Generalization of conditioned avoidance of 10% ethanol to sucrose, quinine and sucrose-quinine mixtures in male and female rats

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Introduction

Food and fluid acceptance are driven by oral cues and reward signals. Ethanol is reported as bitter and sweet by humans[1,2] and generalized to "bitter" and "sweet" compounds by rats[3]. Though humans and rats innately avoid bitter-tasting stimuli, when oral cues are coupled with a post-oral cues, a bitter-tasting stimulus can be learned to be preferred[4].

It has been previously demonstrated that female humans [1,2] and rats [5-7] have a higher propensity to consume ethanol, relative to body weight, than males. Various studies in rat models that use 24-hr intake [8,9], two-bottle tests [10.11], and operant conditioning paradigms[12,13] have demonstrated increased ethanol responses in females compared to males.

One possible explanation for the sex difference in ethanol response is that females treat ethanol as more "sweet" than "bitter", compared to males. Here, male and female rats were conditioned to avoid 10% ethanol. Various concentrations of sucrose, quinine (described by humans and "sweet" and "bitter" respectively), and sucrose-quinine mixtures were presented to test the hypothesis that female rats conditioned to avoid ethanol would generalize the avoidance to sucrose more so than quinine, whereas males would generalize the avoidance to quinine more so to sucrose.

Methods

Forty-seven (24 male and 23 female) Sprague-Dawley adult rats were trained and tested in a lickometer (DiLog Instruments) *(Figure 1)* as described previously elsewhere[14,15]. After training, rats were randomly assigned to one of two groups *(Table 1)*, and conditioned to avoid 10% ethanol by presenting 10% ethanol for 15 minutes at the same time each morning and pairing with intraperitoneal injection of either 0.15 M LiCl (1.33 ml/100 g body weight; unconditioned stimulus; US) or saline (1.33 ml/100 g body weight), control; across four conditioning trials in their home cages *(Table 2)*. Rats were retrained in the lickometer by presenting water in 10-s trials over a 30-min session. The next day, rats were tested in the lickometer by presenting a range of taste stimuli *(Table 3)* in randomized blocks without replacement in 10-s trials over a 30-min session. A 10-s water rinse presentation was interposed between each 10-s trial to minimize any potential carry over effect from the previous stimulus presentation. The rats were able to initiate as many trials as possible during the 30-minute session.

Figure 1: *lickometer*

Table 1: *Training and Testing Schedule*

# Days	Phase
3	Brief access training
2	Rehydration
4	Home cage fluid-access schedule training
7	*Conditioning trials* [1.33 ml/kg LiCl (i.p) or 1.33 ml/kg NaCl (i.p)]
3	Rehydration
1	Brief access testing with **Water**
1	Brief access testing with **Taste Stimuli**
1	Post-test ethanol intake

Table 2: *Conditioning Trials*

	Trial 1		Trial 2		Trial 3		Trial 4
AM	CS 15 min	H_2O 15-min	CS 15 min	H_2O 15-min	CS 15 min	H_2O 15-min	CS 15 min
PM	H_2O 1-hr	H_2O 1-hr	H_2O 1-hr	H_2O 1-hr	H_2O 1-hr	H_2O 1-hr	H_2O 1-hr

Table 3: *Brief-access testing with taste stimuli*

	Test Stimuli (TS)
LSUC	0.03 M Sucrose
HSUC	0.3 M Sucrose
LQUI	0.03 mM Quinine
HQUI	0.3 mM Quinine
HSLQ	0.3 M Sucrose & 0.03 mM Quinine
LSHQ	0.03 M Sucrose & 0.3 mM Quinine
Water	ddH_2O

Means licks to each stimulus were calculated. A suppression score for each LiCl-injected rat was calculated for each taste stimulus during the test session. For a given taste stimulus and LiCl-injected rat, the value was derived by the equation:

$$\text{Suppression score} = 1 - \frac{Rat\ TS\ LiCl}{Group\ TS\ saline}$$

Where Rat TS_{LiCl}=the number of licks of an individual rat in the LiCl-injected group and Group TS_{saline} = the group mean for licks to the taste stimulus for the saline-injected group. A suppression score of 0 indicates equal licks between LiCl-injected and control rats. A suppression score of 1 indicates complete suppression (no licking) of the taste solution. A one-way, one-tailed t-test was then conducted to test the degree of suppression. Correlations were used to compare suppression scores across individual taste stimuli

Results

Figure 2: *Females*

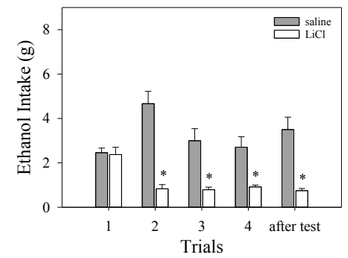
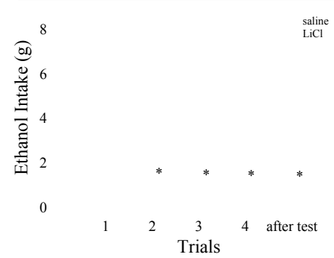


Figure 3: *Males*



Group mean ± SE intake CS on conditioning days 1, 2, 3, and 4, and after testing. Two sample t-tests comparing intake between the two groups revealed 10% ethanol intake for LiCl-injected rats (grey) and saline-injected controls (white) were similar on day 1 for female (p = .42) *(Figure 2)* and male (p = .48) *(Figure 3)* groups. Intake for LiCl-injected groups decreased over trials ($p < 0.005$) and remained significantly lower than controls after testing for both female ($p < .001$) and males ($p < .001$). This confirms the effectiveness of the conditioning procedures.

Figure 4: *Females*

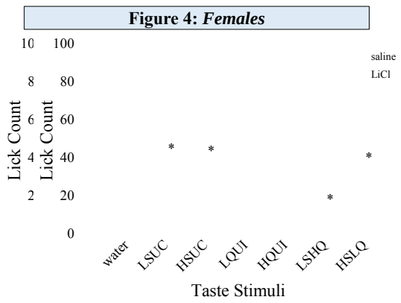
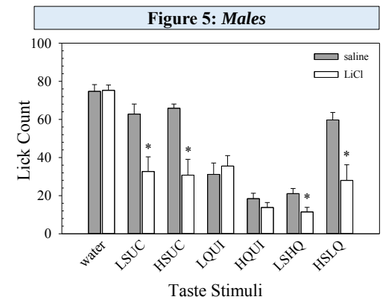


Figure 5: *Males*



Group mean ± SE licks of stimuli on testing day . For LiCl-injected (grey) and saline-injected controls (white). For females, LiCl-injected rats licked significantly less than saline-injected controls to 0.03 M sucrose (p = .002), 0.3 M sucrose (p = .001), 0.03 M sucrose-0.3 mM quinine (p = .007), and 0.3 M sucrose-0.03 mM quinine (p = .001) *(Figure 4)*. Separate two-sample t-tests comparing licks between the two male groups also revealed that male LiCl-injected rats licked significantly less than controls to 0.03 M sucrose (p = .001), 0.3 M sucrose ($p < .001$), 0.03 M sucrose-0.3 mM quinine (p = .004), and 0.3 M sucrose-0.03 mM quinine ($p < .001$). *(Figure 5)*.

Figure 6: *Females*

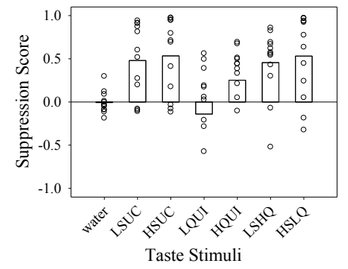
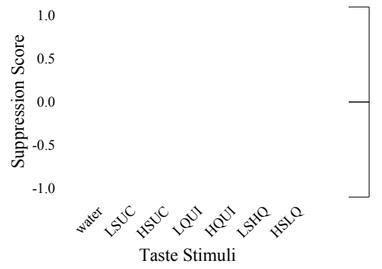


Figure 7: *Males*



Individual suppression scores for LiCl-injected rats. For female rats conditioned to avoid 10% ethanol (*Figure 6*), suppression ratio scores were significantly higher than 0 for 0.03 M sucrose (p = .001), 0.3 M sucrose ($p < .001$) , 0.03 M sucrose-0.3 mM quinine (p = .001), and 0.3 M sucrose-0.03 mM quinine (p = .002). The suppression ratio score for 0. 3 mM quinine did not reach statistical significance (p = .051). Male rats (*Figure 7*) conditioned to avoid 10% ethanol displayed suppression ratio scores significantly higher than 0 for 0.03 M sucrose, 0.3 M sucrose, 0.3 mM quinine, 0.03 M sucrose-0.3 mM quinine, and 0.3 M sucrose-0.03 mM quinine ($p \leq 0.05$).

Correlation coefficients. For LiCl-injected female rats that tended to suppress lick responses to 0.03 M and 0.3 M sucrose, lick responses were also generally suppressed to sucrose-containing stimuli ($p \leq 0.05$). Additionally, LiCl-injected female rats that showed suppressed lick responses to 0.3 M sucrose also tended to suppress lick response to 0.3 mM quinine (p = .04). For males in the LiCl group, rats that tended to suppress lick responses to 0.03 M and 0.3 M sucrose and 0.03 mM quinine also tended to suppress lick responses to sucrose-containing solutions ($p \leq 0.05$). Additionally, suppressed lick responses to 0.3 mM quinine did not significantly correlate with any other taste stimuli.

Discussion

Male and female rats conditioned to avoid 10% ethanol generalized the avoidance to 0.3 M and 0.03 M sucrose, and sucrose-quinine mixtures. These compounds are described by humans as "sweet" and "sweet-bitter" mixtures, suggesting the "sweet" component is the more salient component of 10% ethanol.

- These findings are in line with previous reports that humans report ethanol to taste both "sweet" and "bitter"[16] and that rats generalize conditioned aversions to alcohols, including ethanol, to compounds described by humans as "sweet" and "bitter"[3]. In other reports, rats conditioned to avoid alcohol, generalized the avoidance to mixtures, but less so to single components[17].
- Some discrepancies in the findings across studies may be attributed to methodology (e.g. concentration and types of alcohol and stimuli array). Of note, previous reports test with a single stimulus during extinction, but here an array of stimuli were presented during a single 30-minute session.
- This study only used 10% ethanol as a CS thus including other concentrations of ethanol and additional test stimuli in future studies would provide a more complete qualitative profile of ethanol

Comparable qualitative profiles for 10% ethanol were obtained for male and female rats suggesting both sexes similarly generalize 10% ethanol to sucrose, quinine and sucrose-quinine mixtures. Furthermore, the absence of generalization to 0.03 mM quinine by either male or female rats suggest that 10% ethanol does not have a salient quinine-like component.

- Previous studies that compared 24-hr intake and preference of ethanol showed increased response in female rats, compared to male rats[5-7]. Here comparable qualitative profiles for 10% ethanol were obtained for male and female rats suggesting the sex difference in ethanol response is not primarily driven by differences in the orosensory features of 10% ethanol.
- Taste function can be categorized as identification (e.g. orosensory features of the stimulus) and hedonic components (e.g. postoral cues) [18] thus it remains possible that differences in reward signaling differ between males and females.

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