate the effects of prolonged alcohol drinking
- Investigate how alcohol impacts the expression of c-Fos and activation of CRF1 receptor neurons in the central amygdala (CeA)
- Observe sex differences in CeA activity