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Evaluating the impact of concurrent sucrose availability on operant ethanol self-administration in male and female Long Evans rats

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Abstract

Investigating how environmental factors, such as the availability of non-ethanol alternative reinforcers, influences ethanol self-administration is critical for understanding the pathology of alcohol use disorder (AUD). Here we established the first operant choice paradigm that leverages the strengths of the sipper tube self-administration model to investigate how concurrent access to sucrose altered ethanol self-administration in male and female Long Evans rats. Choice behavior was examined using two distinct paradigms, including a novel adaptation of the response requirement paradigm. Under both a fixed-ratio or response requirement paradigm, we observed that concurrent availability of an alternative reinforcer significantly reduced appetitive and consummatory ethanol drinking-related behaviors. Furthermore, we assessed the sensitivity of the response requirement choice paradigm by administering the pharmacological stressor yohimbine and by altering the taste of the ethanol solution. Yohimbine administration non-selectively increased ethanol and sucrose intake, but not seeking, while taste adulteration decreased ethanol seeking and intake. These experiments demonstrate the utility of two concurrent choice paradigms that can more accurately capture AUD-like phenotypes, such as ethanol-directed choice in the face of alternative reinforcers. Future studies should investigate how models of vulnerability and dependence alter ethanol choice behavior under these paradigms.

Keywords

Choice; Ethanol; Self-administration; Sex differences; Sucrose

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CRediT authorship contribution statement

Olivia A. Ortelli: Writing – original draft, Visualization, Software, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jeffrey L. Weiner:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.addicn.2025.100196.

Introduction

It has been well established that alcohol use has deleterious effects on individual health and society, both among individuals who drink alcohol yet are not diagnosed with alcohol use disorder (AUD) and even more so among those with an AUD diagnosis [1–3]. Research identifying the precise mechanism(s) driving how and why individuals decide to consume alcohol remains incomplete. Specifically, there is a gap in the literature regarding how the brain encodes environmental stimuli and how such information changes alcohol-related behaviors. This is a particularly important area of study, as environmental factors, such as low constraints (i.e., low cost and/or high availability) on alcohol as well as high constraints (i.e., high costs and/or low availability) on alternative, non-alcohol alternatives have a significant influence on AUD (as reviewed in [4,5]). For example, individuals with a greater proportion of alcohol-related reinforcement are more likely to have more heavy drinking days, experience alcohol-related problems, develop AUD, and have worse treatment prognosis compared to those with a lesser proportion of alcohol-related reinforcement [6–8]. To this end, while it remains unknown why certain individuals develop AUD while others do not, it has been reported that people with higher scores on the Alcohol Use Disorder Identification Test also have a greater proportion of alcohol-related reinforcement [9] and we posit that this behavioral phenotype is driven by neurobiological adaptations that are shaped by environmental contingencies.

Specifically, others have contended that the pathological change in behavior that results in symptoms of substance use disorders, broadly, can be explained by greater salience being attributed to the drug-related cues and a reduced ability of non-drug alternatives to serve as effective reinforcers, a phenomenon known as salience misattribution [10]. To this end, there exists evidence of neurobiological differences among those with or without a SUD relating to the processing of both drug- *and* non-drug related cues. For example, numerous human neuroimaging studies have reported that individuals who engage in heavy alcohol drinking have greater subjective responding and reactivity of cortical and subcortical structures for alcohol cues and blunted responses for non-drug rewards compared to healthy controls [11–15]. Taken together, multiple domains of research have established that the presence and accessibility of alternative reinforcers is critical in preventing and treating AUD/heavy drinking [16–18], while the development of AUD results in neurobiological changes that effect salience encoding. However, little is known regarding the causal roles of the brain regions and circuitry that drive these maladaptive changes in behavior.

To this end, preclinical operant paradigms provide an excellent approach to examine how subjects learn and maintain self-administration behaviors for ethanol (i.e., alcohol) and other reinforcers, while simultaneously providing an opportunity to investigate the neural mechanisms driving these behaviors. One particularly well-validated operant ethanol self-administration paradigm is the sipper model [19]. Briefly, rodents are trained to complete a response requirement in order to earn 20 min of uninterrupted access to a sipper tube. Particular strengths of this model include the ability to examine seeking behaviors without the pharmacological effects of ethanol on board (including the sedative effects), as well as the ability to examine voluntary ethanol intake behaviors that are not interrupted or terminated by the experimenter, per se. Many insights have been examined from this model,

such as the unique contributions that specific brain regions and neural circuits have on driving seeking and intake behaviors for ethanol or sucrose solutions [20,21–24]. However, one important limitation is that all studies to date have compared the role of different manipulations on ethanol vs. sucrose behaviors across subjects, as rodents are only ever trained to self-administer one solution. This drastically contrasts with the human experience, as nearly all contexts in which a human can procure and consume alcohol occur where an alternative reinforcer is also available.

To address this knowledge gap, here we sought to modify the sipper model to allow for the investigation of choice behaviors. Choice procedures have enhanced face validity, as humans often have alternative reinforcers available and the individuals who continue to choose alcohol in spite of these alternatives are often the ones who would benefit from clinical and pharmacological interventions [25]. Choice paradigms also have greater sensitivity to potential therapeutic treatments [25]. Despite its translational utility, very few preclinical studies incorporate concurrent choice into their operant paradigms and even fewer have used sucrose, rather than water, as the alternative reinforcer [26–27]. Therefore, it would be particularly important to have an operant paradigm that leverages the strengths of the sipper model while also allowing for within subject analyses when subjects have access to *both* ethanol and sucrose.

Here, we characterized two operant procedures using sipper tubes as the delivery method. We examined how self-administration behaviors changed when only an ethanol-containing solution (10 % ethanol + 2 % sucrose) *or* sucrose (2 %) were available compared to when both solutions were concurrently available. The first procedure was a fixed ratio (FR) 5 schedule that allowed for 15 s of sipper access and an unlimited number of reinforcements earned (Experiment 1), while the second procedure was a variation of the sipper model in which subjects completed a single fixed ratio of 10 (the response requirement) to earn 20 min of access to either or both solutions (Experiment 2). In both experiments, we hypothesized that the concurrent availability of sucrose would decrease ethanol self-administration. To assess the ability of this variation of the sipper model to detect changes following specific experimental manipulations (i.e., paradigm sensitivity), we conducted two additional studies with the same subjects to evaluate how the pharmacological stressor yohimbine (Experiment 3) and manipulating the taste of the ethanol-containing solution (Experiment 4) would alter choice behavior. We hypothesized that yohimbine administration would selectively heighten ethanol seeking and intake behaviors while eliminating sweetness from the ethanol-containing solution would specifically decrease ethanol seeking and intake behaviors.

Methods and materials

Subjects

We assessed operant choice behavior under two different schedules of reinforcement using 24 Long Evans rats (n = 12/sex), with all subjects undergoing all paradigms and manipulations. Rats were aged 21 days upon arrival, with males and females weighing approximately 34.0 ± 1.45 g and 32.9 ± 0.97 g, respectively (Inotiv Laboratories, Indianapolis, IN). All rats were group housed upon arrival (n = 4/cage) for two weeks, until

PND 35 at which time all subjects were single housed. During these two weeks, rats were handled daily. All rats were single housed and maintained on a standard 12-hour light/dark cycle (lights on at 6am). Standard rodent chow (Prolab RMH 3000 Lab Diet 5P00 obtained from Lab Supply, Durham, NC, USA) and water were available *ad libitum* at all times. All animal care procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Wake Forest Institutional Animal Care and Use Committee.

Operant self-administration

Daily (Monday-Friday) operant sessions were performed in commercially available, sound-attenuated Med Associates chambers (Med Associates, East Fairfield, VT) as previously described [28]. Importantly, all chambers were equipped with one lever and retractable sipper tube available on each side wall of the operant chamber. A cue light (ENV-221 M) was housed above the left-side sipper tube while a house light (ENV-215 M) was housed above the right-side sipper tube. Counterbalanced across subjects, one sipper tube was paired with sucrose-only solutions while the other sipper tube was always paired with ethanol-containing solutions. The system was computer controlled and all responses were collected at 2 Hz with MedPC software (Med Associates, St. Albans, VT). Operant self-administration sessions were completed during the first 2 h of the light cycle. At the beginning of each session, the light source associated with the respective active-sipper tube was illuminated and the respective retractable lever extended into the chamber. First, rats were trained on a fixed-ratio (FR) schedule of reinforcement with sipper access being available every other day (e.g., day 1 sucrose-only training, day 2 ethanol training, day 3 sucrose-only training, etc.). Over the span of 22 sessions, the FR was increased from 1 to 5, the sipper duration access was decreased from 10 min to 15 s, the session length was decreased from 2 h to 30 min, and the 10 % sucrose solution was diluted to 2 % sucrose on sipper 1 and 2 % sucrose mixed with 10 % ethanol on sipper 2 (Table S1). Importantly, all subjects had *ad libitum* water access in their home cage when they were not completing their daily operant session.

After subjects were trained, Experiment 1, in which we examined how self-administration behaviors were altered when only one solution was available compared to when both solutions were concurrently available under a FR schedule, commenced (Fig. 1A). All subjects completed 10 sessions of FR no choice sessions (5 sessions/side, all subjects earned at least one reinforcer/session). Next, rats had two days to habituate to FR choice sessions, in which both the house light and cue light were illuminated at the session start and both levers extended into the chamber. Remaining under a FR5 schedule on each lever, rats could complete an unlimited number of FR completions (operationally defined hereon as a reinforcer earned) on both levers within the 30 min session duration. Lever presses on a specific lever did not have to be continuous. Once a FR was completed, both levers retracted and the appropriate sipper extended into the chamber for 15 s, after which both levers re-entered the chamber and another FR5 was required to yield access to a sipper tube. Six additional FR choice sessions were conducted, during which all rats earned at least one reinforcer. To further quantify appetitive strength, a variation of the extinction probe trial (EPT; [29]) was conducted such that both sippers were hung outside the chamber as normal,

both lights illuminated and levers extended into the chamber at the beginning of the session, however, no number of lever presses resulted in procurement of either sipper tube. Another FR choice session was completed the next day to determine whether self-administration behaviors were altered following the choice EPT.

Next, we began training for Experiment 2, such that rats learned how to complete a single FR to earn 20 min of access to either sipper tube (a procedure often referred to as the response requirement; see [19]. Briefly, subjects had 20 min to complete a response requirement and doing so resulted in 20 min of uninterrupted access to a sipper tube. Failure to do so resulted in termination of the session and no solution was procured that day. When transitioning to this schedule, all rats successfully completed a response requirement of 8 to earn 20 min of uninterrupted access to 2 % sucrose on the first day of response requirement training and thus were advanced to a response requirement of 10 to earn 20 min of uninterrupted access to 2 % sucrose + 10 % ethanol the following session. All rats had two additional sessions to habituate to the response requirement 10 schedule (1 session/solution), during which time all subjects demonstrated an ability to complete the response requirement for each solution. Next, an additional 10 response requirement no choice sessions were completed (5 sessions/solution). All rats completed the response requirement for ethanol on at least 4/5 no choice sessions. All rats completed 100 % of sucrose no choice baseline sessions excluding two males who only completed 1 and 0 no choice sucrose baseline sessions, after successfully completing the response requirement on the two training no choice sessions preceding baseline (Fig. S1).

Like Experiment 1, rats had two sessions to acclimate to response requirement choice sessions, during which both levers extended into the chamber and completion of a response requirement results in 20 min of access to that sipper tube. If the other response requirement was later completed, an additional 20 min of access was provided to the newly earned solution. If one response requirement wasn't completed within the first 20 min, the respective lever was retracted. If neither response requirement was completed within the first 20 min, the session was terminated and no solution was procured. Subjects all completed 6 response requirement choice sessions, an EPT, and another response requirement choice session. Individual completion data for the response requirement choice sessions can be observed in Fig. S2.

Experiment 3 examined whether 2 mg/kg yohimbine, a dose commonly used to investigate stress-induced reinstatement of ethanol seeking [30], would alter operant choice behavior under the response requirement paradigm (Fig. 4A). Subjects were acclimated to one week of sterile water i.p. injections 30 min prior to the operant choice session. The next week, rats received a 2 mg/kg i.p. yohimbine injection 30 min prior to the operant choice session on either Tuesday or Thursday (counterbalanced across subjects), and sterile water (vehicle) on all other days. The following Tuesday, rats received either 2 mg/kg i.p. yohimbine or sterile water prior to an EPT choice session. The other condition was then administered two weeks later. Following both EPTs, a normal response requirement choice baseline session was conducted 30 min following an i.p. vehicle injection.

Following Experiment 3, we examined how removing the sucrose from the ethanol solution would alter choice behavior and EPT responding (Experiment 4; Fig. 5A). After 5 sessions off, rats returned into the chambers with only 2 % sucrose available on lever 1 and 10 % ethanol available on lever 2. After 3 days of habituation to these new solutions, 6 response requirement choice sessions were completed followed by an EPT choice session. For the last session, subjects completed a response requirement choice session with 10 % ethanol + 2 % sucrose re-introduced. We hypothesized that rats would be able to identify this solution due to olfaction cues; however, in the case that this was not true, if the response requirement of either solution wasn't completed within 20 min then the sipper was non-contingently entered into the chamber to assess intake behaviors among these subjects with low appetitive drive. All subjects were then euthanized the next morning around the time at which subjects would typically complete operant sessions and brains were harvested and flash frozen.

Drugs

Ethanol solutions were prepared using 95 % ethanol diluted with water to a 10 % (v/v) concentration with the appropriate concentration of sucrose as the solute. Yohimbine HCl (Sigma–Aldrich, St. Louis, MO) was prepared with sterile water at doses of 2 mg/kg body weight in a 2 mg/ml injection volume.

Blood ethanol concentrations

Throughout Experiments 1 and 2, tail bloods were taken to calculate blood ethanol concentrations (BECs) with a commercially available ethyl alcohol enzymatic assay (Carolina Liquid Chemistries Corporation, Brea, CA). Tail bloods were collected 10 min following the completion of the session following one FR no choice session, one FR choice session, one response requirement no choice session, and one response requirement choice session.

Data analyses

Each day, raw MedPC files were uploaded into custom-made R scripts created using the functions available in the R package *medparser* [31] to export appetitive and consummatory variables from the daily session. The majority of our statistical analyses were completed using GraphPad Prism (version 10.2.3). Repeated-measures (RM) three-way ANOVAs were used to determine the main effects of, and interactions among, choice (no choice vs. choice), solution (ethanol vs. sucrose) and sex (male vs. female). When comparing intake (g/kg), RM two-way ANOVAs were evaluated for each solution, as the g/kg is calculated differently for ethanol and sucrose. Sidak's multiple comparison's post-hoc analyses were used when appropriate. Student's *t*-tests were used to assess for sex differences in average percent ethanol choice assessed using the FR choice paradigm (100 * (ethanol FR completions / total FR completions)) or the choice EPT (100 * (ethanol lever presses / total lever presses)). If the assumption of normally-distributed residuals was violated, as assessed using the Shapiro-Wilk test ($p > .05$), then the Mann-Whitney test was used instead. Descriptive statistics are reported as mean ± SEM.

To identify differences in licking microstructures as a function of sex (male vs. female), solution (ethanol vs. sucrose), and yohimbine (vehicle vs. yohimbine), we created cumulative lick curves for each rat by calculating the cumulative licks completed per minute of the 20-minute drinking session. This approach allowed us to examine how rats spent time drinking, regardless of total volume consumed. To determine if licking microstructure changed across session time (minute 0 representing the time at which the sipper extended into the chamber upon completion of the response requirement) while controlling for idiosyncratic individual differences, likelihood ratio tests (LRTs) (using the Kenward-Roger method for calculating degrees of freedom) were used to compare the full (fixed effects: sex, session, sex × session; random effect: subject) and partial (fixed effect: sex; random effect: subject) mixed effects models. The lme4 [32] and pbkrtest [33] packages in R (version 4.3.1) were used to conduct mixed effects models and LRTs, respectively.

Results

Experiment 1: fixed ratio

Our first aim was to determine how self-administration behaviors change when subjects only have availability of one solution compared to when both ethanol and sucrose are concurrently available. When assessing the average number of reinforcers earned, we report a significant main effect of choice ($F(1, 22) = 56.60$, $p < .0001$), indicating that the number of ethanol and sucrose reinforcers earned during choice sessions was significantly less than the number of respective reinforcers earned during no choice sessions (Fig. 1B). We also report a significant solution x choice interaction ($F(1, 22) = 12.72$, $p = .002$) (Fig. 1B). When comparing choice and no choice sessions, we observed a greater reduction in sucrose reinforcers earned than ethanol (average difference, collapsed across sex: 5.53 vs 2.32, respectively). Interestingly, we did not observe significant differences between the number of ethanol vs. sucrose reinforcers earned during no choice sessions among males ($p_{adj} > 0.99$) or females ($p_{adj} > 0.99$), suggesting that when only given the option to procure one solution, rats will expend similar effort to drink either solution. Overall, we did observe that rats earned more total reinforcers during choice sessions compared to no choice sessions ($F(1.70, 37.42) = 4.11$, $p = .03$), perhaps suggesting greater engagement. To this end, rats consumed significantly more total volume (mls) in choice sessions compared to no choice sessions ($F(1, 22) = 95.36$, $p < .0001$), with no observed sex differences ($F(1, 22) = 0.23$, $p > .05$).

Next, we used two-way RM-ANOVAs to assess changes in intake of 10 % ethanol or 2 % sucrose. For ethanol intake, we report a significant main effect of choice ($F(1, 22) = 18.70$, $p < .001$) (Fig. 1C). Likewise, we report a main effect of choice when evaluating sucrose intake (g/kg) ($F(1, 22) = 38.32$, $p < .0001$) (Fig. 1D). There was no main effect of sex nor a significant sex x solution interaction ($ps > 0.05$). Blood ethanol concentrations (BECs) were collected on a representative no choice and choice session. We observed a significant effect of choice ($F(1, 21) = 11.61$, $p < .01$), such that BECs were higher during no choice (72.1 ± 16.45) vs. choice (43.0 ± 0.93) sessions (Fig. 1E). Males consumed 1.25 ± 0.171 g/kg and 0.87 ± 0.112 g/kg, while females consumed 1.25 ± 0.151 and 1.09 ± 0.136 g/kg ethanol, on the no choice and choice sessions when BECs were collected, respectively.

To further analyze how subjects allocated behavior during choice sessions, we calculated the percentage of ethanol reinforcers earned across the choice sessions (100*(ethanol reinforcers earned / total reinforcers earned)). The Mann-Whitney test did not reveal a difference in the average percent of ethanol reinforcers earned when comparing females (median = 74.03 %) and males (median = 71.97 %) (*Mann-Whitney U* = 63, $p = .63$) (Fig. 2A). Interestingly, 7/12 males and 7/12 females allocated over 70 % of their behavior towards ethanol. Of the remaining 5 males, all split their responding between the 2 levers relatively evenly (range: 33.76–67.27 %). On the other hand, only 2 females performed within this range while the remaining 3 females had over 70 % of responding directed towards sucrose.

To quantify solution preference, within-subjects, within the same session, we next ran a variation of the EPT in which both levers were extended into the chamber and the total number of lever presses was recorded; however, no number of lever presses would result in procurement of either sipper tube. When assessing the role of solution (ethanol vs. sucrose) and sex (females vs. males) on lever pressing during the choice EPT, we observed a significant main effect of solution ($F(1, 22) = 11.27$, $p = .003$), but not sex ($F(1, 22) = 0.48$, $p = .49$), such that regardless of sex, subjects directed more lever pressing towards the ethanol-paired lever rather than the sucrose-paired lever. Indeed, 16 of the 24 rats (66.7 %) directed over 70 % of all lever presses towards ethanol (Fig. 2B). While we did not observe significant differences between percent of ethanol-directed lever pressing between males and females ($U = 0.67$, $p = .80$), we do note that 2/12 females directed over 70 % of responding towards sucrose while no males directed >50 % of responding towards sucrose. We report a significant, positive correlation between the average percentage of ethanol reinforcers earned during choice sessions and the percentage of ethanol-directed lever presses during the EPT (Spearman $r = 0.65$, 95 % CI = [0.32, 0.84], $p < .001$) (Fig. 2C).

To this end, we noticed high concordance between many variables obtained throughout the FR no choice, choice, and EPT sessions (Fig. 2D). For example, there was a positive correlation between the number of sucrose reinforcers earned during no choice sessions and the number of sucrose reinforcers earned during the choice sessions ($r = 0.63$, 95 % CI = [0.37, 0.85], $p < .001$). We also report negative correlations between the number of sucrose reinforcers earned during no choice sessions and the percentage of ethanol choice during choice sessions ($r = -0.47$, 95 % CI = [− 0.80, − 0.24], $p < .01$) as well as with the percentage of ethanol-directed lever presses during the EPT ($r = -0.50$, 95 % CI = [− 0.82, − 0.29], $p = .001$).

Experiment 2: response requirement

Rats were then trained to complete a response requirement of 10 on one lever to earn 20 min of access to ethanol and a response requirement of 10 on a second lever to earn 20 min of access to sucrose (Fig. 3A). First, rats completed a single response requirement for one solution, alternating daily for 10 days (5 sessions per solution) (e.g., response requirement no choice). Next, both levers were extended into the chamber and rats could complete both response requirements if desired (e.g., response requirement choice). Most rats consistently earned access to both solutions by completing 10 lever presses on both levers (Fig. S2). When examining total mls. consumed, we observed a significant main effect of choice ($F(1,$

22) = 69.96, $p < .0001$) and sex ($F(1, 22) = 5.30$, $p = .03$), such that males consumed more total volume than females across both session types and both males and females increased total volume consumed (Fig. 3B). Despite drinking more total volume, subjects consumed significantly less ethanol (g/kg) during response requirement choice sessions compared to the no choice sessions ($F(1, 22) = 21.87$, $p < .001$) (Fig. 3C). We did not observe a significant main effect of sex ($F(1, 22) = 0.17$, $p = .67$). Likewise, we observed a significant main effect of choice when comparing BECs collected on a representative no choice vs. choice session, with lower BECs observed during the choice session ($F(1, 20) = 30.74$, $p < .0001$). We also observed a significant main effect of sex, with males having greater BECs than females regardless of session type ($F(1, 20) = 8.76$, $p < .01$). During the no choice session when bloods were collected, males and females consumed 1.22 ± 0.128 g/kg and 1.22 ± 0.133 g/kg ethanol, respectively. During the choice session when bloods were collected, 0.69 ± 0.182 g/kg and 0.69 ± 0.118 g/kg ethanol, respectively. When examining sucrose intake, we also observed a significant main effect of choice ($F(1, 22) = 54.09$, $p < .0001$) and no main effect of sex ($F(1, 22) = 1.55$, $p = .23$), such that males and females both significantly decreased sucrose intake (g/kg) upon the concurrent availability of both solutions (Fig. 3D). These data are consistent with the FR data.

To investigate the relationship between appetitive and consummatory variables during response requirement choice sessions, we evaluated whether the solution that subjects earned access to first influenced their overall session intake. Rats were relatively consistent in what solution they procured first, with 11/24 (54.5 % female) subjects earning ethanol first on the majority of their completed choice sessions, 11/24 (54.5 % female) subjects earning sucrose first, and 2/24 (100 % male) subjects completing sucrose and ethanol on half of the baseline sessions (Fig. S3). When comparing average ethanol intake across the 6 choice sessions, we observed that the rats who completed ethanol first on most sessions had significantly higher average ethanol intake than the rats who completed sucrose first on most sessions ($t(20) = 3.56$, $p = .002$) (Fig. 3E). Likewise, we observed that the rats who completed sucrose first on most sessions had significantly higher average sucrose intake than the rats who completed ethanol first on most sessions ($U = 9$, $p < .001$) (Fig. 3F).

We then conducted another choice EPT. Unlike the choice EPT that followed the FR choice sessions, 50 % of rats allocated between 30 and 70 % of lever pressing towards ethanol whereas most rats allocated over 70 % of lever pressing towards ethanol before. We did not observe any sex differences in the percentage of ethanol lever pressing during the EPT following the response requirement choice sessions ($t(22) = 0.66$, $p = .52$) (Fig. 3G). While ethanol-directed lever pressing during the EPT changes, it does appear that this change was systematic with response requirement choice behavior as we report a significant positive correlation between the total number of ethanol-directed lever presses that occurred across the 6 response requirement choice sessions before the choice EPT and the percentage of ethanol-directed lever presses during the choice EPT, such that the rats who did not meet the response requirement for ethanol across all 6 choice sessions (e.g., < 60 lever presses; $n = 12$) also had <50 % ethanol-directed responding during the choice EPT (Spearman $r = 0.63$, 95 % CI = [0.29, 0.83], $p = .001$).

Given that most studies using the response requirement paradigm with a single reinforcer have reported no correlation between average intake and subsequent EPT responding ([29,34–36]), we evaluated the correlation between intake of ethanol or sucrose with percent ethanol EPT responding. We observed a significant positive correlation between average ethanol intake across baseline choice sessions and percent ethanol responding during the choice EPT ($r(22) = 0.48$, $p = .02$) and a significant negative correlation between average sucrose intake across baseline choice sessions and percent ethanol responding during the choice EPT ($r(22) = -0.53$, $p = .008$).

Experiment 3: yohimbine

To determine whether a pharmacological stressor might alter appetitive or consummatory behaviors during the concurrent choice variation of the sipper model, we administered (i.p.) 2mg/kg of the alpha-2 antagonist, yohimbine (Fig. 4A). All but one rat completed the ethanol response requirement during both the vehicle and yohimbine sessions. A subset of rats didn't complete the sucrose response requirement during the vehicle session ($n = 2$; 1/sex), yohimbine session ($n = 1$ female), or either session ($n = 2$; 1/sex). Ethanol intake significantly increased when comparing vehicle vs. yohimbine choice sessions ($F(1, 22) = 12.38$, $p = .002$), with no main effect of sex observed ($F(1, 22) = 0.0003$, $p = .99$) (Fig. 4B). We also observed an increase in sucrose intake (yohimbine: $F(1, 22) = 6.27$, $p = .02$), with no significant main effect of sex ($F(1, 22) = 4.00$, $p = .058$) (Fig. 4C). We did not observe any significant yohimbine x sex interactions ($ps > 0.05$). Yohimbine's non-specific increase in intake was also demonstrated by a significant increase in total volume (mls) consumed ($F(1, 22) = 25.90$, $p < .0001$). To evaluate whether there was a yohimbine x solution interaction, we assessed time spent drinking (operationally defined as cumulative time, in seconds, that contact was being made with the sipper tube). We observed a significant main effect of solution ($F(1, 22) = 10.00$, $p < .01$), such that subjects spent more time drinking sucrose than ethanol. We also observe a significant main effect of yohimbine ($F(1, 22) = 31.15$, $p < .0001$), but no significant main effect of sex nor any significant interactions (Fig. 4D).

Given this non-specific increase in intake, we next examined whether yohimbine resulted in solution-specific alterations in lick patterns. Yohimbine resulted in an increase in lick bouts (operationally defined as approach to sipper tube with at least 20 s of no activity on that sipper) ($F(1, 22) = 29.73$, $p < .0001$) (Fig. 4E). We also observed a main effect of solution ($F(1, 22) = 17.99$, $p < .001$) and no main effect of sex nor any significant interactions ($ps > 0.05$). When observing lick rate among the rats who completed the response requirement across both sessions (operationally defined as licks/total time spent making contact with sipper (min.)), we report significant main effects of sex ($F(1, 22) = 6.02$, $p = .02$), solution ($F(1, 22) = 8.37$, $p < .01$), and yohimbine ($F(1, 22) = 12.44$, $p < .01$) (Fig. 4F). Regardless of sex, lick rates for ethanol (311.1 ± 17.62) were greater than those for sucrose (274.7 ± 18.51) and for both solutions, females had greater lick rates than males. For both sexes and both solutions, yohimbine resulted in decreased lick rates.

To further visualize how yohimbine altered consummatory behaviors, cumulative lick curves were generated for each completed consummatory phase. Here, we can appreciate that

rats complete most of their ethanol consumption during the first few minutes of sipper presentation while sucrose consumption occurs at a steadier pace throughout the entire 20 min of access (Fig. 4G). Indeed, the LRT comparing the full model with sex, yohimbine, solution, and time as main effects (subject as random effect) with the partial model omitting solution was significantly different ($p < .0001$), suggesting different lick patterns across both solutions, regardless of sex or yohimbine administration. Further analyses identify an important role of solution. While males and females had comparable lick patterns when consuming sucrose, females had steeper cumulative lick curves for ethanol compared to males (LRT with full model including all main effects and sex x solution interaction vs. partial model omitting interaction: $p < .0001$). Regardless of sex and solution, yohimbine resulted in a further blunting of the cumulative lick curves (LRT with full model including all main effects vs. partial model omitting yohimbine: $p < .0001$). It appears that yohimbine's effects on licking patterns more robustly impacted ethanol drinking (LRT comparing a full model including all main effects and the yohimbine x solution interaction compared to a partial model omitting this interaction: $p < .0001$); however, this may be due to a floor effect as vehicle sucrose drinking was already very consistent across the 20 min of drinking access.

So far, these data suggest that yohimbine is primarily altering intake but not seeking processes. To further test this hypothesis, all subjects completed additional EPTs following a vehicle or yohimbine i.p. injection, administered in a counterbalanced order. When assessing percent of ethanol-directed lever pressing during the choice EPTs, a two-way ANOVA revealed a significant sex x yohimbine interaction ($F(1, 22) = 5.25$, $p = .03$). Follow-up multiple comparisons tests did not identify significant sex differences after correcting for multiple comparisons; however, females treated with vehicle tended to have less ethanol-directed lever pressing (47.8 % ± 9.35 %) compared to females treated with yohimbine (55.8 % ± 6.93 %) and males treated with vehicle (60.9 % ± 7.23 %) or yohimbine (57.0 % ± 7.71 %) (Fig. 4H). Taken together, these data suggest that yohimbine administration robustly alters consummatory processes, with a particularly strong effect on ethanol licking microstructures, without dramatically altering seeking behaviors.

Experiment 4: response requirement choice with unsweetened ethanol

Adulterating the taste of ethanol is a commonly used approach to assess ethanol-related "consequences". Rather than adding quinine, a bitter tastant, to the ethanol solution, we assessed how choice behavior would change when subjects were given the option to self-administer 2 % sucrose and 10 % ethanol, rather than a 10 % ethanol + 2 % sucrose solution. To determine if removing the sweetness from the ethanol solution would alter choice behavior, we compared the average intake of each solution across 6 recent sweetened ethanol + sucrose choice sessions and subsequent unsweetened ethanol + sucrose choice sessions (Fig. 5A). We observed a significant main effect of sweetened ethanol availability ($F(2, 44) = 34.23$, $p < .0001$) (Fig. 5B). Males (− 68.02 % ± 6.26 %) and females (− 69.48 ± 5.18 %) showed dramatic reductions in the percent change of ethanol intake when the ethanol was not sweetened. However, intake returned back to (Sidak's post-hoc tests in females: mean difference = _ 0.03, 95 % CI = [− 0.37, 0.31], $p_{adj} = 0.99$) and even surpassed (Sidak's post-hoc tests in males: mean difference = 0.35, 95 % CI = [0.02, 0.69], $p_{adj} = 0.04$)

sweetened ethanol intake during the single session when 2 % sucrose was re-introduced into the ethanol solution following the 6 sessions of unsweetened ethanol self-administration. The change in the taste of the ethanol solution also altered sucrose intake ($F(2, 40) = 4.86$, $p = .01$) (Fig. 5C). While post-hoc analyses failed to identify significant differences across the three session types, we observed numerical *reductions* in 2 % sucrose intake during the single session when sweetened ethanol was reintroduced, compared to the average sucrose intake observed during the six choice sessions preceding this session with the alternative solution of unsweetened ethanol among both females (mean difference = − 0.13, 95 % CI = [− 0.28, 0.02], $p_{adj} = 0.11$) and males (mean difference = − 0.14, 95 % CI = [− 0.30, − 0.02], $p$adj = 0.10)

To assess how altering the taste of the ethanol solution would affect the motivation to procure it, we conducted a final choice EPT. A percent-change analysis revealed one extreme outlier (ROUT method; $Q = 0.5$ %) so the data for this subject were excluded. We observed a significant reduction in the percentage of ethanol-directed lever presses ($F(1, 21) = 17.76$, $p < .001$), as well as a significant sex x sweetened ethanol availability interaction ($F(1, 21) = 5.77$, $p = 03$) (Fig. 5D). Specifically, we observed a greater reduction in the percent of ethanol-directed lever pressing among females (% change = − 35.95 % ± 14.29) compared to males (% change = − 8.52 % ± 6.06 %). Indeed, post-hoc analyses revealed a significant reduction in ethanol-directed lever pressing for females ($p_{adj} < 0.001$) but not males ($p_{adj} = 0.37$).

Discussion

The current experiments are the first to demonstrate that male and female Long Evans rats can readily acquire an operant self-administration paradigm for ethanol and a non-drug reward using the sipper paradigm. Specifically, we report that under two different concurrent choice operant paradigms, concurrent availability of a non-ethanol alternative reinforcer was sufficient to decrease ethanol self-administration, replicating the findings of previous preclinical [26–27, 37–40] and clinical [7–8] studies. To further characterize the paradigm detailed in Experiment 2, Experiment 3 extended our understanding of how acute stress may alter self-administration behaviors by revealing that yohimbine administration resulted in increased ethanol and sucrose intake without altering seeking behaviors. Finally, Experiment 4 further highlighted how altering the taste of the ethanol-containing solution can alter both appetitive and consummatory behaviors.

Experiment 1 detailed the first experiment to conduct concurrent operant self-administration of ethanol and sucrose under a traditional FR 5:5 schedule of reinforcement using 15 s of access to a sipper tube per reinforcer. Here, we reported that rats would readily self-administer either ethanol or sucrose while only one was available (e.g., no choice), self-administration of both solutions significantly decreased when both solutions were concurrently available (e.g., choice sessions). We were surprised that during FR choice sessions, most rats allocated the majority (≥70 %) of their behavior towards the ethanol-containing solution despite there being no significant difference in procurements of ethanol vs. sucrose during no choice sessions. This finding highlights how the reinforcing strength of a reinforcer can change when environmental contingencies also change (e.g., no choice vs.

choice sessions). Future studies aim to further characterize this relationship by investigating how manipulating the price of one reinforcer alters self-administration of that reinforcer (own-price demand), as well as the self-administration of the concurrently available reinforcer (cross-price demand).

Our findings align with previous choice studies which have demonstrated that ethanol is a powerful reinforcing stimulus compared to other non-drug reinforcers, such as social interaction with other rats [37–38]. However, other choice studies comparing self-administration of an ethanol-containing solution to that of another oral reinforcer have not observed a comparable distribution of rats with a preference for ethanol [27,39,41]. Methodological differences might explain this incongruence. Specifically, these previous studies used saccharin (0.10–0.20 %), which is known to be orders of magnitude sweeter than equivalent sucrose concentrations, or higher concentrations of sucrose (4–12 %). Additionally, only Alaux-Cantin et al. (2021) used an ethanol-containing solution with a matched sucrose concentration as the alternative reinforcer. Our experimental design comparing ethanol-containing and non-containing solutions with the same concentration of sucrose may have reduced the influence of the inherent differences in sweetness between the two solutions. It is likely that increasing the concentration of sucrose would have decreased ethanol preference, especially given that the subjects used in the current study were not physiologically dependent on ethanol. Additional studies are also necessary to investigate how manipulating the concentration of ethanol would alter choice behavior.

An additional methodological difference that likely influences self-administration behaviors includes the experimenter-decided access to each reinforcer. In Experiment 1, we allowed for 15 s of access to the appropriate sipper tube per completion of the FR5. During this time, subjects were allowed to drink as much solution as they wanted. On average, rats complete ~100 licks per 15 s of access, which we estimate results in ~0.3–0.5 mls/reinforcers, significantly more than the typical 0.1 mls/reinforcer often used in self-administration studies. It is possible that allowing for greater consumption per reinforcer enhances the pharmacological effects of ethanol. Indeed, we report blood ethanol concentrations as high of 150 mg/dL further highlighting that subjects self-administering ethanol were in fact reaching intoxicating levels during daily sessions.

Next, we leveraged the strengths of the sipper-tube model [19] using a variation that allowed for the assessment of ethanol-directed choice behavior under a paradigm that permits “unlimited” solution availability for 20 uninterrupted minutes following the completion of 10 lever presses. Many insights have been gained from the sipper model, such as how taste, ethanol concentration [28,42], and stress [43–44] alter self-administration, as well as the neural mechanisms underlying discrete appetitive and consummatory ethanol and sucrose self-administration behaviors [20,21,23,24,45–50]. Despite these advances, no study has yet used this paradigm to assess concurrent self-administration of ethanol and a non-drug reinforcer within-subjects and within-session. Like Experiment 1, we observed that concurrent availability of ethanol and sucrose decreased intake of both solutions, despite that subjects had to expend the same amount of effort to procure either solution in no choice vs. choice sessions. Moreover, we observed that subjects consumed significantly more total volume during choice sessions, which informs our understanding of traditional no

choice sessions under this paradigm. Understanding why subjects terminate consummatory processes, as well as what neural mechanisms regulate consummatory processes during no choice sessions, and how these behaviors and mechanisms are altered during choice sessions will provide insights into our understanding of AUD symptoms, such as loss of control over drinking.

Following choice sessions in Experiments 1 and 2, we conducted a variation of the EPT [29] such that both levers extended into the chamber yet no number of lever presses resulted in any procurement of solution. Recently, we demonstrated that the EPT is strongly correlated with breakpoints derived from an across session progressive ratio schedule [36]; however, how EPT responding is related to measures of choice has yet to be empirically assessed. In Experiment 1, which used the FR5:5 schedule of ethanol/sucrose sipper tube access, we observed a significant positive correlation between percent of lever pressing directed towards ethanol during the EPT and average ethanol choice (%) completed during the choice sessions. Likewise, in Experiment 2 we observed a significant correlation between cumulative ethanol-directed lever pressing across the 6 choice sessions and the percent of ethanol-directed lever pressing during the EPT. We believe that it is a strength of the EPT to capture these congruent relationships across both experiments, with the same subjects yet different schedules of reinforcement, as the differences in schedule may have potentially influenced the associations between the lever and the sipper tube. Specifically, subjects only needed to complete 10 ethanol-directed lever presses per session during the response requirement choice sessions, which was much less than the mean number of daily ethanol-directed lever presses that occurred under the FR schedule (43.35 ± 3.28 ethanol-directed lever presses per FR session), which inherently links frequent lever pressing to sipper availability. The reduction in ethanol-directed lever pressing during the response requirement choice sessions might explain why the percent ethanol responding observed during the EPT was much higher in Experiment 1 compared to Experiment 2. However, it is also possible that the uninterrupted access to ethanol and sucrose during the response requirement sessions enhanced the reinforcing value of sucrose in some subjects. Taken together, these findings provide additional support for using the EPT as a single-session approach to quantify appetitive strength, as both breakpoint and percent choice (e.g., preference) are two of the most common measures of reinforcer value [51].

Following the EPT in Experiment 2, we assessed whether EPT responding was related to average intake behaviors observed during the preceding choice sessions. Historically, there has not been an observed relationship between EPT responding and ethanol intake the day before the EPT [52]. Moreover, we recently reported non-significant correlations between average ethanol or sucrose intake and EPT responding using a sample of both male and female Long Evans rats [36]. Interestingly, here we do report a significant, positive correlation between percent ethanol lever pressing during the EPT and average ethanol intake as well a significant, negative correlation between percent ethanol lever pressing during the EPT and average sucrose intake during the preceding choice sessions. While these data contrast with those obtained from EPTs used to assess the reinforcing efficacy of a single reinforcer, they are congruent with additional data collected in Experiment 2. Specifically, we observed that subjects who more often procured either solution first (e.g., shorter latency to complete) consumed significantly more of that solution compared

to subjects who were more likely to procure the other solution first. The contrasting findings further emphasize how environmental contingencies (e.g., no choice vs. choice) can alter self-administration behaviors. These results also add context to previously completed studies that reported a specific effect of a pharmacological manipulation on EPT responding without altering intake behavior [20,21,48,50,53]. Whether these results replicate in a choice paradigm remains to be tested and is critically important for an enhanced translational understanding of the neural processes underlying ethanol self-administration. Indeed, future studies investigating the neural mechanisms driving specific facets of appetitive vs. consummatory behaviors during the choice paradigm will be imperative to develop a greater understanding of how the brain informs decisions to self-administer ethanol in spite of the availability of alternative reinforcers.

In Experiment 3, we administered 2 mg/kg of yohimbine, an alpha-2 antagonist known to elicit anxiogenic effects in both humans and rodents [30]. We observed a significant increase in ethanol and sucrose intake, consistent with a previous study investigating the effects of yohimbine administration on ethanol self-administration using the sipper tube model [43]. These results also mirror a recently published paper from our lab which demonstrated that this same dose of yohimbine significantly increased ethanol intake in a unique non-operant self-administration paradigm designed to measure ethanol seeking with negative consequences [54]. Many studies have assessed the role of yohimbine on ethanol and sucrose reinstatement (e.g., lever pressing without procurement across multiple sessions) and have reported increases in responding for either reinforcer alone [55–56]. Moreover, others have observed a yohimbine-induced increase in sucrose (pellet) intake which also aligns with our results [57]. To our knowledge, the current study is the first to report a simultaneous increase in sucrose intake alongside ethanol intake. While alpha-2 antagonists can modulate the pharmacokinetic/pharmacodynamics profile of ethanol [58] the simultaneous effects of yohimbine on ethanol *and* sucrose consumption suggests that any changes in ethanol metabolism caused by yohimbine were likely not a driving factor of our results. Given that yohimbine recruits multiple neurotransmitter systems [59], the specific mechanism underlying the current findings remains unclear. While speculative, the specificity of our results to consummatory processes might suggest that yohimbine's effects on the orexin system might be driving our results [55]. This hypothesis can be tested in future studies by pre-treating subjects with an orexin antagonist.

In the current experiment, we were also able to detail the licking microstructures observed under vehicle and yohimbine conditions and observed that yohimbine administration resulted in steadier drinking throughout the entirety of the session. These effects were especially apparent in ethanol drinking, as front-loading (a pattern in which intake is skewed towards the onset of sipper access; see [60]) was specifically observed for ethanol during vehicle treatment, especially among female subjects. While it has previously been reported that female rodents engage in more front-loading behaviors than males [61], this is the first study, to our knowledge, to detail licking microstructures to this level of specificity and demonstrate solution-specific cumulative lick patterns. Moreover, we anticipate that the continued use of lick microstructure analyses in future studies will provide novel insights pertaining to how a manipulation might be altering consummatory behaviors. For example, the observed yohimbine-induced shift in lick patterns observed here highlights how this

acute stressor specifically altered subjects' ability to terminate drinking bouts. Importantly, all of the results discussed are specific to the single dose (2mg/kg) tested in this experiment. To fully understand how yohimbine alters choice behavior in this paradigm, future studies testing a full range of doses is necessary.

In our final experiment, we evaluated how removing the 2 % sucrose from the ethanol solution would alter choice behavior and choice EPT responding. We report that intake of the ethanol-containing solution significantly decreased when the sucrose was removed and this observation was reversed when the sucrose was reintroduced into the solution. Intake of the concurrently available sucrose solution followed the inverse trend as the ethanol-containing solution was manipulated. At this point in the experiment, all subjects had months of experience consuming a sweetened ethanol solution. Therefore, removing the sucrose from this ethanol-containing solution can be thought of as similar to the common approach of adding quinine, a bitter tastant, to an ethanol-containing solution. Much research has investigated how quinine adulteration impacts ethanol-directed behavior and the underlying mechanisms of these changes [62], which might also play a critical role in the transition from recreational to pathological alcohol drinking in humans. Interestingly, many studies using quinine as an ethanol deterrent demonstrate that females are more resistant to quinine's effects compared to males [63]. We did not observe a similar finding when altering the taste of the ethanol-containing solution by removing sucrose in the current study. While males and females continued to earn access to the sipper tube, both sexes demonstrated comparable reductions in intake. Interestingly, we did observe a profound sex difference in appetitive behavior during the EPT that followed the removal of sucrose from the ethanol solution as this manipulation significantly decreased the reinforcing value of the ethanol-containing solution in female, but not male, subjects. While a previous study reported that female rats have greater motivation to procure sucrose compared to males (albeit not tested at the 2 % concentration) [64], it remains interesting that we only observed this behavior after altering the taste of the ethanol-containing solution. To this end, it is worth noting that this finding was the only significant sex difference observed across all experiments, as males and females demonstrated comparable appetitive and consummatory behaviors for both solutions as well as comparable ethanol-directed choice behavior, overall. These results add to the growing and mixed literature pertaining to sex differences in ethanol and sucrose self-administration [36,64–66].

Here, we described two approaches that allow for the assessment of ethanol-directed choice behavior in the presence of an alternatively available oral reinforcer. Choice experiments allow for a deepened understanding of how environmental contingencies, such as the presence of alternative reinforcers, alters ethanol self-administration and ultimately inform our understanding of the development and maintenance of AUD. Indeed, self-administration behaviors significantly differ when a choice is available. This distinction is important because pharmacological treatments likely will not have the same effects in no-choice versus choice conditions, as we demonstrate that ethanol self-administration is not equivalent under both conditions. These findings have implications for understanding how treatments for AUD may vary depending on the conditions under which self-administration is assessed. Throughout all experiments, we observed that despite the availability of an alternative reinforcer, some subjects still demonstrated nearly exclusive ethanol-directed choice. Future

studies are necessary to explore the mechanisms that might underlie these individual differences in choice behavior to further understand why certain individuals may have greater risk for developing AUD than others. For example, understanding why some individuals have greater preference for ethanol under choice conditions and how these preferences are influenced by changes in effort requirement (e.g., price) would enhance our understanding of both biological and environmental factors contributing to AUD. Additionally, future studies will investigate how preclinical models of vulnerability (e.g., early life stress) and dependence alters behavior in these paradigms, as well as the underlying neurobiology, to further develop our understanding of AUD. Importantly, we believe these findings are aligned with the call for focusing policies on environmental strategies to help reduce alcohol use [4]. Additionally, a continued understanding of the neurobiological processes that underlie ethanol-directed choice and how these processes adapt to changes in the environment will ultimately help advance the development of novel pharmacotherapies, providing additional support for a subset of treatment-seeking individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

Data will be made available on request.

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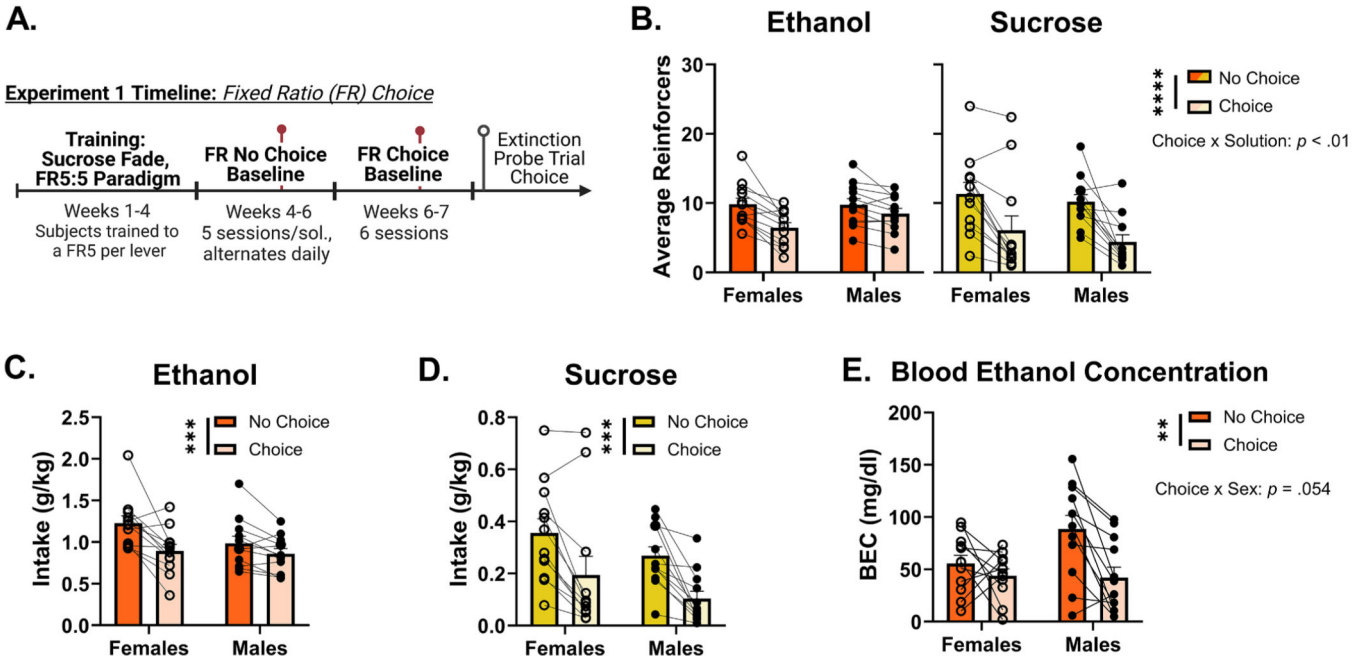

Fig. 1.

Experimental timeline, created using Biorender.com **(A)**. Sessions in which blood ethanol concentrations (BECs) were collected are noted by the red (closed circle) hashes while the extinction probe trial session is noted by the open-circle hash. Under a fixed-ratio (FR) 5:5 schedule, concurrent availability of both ethanol and sucrose (choice) results in significant reductions in the number of ethanol and sucrose reinforcers earned **(B)**, intake consumed **(C-D)**, and BEC reached **(E)** compared to when only ethanol or sucrose is available (FR5, no choice). All asterisks represent main effects of repeated-measure ANOVAs: $^{**}p < .01$, $^{***}p < .001$, $^{****}p < 0.0001$. Error bars denote SEM.

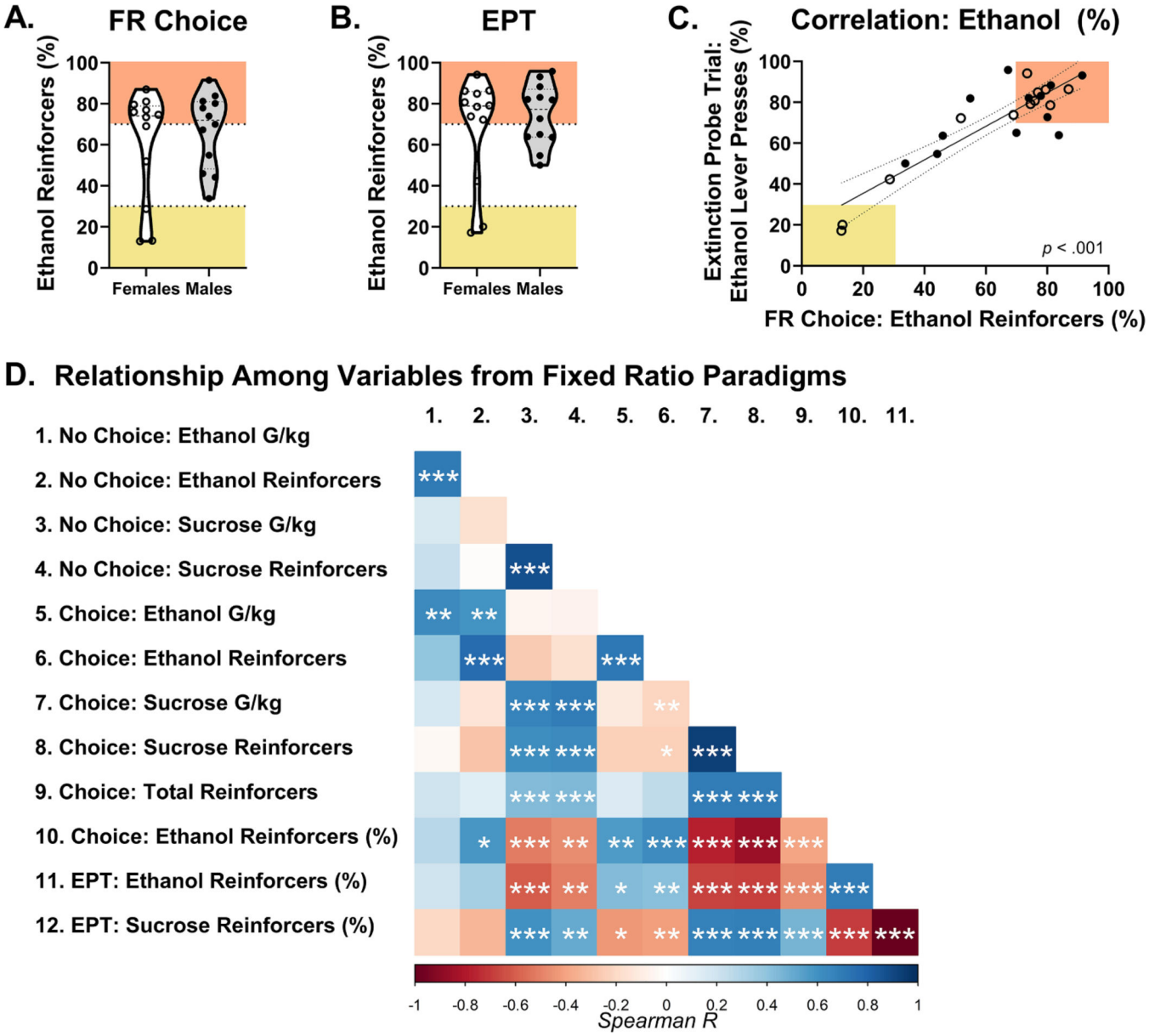

Fig. 2.

Percent of average ethanol reinforcers earned during fixed-ratio (FR) 5:5 choice sessions **(A)** and percent of ethanol-directed lever pressing during a choice extinction probe trial (EPT) **(B)**. The Spearman-rank correlation between these variables is reported **(C)**. Spearman-rank correlations among FR no choice and choice session variables are reported **(D)**, with Spearman *R* values represented in color (legend across x-axis) and the corresponding *p*-value represented via the noted asterisks: **p* < .05, ***p* < .01, ****p* < .001. Open symbols represent female subjects while closed symbols represent male subjects.

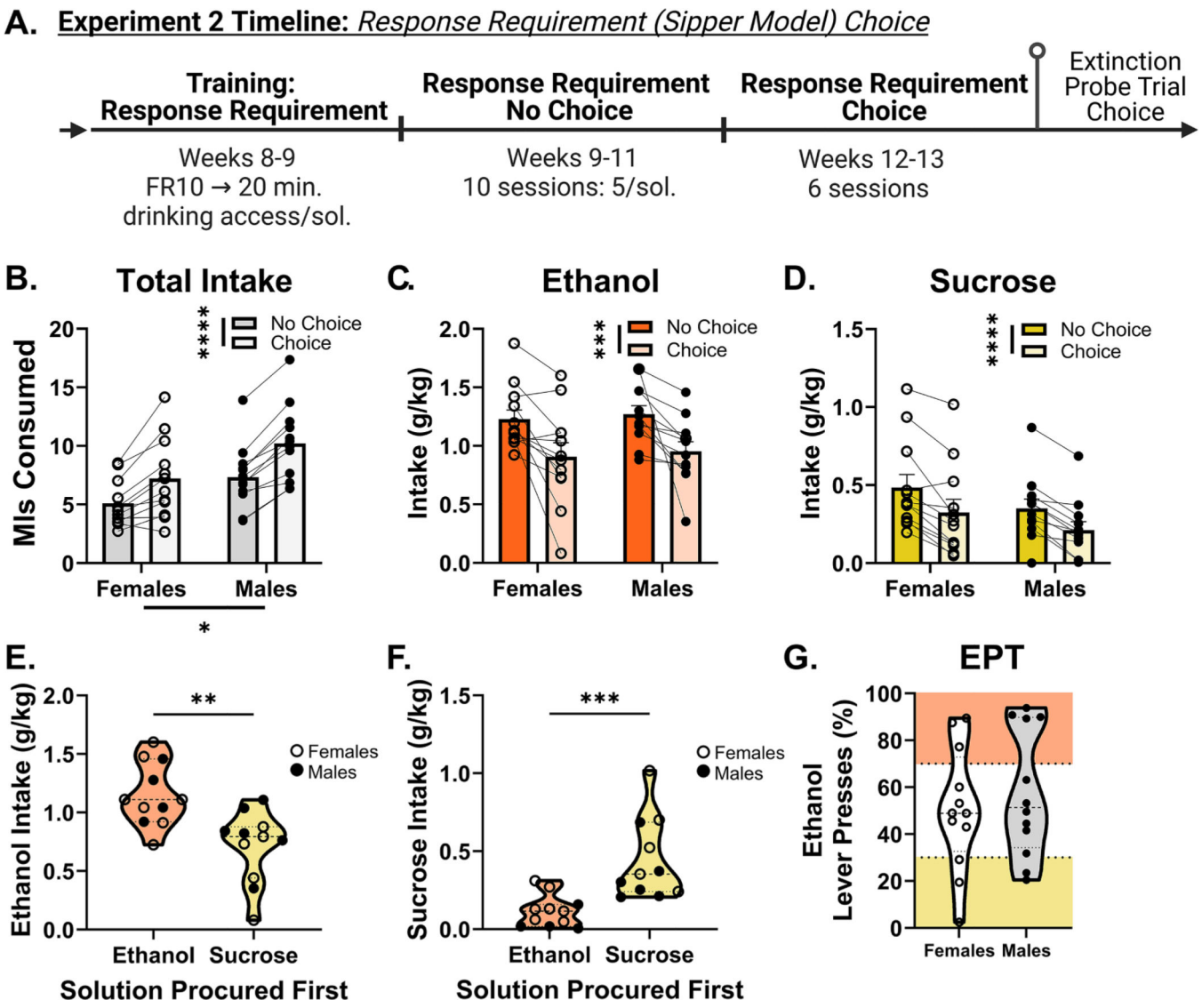

Fig. 3.

Experimental timeline, created using Biorender.com **(A)**. Under a response-requirement 10 paradigm, in which completion of 10 lever presses results in 20 min of uninterrupted access to the respective solution, concurrent availability of ethanol and sucrose (choice) results in greater total volume consumption **(B)** and subsequent decreases in ethanol **(C)** and sucrose **(D)** intake (g/kg). We observed that the subjects who procured ethanol first on most (>50 %) of the completed choice sessions had significantly higher ethanol intake **(E)** and significantly lower sucrose intake **(F)** compared to the subjects who procured sucrose first during most choice sessions (n = 11/group). Ethanol-directed lever pressing during an extinction probe trial did not reveal any sex differences following a history of no choice and choice response requirement sessions **(G)**. Open symbols represent female subjects while closed symbols represent male subjects. All asterisks represent the p-value associated with the main effects of repeated-measure ANOVAs **(B-D)**, student's t-test **(E)**, or

the Mann-Whitney U test **(F)**: $^{**}p < .01$, $^{***}p < .001$, $^{****}p < 0.0001$. Error bars denote SEM.

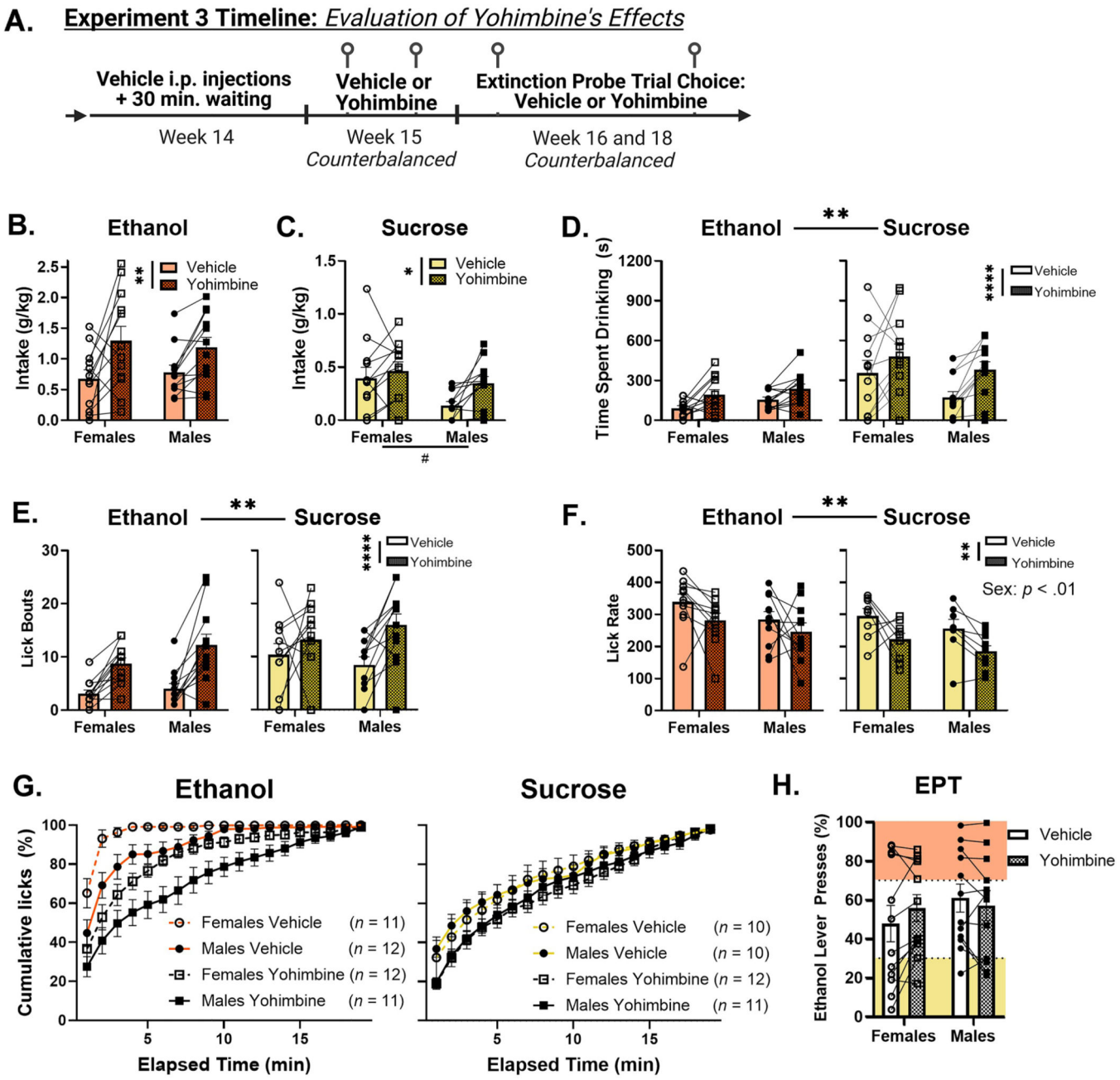

Fig. 4.

Experimental timeline, created using Biorender.com **(A)**. Yohimbine's effects on ethanol intake (g/kg) **(B)**, sucrose intake (g/kg) **(C)**, time spent drinking both solutions **(D)**, number lick bouts for each solution **(E)**, and lick rate of each solution **(F)** were assessed. Additionally, we evaluated the cumulative licks (%) per minute of sipper access across both solutions, sexes, and yohimbine administration (vs. vehicle) **(G)**. Sex-specific effects of percent ethanol lever-pressing during a choice extinction probe trial (EPT) were also evaluated **(H)**. All symbols/asterisks represent main effects of repeated-measure ANOVAs: #$p < .10$, *$p < .05$, **$p < .01$, ****$p < .0001$. Error bars denote SEM.

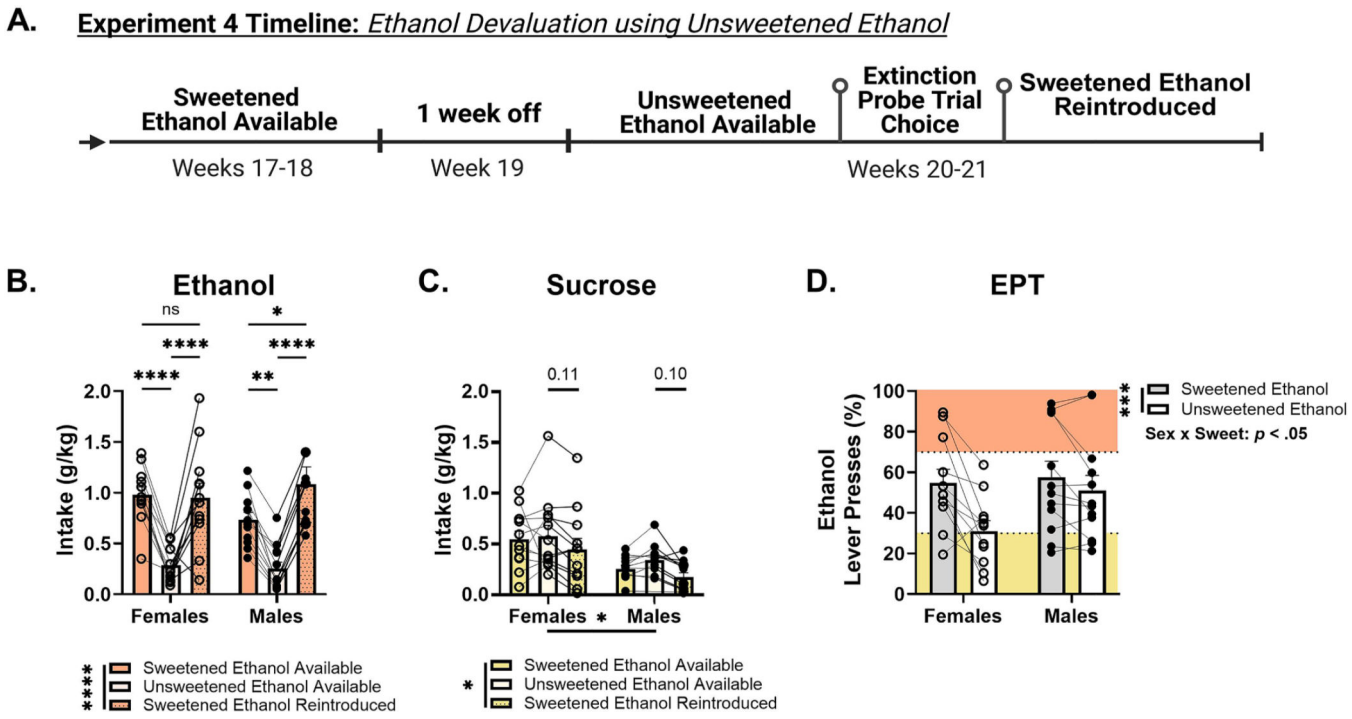

Fig. 5.

Experimental timeline, created using Biorender.com **(A)**. Effects of unsweetened ethanol availability during concurrent access to ethanol and sucrose, as well as a single-session reintroduction to the sweetened ethanol solution (10 % ethanol + 2 % sucrose) resulted in significant changes in ethanol **(B)** and sucrose **(C)** intake (g/kg). Additionally, manipulating the taste of the ethanol solution resulted in female-specific decreases in ethanol-directed lever pressing during a choice extinction probe trial (EPT) **(D)**. All asterisks to the right of legends represent main effects of repeated-measure ANOVAs while asterisks/numbers above bars represent *p*-values derived from post-hoc analyses: *$p < .05$, **$p < .01$, ****$p < .0001$. Error bars denote SEM.