Alcohol Relapse and Interoceptive Neural Circuitry

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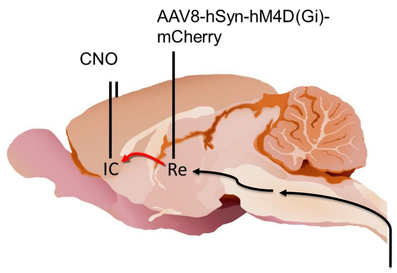
Introduction

Disorders involving drugs of abuse, including Alcohol Use Disorder (AUD), are increasingly prevalent in society, despite widespread knowledge of the harmful effects of addiction. Interoception, the body's perception of its internal state, can be a powerful driver of such drug-seeking behavior[1]. Alcohol produces interoceptive effects that contribute to the emergence and continuation of AUD. Further, alcohol-related environmental cues (e.g. a bar, liquor bottles, etc) are reported to produce interoceptive effects reported as jitteriness, tension, and butterflies in the stomach[1]. Thus, the presence of alcohol-related cues can drive an interoceptive state that contributes to alcohol-seeking and relapse. We hypothesize that this interoceptive information is carried to the primary interoceptive brain region, the anterior insular cortex (IC), through the nucleus reuniens (RE), a midline thalamic nucleus we have previously shown to play a role in the interoceptive effects of alcohol[2]. In this experiment using a rat model, we silenced the RE-IC neural pathway using a chemogenetic approach to determine how this important interoceptive circuit contributes to alcohol relapse.

DREADD Method and Background

A.

Silencing RE-IC projection with DREADDs



Vagus nerve pathway carrying interoceptive information about the body state

B.

Silencing the RE-IC projection disrupts interoceptive effects of alcohol

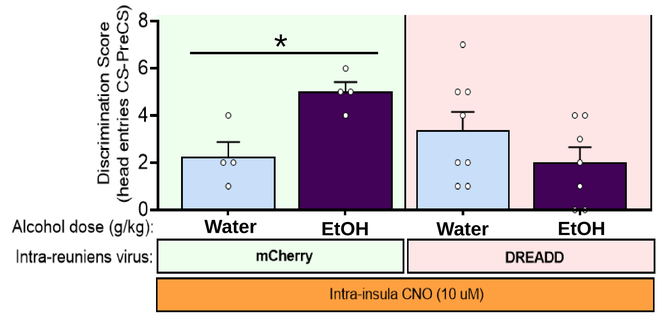
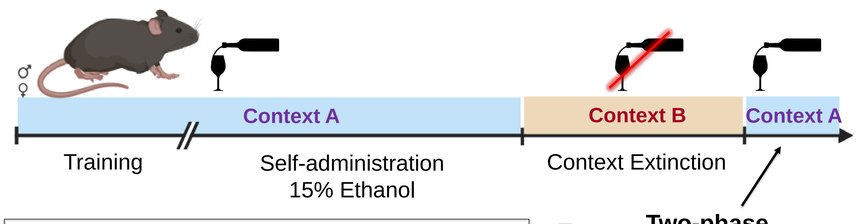


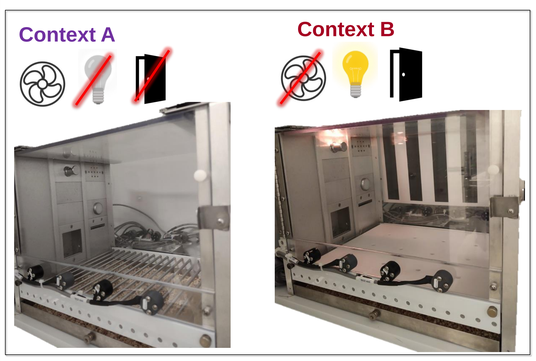
Figure 2: In these experiments we used DREADD technology, in which neurons express artificial designer receptors that are activated only by an artificial designer drug (CNO). **(A)** To silence the neural pathway from the nucleus reuniens (RE) and the anterior insular cortex (IC), we injected an inhibitory Gi DREADD virus into the nucleus reuniens and activated these receptors with CNO infused into the IC. **(B)** In a previous experiment using a drug discrimination task, rats in the control group (left) were able to discriminate between water and alcohol. In the group where the RE-IC projection was silenced (right) , rats were unable to discriminate between water and alcohol, indicating disrupted interoceptive processing of the alcohol cue. Thus, this identified the RE to IC pathway as critical in modulating the sensory cues of alcohol.

Behavioral Methods

A.

Rats are trained to lever press for alcohol, then are tested in a context reinstatement procedure





B. **Two-phase reinstatement test:**

Phase 1: Seeking
Lever presses result in normal cues but no delivery of alcohol

Phase 2: Re-initiation of Drinking
Lever presses result in normal cues and alcohol delivery: rats can drink alcohol freely

C. **Lever responses on training, extinction, and test days**

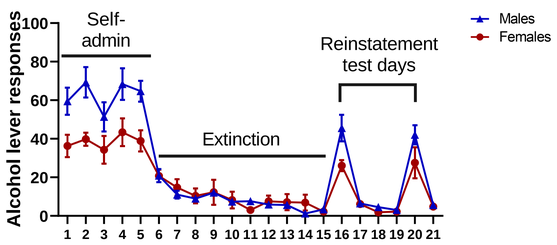


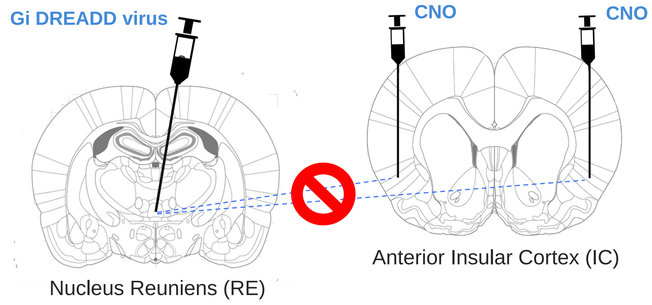
Figure 1: (A) Rats were trained to press a lever to self-administer alcohol in daily 30 minute sessions. Self-administration always occurred in Context A. Next, rats underwent extinction in the distinct Context B for 10 days, where lever presses did not result in alcohol delivery **(B)** On the test day, rats were put back into Context A and were tested in a two-phase reinstatement test, where in the initial seeking phase lever responses did not result in delivery of alcohol. After 15 min, alcohol was automatically delivered, and subsequently lever presses resulted in alcohol delivery. **(C)** Representative data showing lever responses across the experiment.

Silencing the RE-IC Projection in Alcohol Relapse

A.

On test days:

Experimental Group: CNO Injection	Control Group: Vehicle Injection (Veh)
Activates the DREADD virus which inhibits the key RE-IC pathway	**Does not activate the DREADD virus, leaving the RE-IC pathway active.**



Chemogenetic silencing of RE to IC projections altered relapse in males

B.

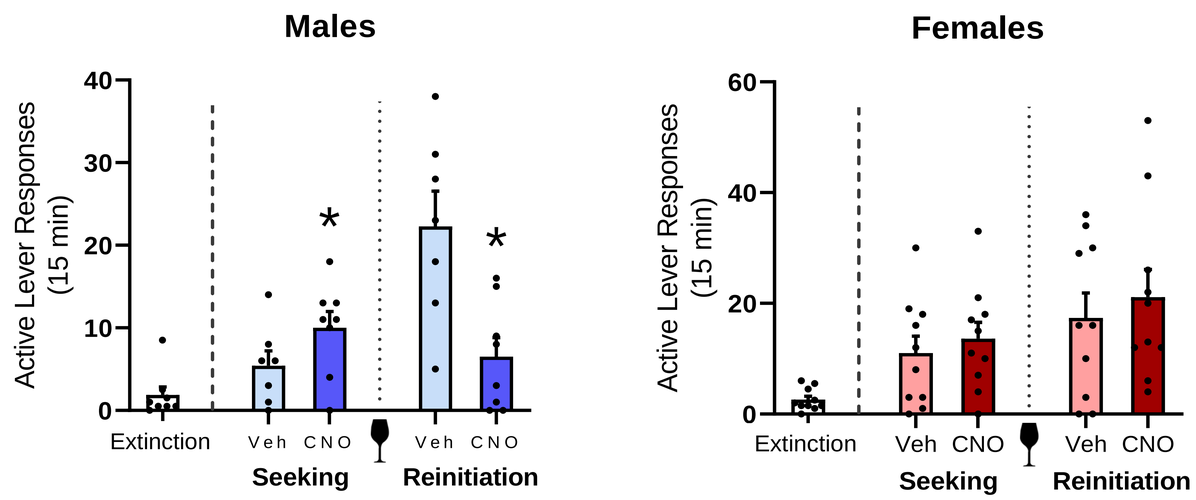


Figure 3: (A) On reinstatement test days we microinjected CNO into the IC which inhibited the Re to IC projection by activating the inhibitory Gi DREADD receptors. **(B)** Male rats that received CNO demonstrated increased alcohol seeking during seeking phase of reinstatement testing compared to the vehicle condition group. Male rats that received CNO showed diminished alcohol drinking during the re-initiation phase compared to the vehicle condition group. No significant alcohol seeking or drinking effects were observed in female rats. The disparity between alcohol seeking and actual alcohol consumption in male CNO rats suggests a difference between interoceptive effects before and after actual alcohol drinking in males.

Reference

1. Lovelock, D.F., Tyler, R.E., and Besheer, J. Interoception and alcohol: Mechanisms, networks, and implications. Neuropharmacology, 2021. 200: p. 10887.
2. Jaramillo et. Al. Silencing the insular-striatal circuit decreases alcohol self-administration and increases sensitivity to alcohol. Behavioral Brain Research, 2018. 348: p.74-81

Acknowledgements

Thanks to NIAAA grant AA026537 and the Bowles Center for Alcohol Studies, especially Dennis Lovelock and Dr. Joyce Besheer, for making this work possible.

Conclusions

- Inhibition of the nuclear reuniens to insular cortex pathway was found to promote alcohol-seeking and diminish alcohol drinking upon context reinstatement and alcohol re-initiation in males only. Seeking and consumption were unaffected in female rats.
  - The reduced drinking exhibited by CNO male rats during the re-initiation phase could be attributed to a lack of interoceptive feedback when the alcohol is in the rats' system, discouraging them from consuming more while in the vehicle rats the alcohol interoceptive cues were intact and facilitated alcohol consumption
  - The lack of similar effects in females could imply a different interoceptive neural processing pathway that explains the sex-differences in cue-based drinking and relapse.
- Overall, this experiment builds a framework for understanding the neural circuitry behind interoceptive cues and alcohol relapse, and how interoceptive effects based on alcohol-associated contexts can affect both the seeking and actual consumption of alcohol.