Investigating individual differences in aversion-resistant drinking behavior

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Introduction

- Aversion-resistant alcohol drinking, characterized by persistent consumption despite negative consequences, emerges in only a minority of those who drink alcohol and is a key feature of alcohol use disorder (AUD).
- The neurobiological basis of individual differences in aversion-resistant drinking is understudied. Furthermore, the contributions of innate differences vs. alcohol experience to giving rise to such individual differences remain unknown.
- This project classifies individual differences in aversion-resistant drinking through the implementation of alcohol self-administration (SA) paired with random foot shocks in rats, resulting in distinct subgroups of shock-sensitive, intermediate, and shock-resistant rats. To explore brain activity differences underlying these behavioral traits, we first employ c-Fos immunohistochemistry to measure neuronal activation in the prelimbic region (PrL) of the medial prefrontal cortex, a known center for behavioral inhibition. Next, we investigate individual differences in aversion-resistant drinking after extended alcohol history using chronic intermittent ethanol (CIE) exposure.
- We hypothesize that shock-resistant rats will show decreased neuronal activation in the PrL during punished SA compared to shock-sensitive and intermediate rats. Also, that extended alcohol history will increase shock resistance during punished SA in a subset of vulnerable rats, thereby increasing the proportion of rats meeting the criteria for shock-resistant classification.

Methods

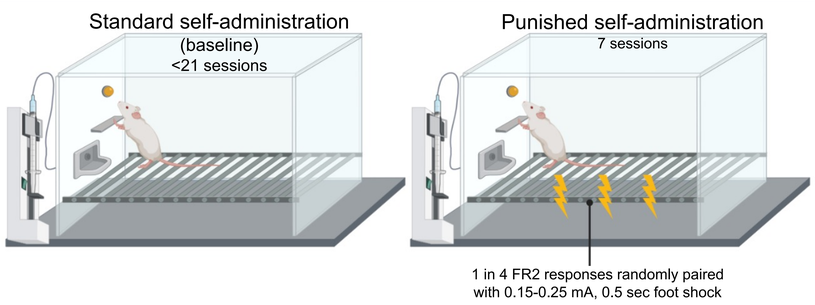

Figure 1. Modeling aversion-resistant drinking in rats. Male Wistar rats are trained to self-administer a 15% alcohol solution using a fixed ratio 2 (FR2) schedule of reinforcement in 30 min/day sessions, then they undergo punished SA. The percent change from baseline is averaged over the last 3 punished sessions (0.25 mA). Rats are categorized via interquartile range **as shock-sensitive** (**SS**; bottom 75%), **intermediate** (**I**; middle 50%), or **shock-resistant** (**SR**; top 25%).

Experiment 1

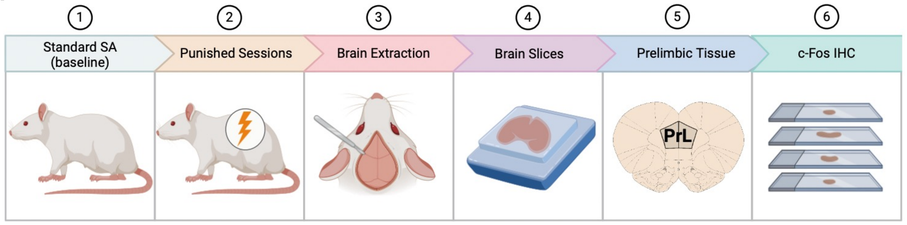

Figure 2. Timeline for experiment 1. Following the completion of SA training and baseline sessions, the rats proceed to undergo seven punished SA sessions. Based on their aversion response, they are subsequently classified SR, I, or SS. 90 minutes after the initiation of the last punished session, their brains are extracted and sliced. Brain sections containing the prelimbic cortex underwent processing for c-Fos immunohistochemistry and c-Fos+ cells were manually quantified.

Experiment 2

Figure 3. Timeline for experiment 2. Following the completion of punished SA, rats were transferred to chambers within an alcohol vapor inhalation system. Vapor exposures lasted 16 hrs/day for 4 days. After each daily vapor exposure, tail blood was collected from 2 rats and blood alcohol concentration was analyzed to ensure rats were reaching target BACS (150-200 mg/dl). Rats underwent a total of 3 weeks of vapor exposure. After each week of vapor exposure, rats completed 3 sessions of standard (unpunished) SA. Following the last week of vapor exposure, rats returned to punished SA for 5 sessions. Brains were extracted and collected 72 hours after the last punished session for gene expression analysis.

Shock-resistant alcohol self-administration emerges in a subset of rats

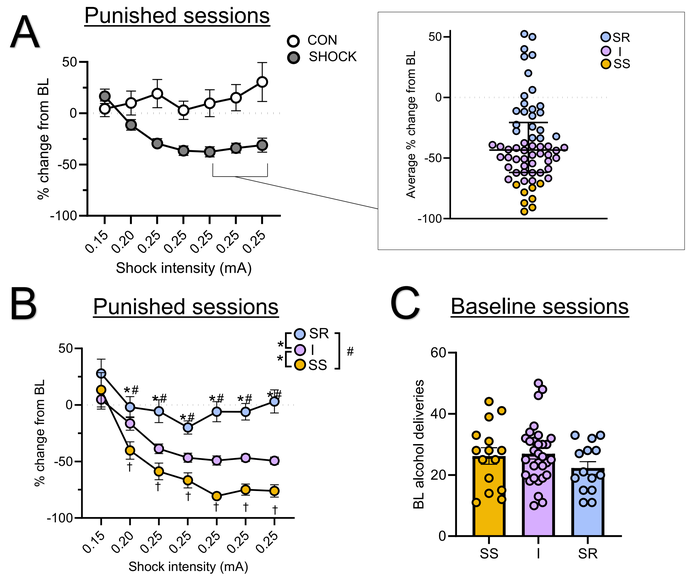

Figure 4. Individual differences in responding for alcohol under punishment. (A) male rats showed decreased responding for alcohol when foot shocks were paired with alcohol delivery; there was striking individual variation among the rats. **(B)** Analysis of individual differences in percent change from baseline yields distinct subgroups of SS, I, and SR rats. Significant differences from controls not shown; * denotes significant differences ($p<0.05$) between I and SS or SR subgroups; # denotes significant differences ($p<0.05$) between SS and SR subgroups. **(C)** Baseline responding for alcohol is similar across subgroups, indicating that baseline intake does not predict the emergence of aversion-resistant drinking.

Experiment 1: Reduced neuronal activation in shock-resistant rats during punished SA

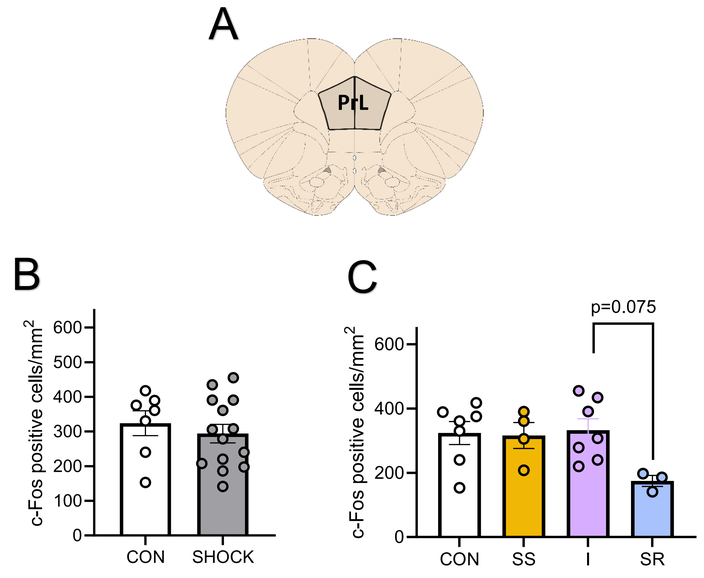

Figure 5. Neuronal activation in SS, I, and SR male rats during punished SA. (A) Brain region that was quantified **(B)** No difference in neuronal activation between control rats and rats that underwent punished SA. **(C)** Among the subgroups, SR rats exhibited decreased c-Fos immunoreactivity (IR) in PrL neurons compared to both I and SS rats. This reduction suggests diminished neuronal activation during punished SA in the SR subgroup.

Experiment 2: Increased self-administration in shock-sensitive rats after CIE

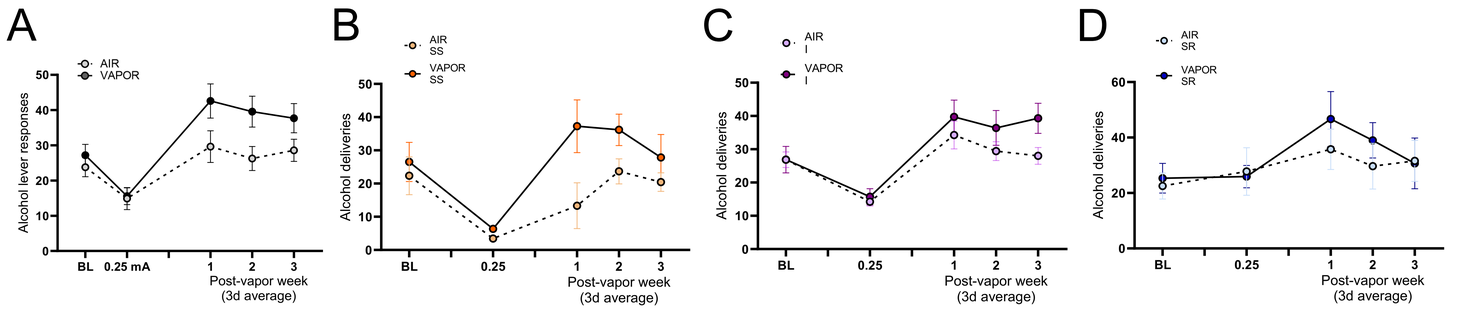

Figure 6. Self-administration between CIE cycles. (A) Average value of three days of standard SA observed between vapor cycles. Male rats exposed to vapor exhibited an increase in lever presses compared to those exposed to air. **(B)** Alcohol deliveries in air SS rats were notably lower than those in vapor SS, means $p<0.05$. **(C, D)** There was no difference in SA rates between vapor-exposed rats and controls in I or SR subgroups.

Conclusions

- When foot shock is combined with alcohol delivery, male rats exhibit individual differences in operant SA behavior. This variability is observed although alcohol history is equivalent and initial responding to alcohol at baseline is comparable.
- Diminished activity in the prelimbic cortex may contribute to shock-resistant SA behavior.
- Shock-sensitive (SS) rats exposed to vapor exhibited increased self-administration. While overall ethanol vapor exposure induced a small, nonsignificant increase in self-administration, these effects varied across subgroups.

Future Directions

- Examine punished SA after vapor exposure to determine if extended alcohol history promotes SR drinking in rats previously sorted as SS and I.
- Investigate gene expression differences between subgroups and air vs. vapor conditions to uncover baseline gene expression distinctions among subgroups (air SS, I, and SR) and explore how these differences may be influenced by vapor exposure.
- Evaluate neuronal activation in regions that receive projections from PrL in the same group of rats to gain further understanding of their involvement.

Acknowledgements

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