Behavioral conditioning effects of ingested alcohol in female rats and exploration of estrous cycle in drinking

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Rationale

The conditioning effects of alcohol may contribute to the progressive loss of perceived control over use that characterizes alcohol use disorder. In younger generations, women drink as much as men. However, women may progress to alcohol use disorder more quickly than men. In order to explore the neurobiological basis behind this unique vulneriability, it is important to first develop a model of the conditioning effects of self-administered alcohol in female rats.

Methods

Subjects

Female Long-Evans rats were obtained from Envigo at 200-225 g and housed in separate shoe box-style plexiglass cages on a metal rack. Metal wire cage tops were used to allow water bottle placement on top of the cage. Cages contained a Bio-Serv Gummy Bone and Sani-Chips bedding. The room was humidity and temperature controlled (22±2 ºC). The light cycle was 12 hr on/12 hr off. Drinking sessions always began 3-4 hr after lights on. Tap water and standard rat chow were freely available in the homecage and replenished daily before lights off. Conditioning sessions took place in an adjacent room.

Pre-Conditioning Phase

24 hr homecage two-bottle choice sessions (15% ethanol v/v in tap water or tap water) on MWF for 5 weeks. Two water bottles provided on TTSS. Ethanol and water bottle placement on cage top alternated across sessions.

Conditioning Phase

Discrete-trial sessions in a MedAssociates conditioning chamber for 12 days (1 session/day). Each session consisted of 8 trials on a 280 s variable intertrial interval (ITI). Each trial consisted of 20 s illumination of a chamber houselight. For group Paired, a retractable sipper was presented 10 s into illumination to allow 10 s access to 15% ethanol v/v in tap water. For group Unpaired, 10 s access to the sipper was presented halfway into the variable ITI. All trials were videotaped and scored for appetitive behavior using a sampling method.

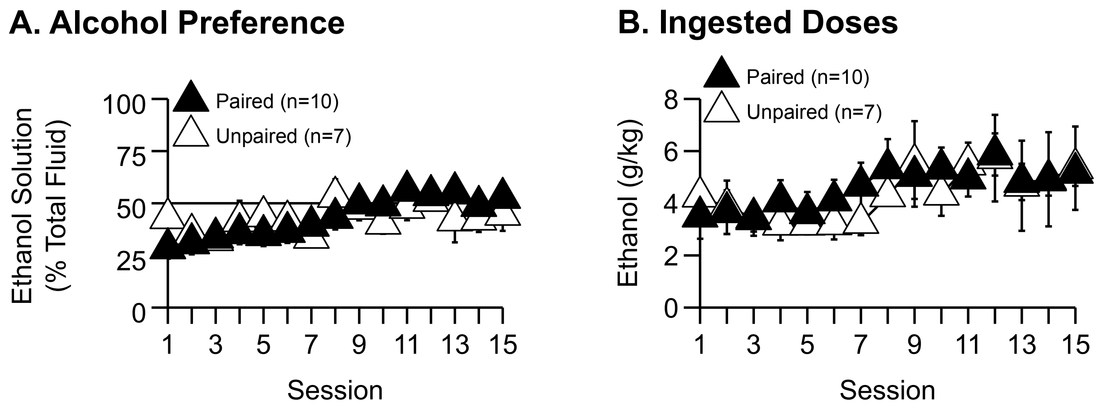
Post-Conditioning Phase

0.5 hr homecage two-bottle choice sessions (15% ethanol v/v in tap water or tap water) for 8 days (1 session/day). Two water bottles provided before and after sessions. Ethanol and water bottle placement on cage top alternated across sessions.

Estrous Cycle Tracking Phase

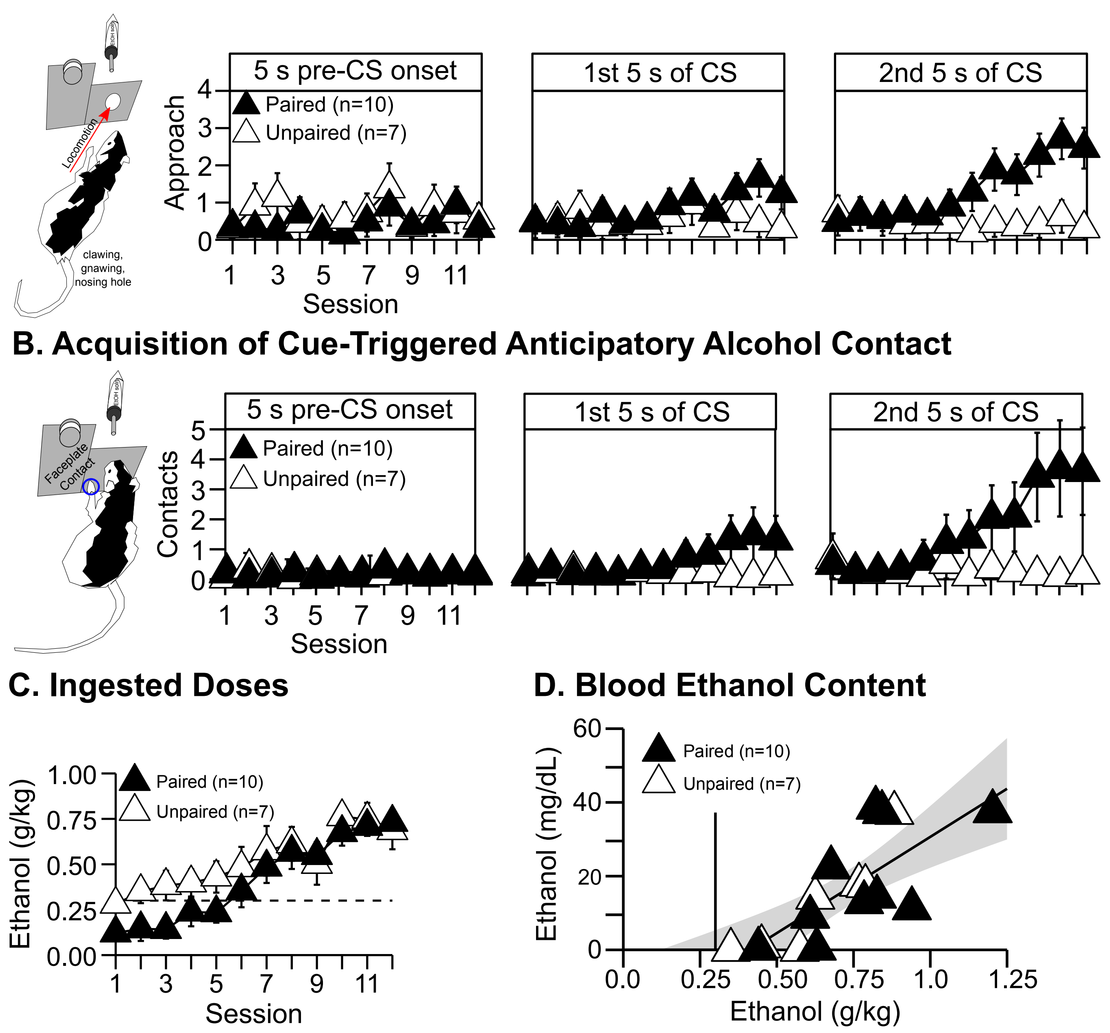
0.5 hr homecage two-bottle choice sessions (15% ethanol v/v in tap water or tap water) for 8 days (1 session/day). Two water bottles provided before and after sessions. Ethanol and water bottle placement on cage top alternated across sessions. 18 hr before every session, group Lavaged underwent vaginal lavage for estrous cycle stage determination. Group Control did not receive additional handling. Estrous cycle stage was determined by using light microscopy to visualize and identify cell types in each lavage sample.

Figure 1. Pre-Conditioning



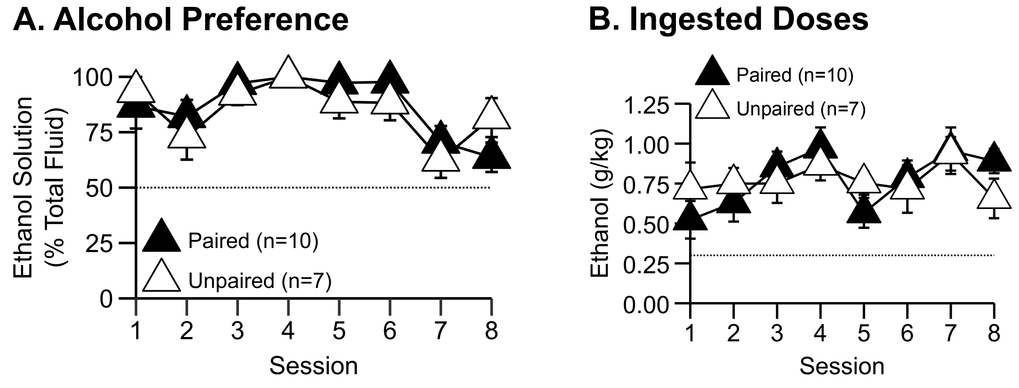
Group mean±sem on rats' fluid intake from the ethanol bottle as a percentage of total fluid intake (ethanol bottle plus water bottle) across 24 hr homecage two-bottle choice sessions (Panel A) and ingested ethanol doses for the same (Panel B). Panel A-B: Black and white-filled triangles represent rats trained with the CS explicitly paired and unpaired with ethanol sipper access, respectively. The CS was never presented.

Figure 2. Conditioning



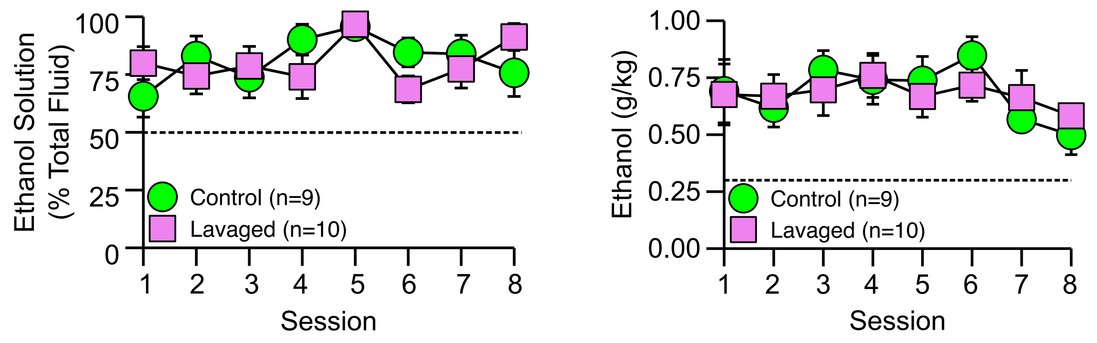
Group mean±sem on rats' mean frequency of sipper site approach state (Panel A) and sipper site contacts (Panel B) across 8 trials per session paneled by phases of houselight illumination (conditional stimulus; CS). Panel C: Group mean±sem on rats' total ingested dose per session. Panel D: Relationship between ethanol detected in blood 10-15 min after a conditioning session and ingested dose in that session. Ethanol level was determined via GC-FID. Blood sampled from lateral saphenous vein. Panel A-D: Black and white-filled triangles represent rats trained with the CS explicitly paired and unpaired with ethanol sipper access, respectively.

Figure 3. Post-Conditioning



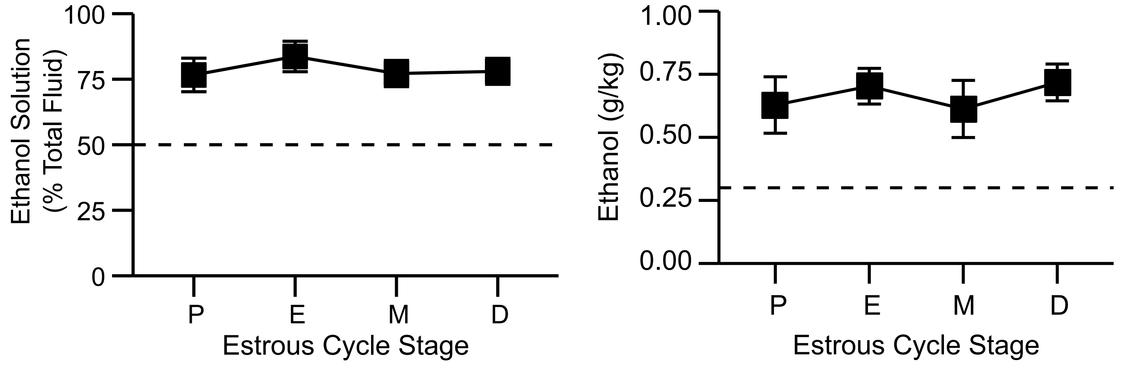
Group mean±sem on rats' fluid intake from the ethanol bottle as a percentage of total fluid intake (ethanol bottle plus water bottle) across 0.5 hr homecage two-bottle choice sessions (Panel A) and ingested ethanol doses for the same (Panel B). Panel A-B: Black and white-filled triangles represent rats trained with the CS explicitly paired and unpaired with ethanol sipper access, respectively. The CS was never presented.

Figure 4. Estrous Cycle Tracking



Group mean±sem on rats' fluid intake from the ethanol bottle as a percentage of total fluid intake (ethanol bottle plus water bottle) across 0.5 hr homecage two-bottle choice sessions (Panel A) and ingested ethanol doses for the same (Panel B). Panel A-B: Pink-filled squares represent rats that were subject to vaginal lavage 18 hr before the session and green-filled circles represent rats that did not get additional handling.

Figure 5. Estrous Cycle



Panel A: Black-filled circles represent mean±sem across rats' fluid intake from the ethanol bottle as a percentage of total fluid intake (ethanol bottle plus water bottle) in 0.5 hr homecage two-bottle choice sessions on average across stages of the estrous cycle. Panel B: Ingested ethanol doses for the same. Panel A-B: Cycle stage determined 5 hr post session based on cell types in lavage sample.

Conclusions

1. Cue-triggered alcohol-seeking states in female rats reflect associative learning & memory.

2. Conditioning may have increased preference for the ethanol bottle, and established a preferred ethanol dose level. However, a controlled study with detailed measurement of free-choice drinking before and after conditioning is needed.

4. Estrous tracking did not affect drinking the next day, so it may be possible to track estrous across all phases of future experiments without affecting alcohol-related behavior.

5. Drinking did not appear to be a function of estrous cycle stage.

Acknowledgements

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