Modeling Pavlovian alcohol seeking in rats using a retractable sipper to study both appetitive and consummatory behavior

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Rationale

- Alcohol-associated cues may promote problematic drinking in individuals at risk for alcohol use disorder (AUD) and represent a relapse liability in AUD treatment.
- Pavlovian conditioning procedures in rodents provide a preclinical model suitable for studying neural mechanisms for alcohol-associated cue learning & memory.
- Existing paradigms for modeling Pavlovian alcohol seeking behavior use a fluid port to dispense alcohol and can measure only changes in appetitive behavior (anticipatory port entries).
- In order to measure changes in both appetitive and consummatory (licking) behavior, we developed a paradigm in which time-limited access to alcohol is granted via a retractable sipper coupled to a lickometer.

Methods

Subjects: 16 singly-housed, adult male Long-Evans rats on ad libitum food and water

Alcohol acclimation: 5 weeks of access to unsweetened alcohol for 24 hr in the homecage every Monday, Wednesday, and Friday. Rats ingested 3.2 ± 0.3 g EtOH/kg bodyweight/24 hr over the last week. [1 rat dropped for failure to drink]

Pavlovian conditioning

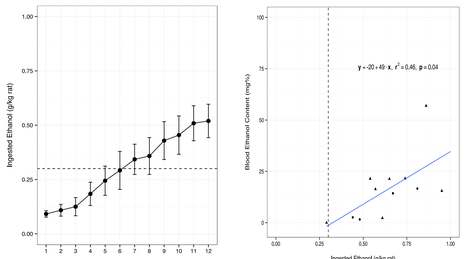
Day 1: Habituation (8 trials of 20-s light presentation)
Day 2-14: Training (8 trials of 20-s light presentation with 10-s access to alcohol sipper) [3 rats dropped for drinking below 0.30 g EtOH/kg bodyweight]
Day 15-26: Extinction (12 trials of 20-s light presentation with 10-s access to dry sipper)
Day 28: Long-term Memory Test (4 trials of 20-s light presentation with 10-s access to dry sipper)
Day 30: Reinstatement Test (4 trials of 20-s light presentation with 10-s access to dry sipper; EtOH odor in chamber)

Behavior Measurement

Appetitive behavior was measured as the absolute frequency of "approach to sipper site" determined from video recording analysis using an observational sampling method. "Approach to sipper site" was defined as the rat attending to or moving or turning toward or exploring the hole through which the sipper is inserted. Observations were made every 1.25s starting 5s prior to and throughout illumination of the houselight.

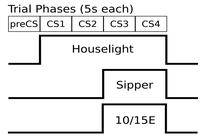
Consummatory (licking) behavior was measured using the lickometer. Both latency (s) to first lick and number of licks per trial were tracked.

Drinking & Post-Training Session BEC

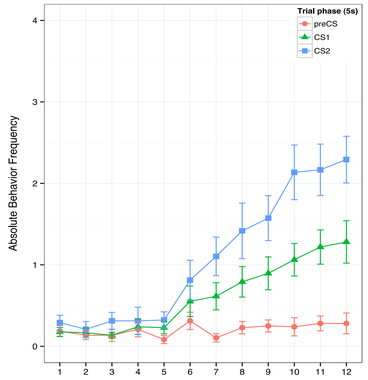


[Left panel] Mean (± SEM) ingested EtOH (g/kg) across rats per session. The horizontal line at 0.3 g/kg represents the minimum dose expected to result in detectable blood EtOH content (BEC). [Right panel] After retraining, blood samples were taken from 12 rats ~~after the 8th drinking opportunity~~ and analyzed for EtOH content using a gas chromatography system with flame ionization detection. Ingested EtOH and post-session BEC (mg%: mg EtOH/dL blood) are shown for each rat (circles: saphenous vein samples at 10min; triangles: trunk samples taken at 5min). Simple regression revealed a significant linear relationship between ingested EtOH and post-session BEC despite uncontrolled stomach content.

Training

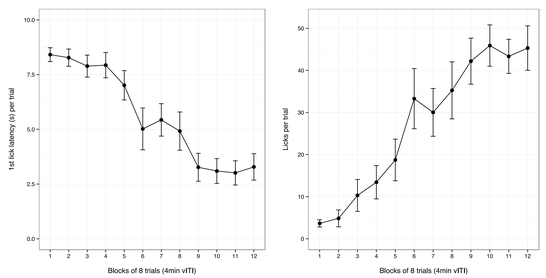


Approach



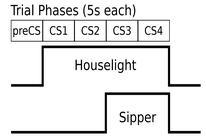
Across daily conditioning sessions, there was a temporally selective increase in approach to the sipper site. Approach to sipper site pre-CS (before houselight illumination) remained negligible, whereas it grew appreciably during the CS period, especially during the 5s immediately prior to sipper insertion (~~termed~~ CS2). Data represent the mean frequency ± SEM across rats (n=12) per session. Given that only 4 observations are made per 5s trial phase, the maximum behavior frequency would be 4.

Licks

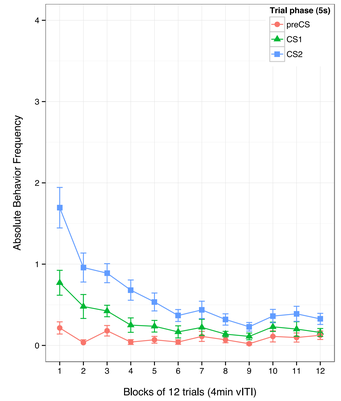


[Left panel] Across daily conditioning sessions, latency (s) to make contact (1st lick) with the sipper upon presentation decreased. Data represent mean latency per trial ± SEM across rats (n=12) per session. [Right panel] Licks per trial increased across conditioning sessions. Data represent mean licks per trial ± SEM across rats (n=12) per session.

Extinction

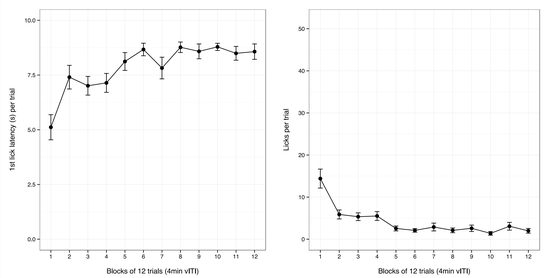


Approach



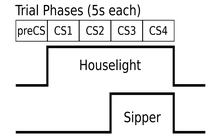
Approach to the sipper site during the CS period decreased rapidly across daily extinction sessions. Data represent the mean frequency ± SEM across rats (n=12) per session. Given that only 4 observations are made per 5s trial phase, the maximum behavior frequency would be 4. During extinction, ethanol solutions were completely absent from the chambers.

Licks

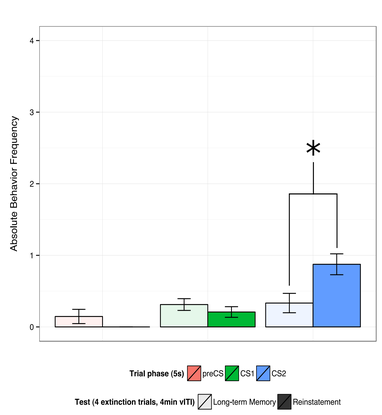


[Left panel] When licking the sipper no longer delivered fluid, the latency (s) to make contact upon presentation increased rapidly. Data represent mean latency per trial ± SEM across rats (n=12) per session. [Right panel] Licks per trial dropped dramatically during the first extinction session and decreased slowly thereafter. Data represent mean licks per trial ± SEM across rats (n=12) per session.

Testing

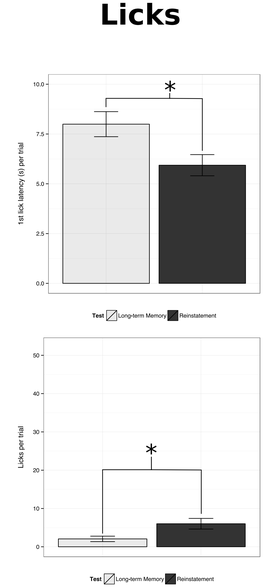


Approach



Efficacy of extinction training was evaluated across two test sessions given 48hr and 96hr after the last extinction session. The pattern of approach to the sipper site across trial phases differed by test session (phase x test interaction: F2,22=7.3, Greenhouse-Geisser corrected p=.0036). The first test, "Long-term Memory," assessed the level of spontaneous recovery of conditioned approach. The second test, "Reinstatement," assessed the ability of EtOH odor to reinstate conditioned approach above the level of spontaneous recovery Pre-CS and CS1 phase approach did not differ by session (t1,11=1.5, p=.17; t1,11=1.05, p=.32) whereas approach during the CS2 phase was greater in the "Reinstatement" relative to "Long-term Memory" test session (t1,11=2.8, p=.017). Data represent mean frequency ± SEM across rats (n=12) per session.

Licks



[Top panel] Rats (n=12) were quicker to make contact with the sipper upon presentation during the "Reinstatement" relative to "Long-term Memory" test session (t1,11=2.8, p=.017). [Bottom panel] Rats also made a greater number of licks per trial during the "Reinstatement" relative to "Long-term Memory" test session (t1,11=2.4, p=.03).

Findings

- 12 sessions of 8 conditioning trials were sufficient to establish reliable houselight-cued anticipatory approach to the sipper site as well as rapid initiation of licking and vigorous licking upon sipper presentation. By the 9th session, ingested ethanol doses were above the minimum expected to result in detectable BEC.
- After 12 sessions of 12 extinction trials, conditioned approach and licking were both suppressed. No spontaneous recovery of conditioned approach and licking were seen during the test given 48 hrs after last extinction session. However, when the EtOH odor was present 48hr later, anticipatory approach and licking behavior were significantly reinstated.

Innovation

In being able to separate anticipatory approach to the alcohol access site from access attempts (and consumption), our paradigm provides a rich model of Palvovian alcohol seeking behavior for future studies.