



Published in final edited form as:

Alcohol. 2017 February ; 58: 19–22. doi:10.1016/j.alcohol.2016.11.005.

Initial subjective reward to alcohol in Sprague-Dawley rats

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Abstract

Initial subjective response to the rewarding properties of alcohol predicts voluntary consumption and the risk for alcohol use disorders. We assessed the initial subjective reward to alcohol in rats using a single exposure conditioned place preference (SE-CPP) paradigm. Sprague-Dawley rats demonstrate preference for a context paired with a single systemic injection of ethanol (1.0 g/kg, delivered intraperitoneally). However, expression of SE-CPP in males depended on pairing ethanol with the first exposure of two (ethanol; saline) to the conditioning apparatus and procedures, while conditioning day did not appreciably affect SE-CPP in females, consistent with the view that females experience heightened addiction vulnerability. This model offers researchers a high throughput assay for investigating factors that influence alcohol reward and may point the way toward more effective prevention and treatment efforts.

Keywords

Conditioned place preference; Alcohol use disorder; Ethanol; Sex differences; Susceptibility; Novelty

1. Introduction

The response to one's initial drug experience has long been studied in the clinic as a predictor of abuse liability (Haertzen, Kocher, & Miyasato, 1983; Schuckit, 1984; de Wit, 1998). The subjective response to alcohol has been defined as an endophenotype in the etiology of alcoholism reflecting its capacity to predict future alcohol use and misuse (King, de Wit, McNamara, & Cao, 2011; Ray, Mackillop, & Monti, 2010). Better understanding of the underlying neurobiology of the initial subjective response to alcohol will aid researchers in detecting relevant biomarkers and further the development of more effective intervention and prevention strategies. However, there are few animal models for assessing initial subjective reward, impeding progress in identifying the underlying mechanisms involved (Lynch, Nicholson, Dance, Morgan, & Foley, 2010).

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We developed a method for assessing initial subjective reward to alcohol (ethanol) using a single exposure conditioned place preference paradigm (SE-CPP). We found reliable place preference in three strains of mice to a context paired just one time with a moderate dose of ethanol (Grisel et al., 2014). Although assessing genetic influences using inbred mice in the SE-CPP is useful, there are a wide array of selected lines for modeling facets of human alcoholism in rats, which help make this species especially appealing to basic researchers (Ciccocioppo, 2013). Moreover, rats have long been appreciated by those investigating social and developmental influences on complex behaviors, such as addiction (Iannaccone & Jacob, 2009; Nylander & Roman, 2013; Varlinskaya & Spear, 2015). Therefore, we sought to extend the model to male and female Sprague-Dawley (outbred) rats. Historically, obtaining CPP to ethanol in rats has proven difficult (Cunningham, 1981) with few positive results involving multiple ethanol exposures (Bozarth, 1990; Morales, Varlinskaya, & Spear, 2012). Here, we demonstrate SE-CPP to ethanol in Sprague-Dawley rats and provide evidence for a simple and generalizable model capable of evaluating innate liability to ethanol reward in rats and mice.

2. Methods

2.1. Subjects

Adult (65e90 days old) male and female Sprague-Dawley rats (Charles-River; Kingston, NY, USA) were group housed by sex two to three per polycarbonate cage under standard conditions with free access to food and water and maintained on a 12:12 light-dark cycle (lights off at 06:00 pm). All subjects were conditioned and tested 3e6 h into their dark (active) cycle. All procedures were approved by the Bucknell University Animal Care and Use Committee and met the National Institutes of Health guidelines for ethical and humane animal research.

2.2. Conditioned place preference procedure

We employed an unbiased 3-compartment conditioned place preference apparatus, which is identical to that previously described (Grisel et al., 2014) except enlarged for use in rats (127 30 46 cm). A neutral center compartment separates chambers that have distinct tactile floor cues. The protocol uses a 3-day procedure, including conditioning on days 1 and 3, and testing on day 5, conducted on alternate days across a 5-day period. On days 1 & 3 subjects were weighed and transported to the testing area across the hall from the colony room, and half immediately received intraperitoneal (i.p.) injections of ethanol (1.0 g/kg, 20% by volume in saline) and the other half received equivolume saline before being relegated to one compartment of the apparatus for a 30-min conditioning session; each animal received ethanol on one conditioning day and saline on the other (or vice versa). To avoid potential confounds of social interaction following conditioning, animals housed together received the same treatment on each day but otherwise day of ethanol administration and compartment type were counterbalanced. On day 5 all subjects received saline injections, and were immediately placed into the neutral center compartment, which allowed free access to move throughout the apparatus. Behavior was recorded for 30 min by a ceiling-mounted camera and scored later by blind observers with an inter-rater reliability of 0.995, measured by

Pearson's r . The apparatus was cleaned with a dilute, low-residue solution and dried between each subject's conditioning and test sessions.

2.3. Statistical analyses

We wanted to characterize the response to SE-CPP in male and female rats in our 5-day paradigm, so first we conducted a two-way ANOVA with sex and conditioning day as between-subjects factors and preference scores [(Total time on ethanol-side)/(Total time on ethanol-side + Total time on saline-side)*(100)] as the dependent factor. Then, to address our initial hypothesis that male and female rats would exhibit a SE-CPP to ethanol we compared preference scores to the null hypothesis value of 50% using t-tests. Statistical significance was set at $P < 0.05$.

3. Results

The two-way ANOVA revealed no main effect of sex ($F_{(1,35)} = 0.293$, $P = 0.592$), no main effect of day of ethanol administration ($F_{(1,35)} = 2.48$, $P = 0.125$), but there was a significant interaction between sex and day of ethanol administration ($F_{(1,35)} = 5.494$, $P < 0.05$; Fig. 1). To determine which factors influenced SE-CPP, we followed this up with four separate t-tests comparing preference scores in the following groups: females day 1, females day 3, males day 1, and males day 3, to the null hypothesis value of 50% (equal time in ethanol-paired and saline-paired contexts). We adjusted for multiple tests using a Bonferroni correction and alpha levels of 0.0125. Males that received ethanol on conditioning day 1 showed a significant place preference (Mean \pm SEM = 58.20 ± 2.70 , $t_{(9)} = 3.036$, $P < 0.0125$; Fig. 1), but did not if they received ethanol on conditioning day 3 (Mean \pm SEM = 48.29 ± 2.18 , $t_{(8)} = 0.785$, $P = 0.23$; Fig. 1). Conversely, females showed a place preference if they received ethanol on conditioning day 3 (Mean \pm SEM = 55.58 ± 1.93 , $t_{(7)} = 2.888$, $P < 0.0125$; Fig. 1), but not if they received ethanol on day 1 (Mean \pm SEM = 53.64 ± 2.93 , $t_{(8)} = 1.241$, $P = 0.125$; Fig. 1). Overall Sprague-Dawley rats demonstrated conditioned place preference to a single i.p. injection of ethanol, as indicated by a preference score significantly above 50%, i.e., no preference ($t_{(35)} = 2.946$, $P < 0.01$; Fig. 2). Furthermore, preference scores assessed separately by sex, but collapsing across day, revealed that the ethanol place preference was evident in females ($t_{(16)} = 2.583$, $P < 0.05$; Fig. 2) and marginally significant in males ($t_{(18)} = 1.692$, $p = 0.054$; Fig. 2).

4. Discussion

We found that adult male and female Sprague-Dawley rats exhibit a significant conditioned place preference to a single, moderate dose (1.0 g/kg) of ethanol. Our results extend previous findings of SE-CPP to rats, and suggest SE-CPP as a generalizable model for assessing and investigating initial subjective reward to ethanol in rodents. Subjects received the systemic injection of ethanol on either day 1 or 3 of the study, and equivolume saline on the other, immediately before a 30 min conditioning session (and remain undisturbed on days 2 & 4). On the test day (5) both male and female rats preferred the ethanol paired context. However, researchers interested in using this model in rats should consider the potential influence of sex differences in conditioning, as male rats only showed a significant place preference when receiving ethanol on the first conditioning day. Females were less

affected by conditioning day, though if anything, preference was stronger when ethanol was administered on day 3 (see Fig. 1). We did not see any evidence for sex differences using this paradigm in any of the three strains of mice tested (Grisel et al., 2014).

Male and female rodents exhibit subtle but significant differences on various measures of addictive-like behavior suggesting that females may experience heightened vulnerability (Carroll & Lynch, 2016). One well-documented difference is that female rodents tend to drink more alcohol than males (e.g., Lancaster & Spiegel, 1992). Similarly, females might be more prone to developing an ethanol place preference for some of the same reasons they show faster acquisition of self-administration than males for low doses of various drugs of abuse (Carroll, Lynch, Roth, Morgan, & Cosgrove, 2004), and develop greater preference for cocaine over food compared to males (Perry, Westebroek, & Becker, 2013). Gonadal hormones might also influence the rewarding properties of ethanol differently in male and female rats. Using a biased, multiple exposure paradigm, Torres, Walker, Beas, and O'Dell (2014) reported that adolescent and adult female rats showed a preference only at 1.0 g/kg (the same dose used in the present study), which did not depend on estrous cycle, and males and ovariectomized females did not show a preference at any dose tested (0–2.5 g/kg).

Female rats display greater striatal dopamine (DA) release to various drugs of abuse, including ethanol, relative to males (Blanchard et al., 1993; for review see; Becker, Perry, & Westebroek, 2012). Increased DA signaling might function to enhance the salience of the ethanol-paired context to a greater extent in females than males, as in the present study expression of preference in males was contingent on a combination of ethanol and exposure to a novel apparatus and testing procedures (Fig. 2, day 1 males). Similarly, in a single trial paradigm, Bevins (2001) reported that a sub-threshold dose of cocaine, insufficient to support a CPP alone, facilitated a CPP when paired with a novel object, which involves a DA-dependent mechanism (Besheer, Jensen, & Bevins, 1999). Consistent with these findings, Morales et al. (2012) found that male Sprague Dawley rats demonstrated an ethanol CPP, following multiple drug pairings, only if the rats were not pre-exposed to the testing apparatus prior to conditioning, suggesting males might habituate more readily than females. Thus, male rats in our study might have habituated to the testing apparatus by day 3, accounting for the absence of a CPP and suggesting a combination of novelty to the apparatus and ethanol might be necessary to facilitate ethanol-CPP induction in male rats, but not in females.

We observe an ethanol CPP when rats receive a single moderate dose of ethanol using a single exposure, high throughput protocol. Place conditioning procedures have become increasing popular tools for assessing the rewarding effects of drugs and non-drug reward (see Tzschentke, 2007 for a comprehensive review) and the SE-CPP model offers researchers an expedient tool for studying molecular and cellular mechanisms underlying initial ethanol experiences (Franklin et al., 2009; Melis, Camarini, Ungless, & Bonci, 2002) as well as genetic and environmental influences of ethanol reward in rats and mice (Ciccocioppo, 2013).

Acknowledgements

This work was supported by the National Institute on Alcohol Abuse and Alcoholism, United States (R15 AA022506). We thank Samuel Baum for his help analyzing behavioral videotapes.

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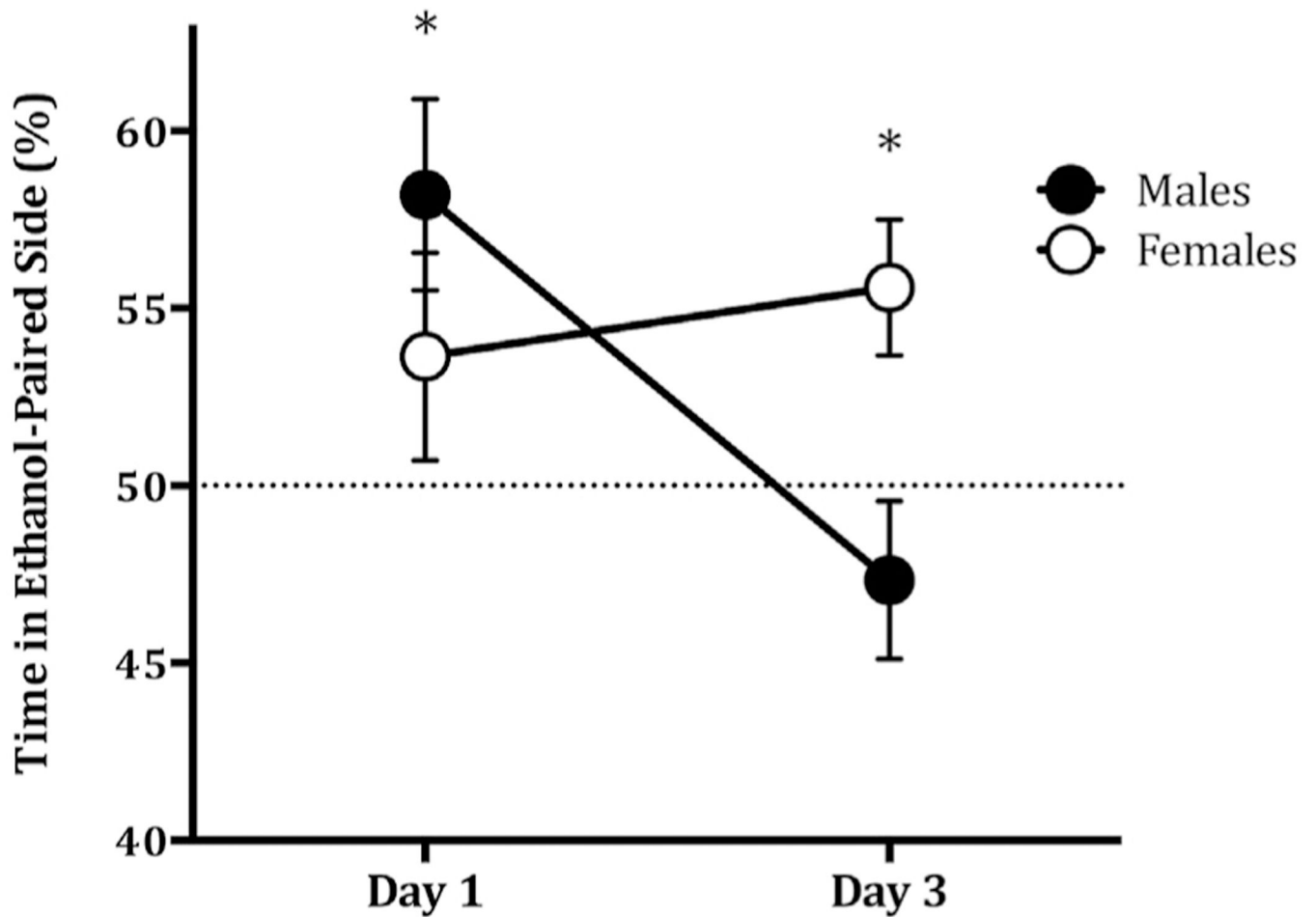


Fig. 1. Mean ± SEM % place preference of a Sex x Ethanol administration day interaction. Percent preference for male (closed circles; day 1 $n = 10$, day 3 $n = 9$) and female (open circles; day 1 $n = 9$, day 3 $n = 8$) rats shown separated by day of ethanol administration. A two-way ANOVA revealed no main effect of sex and no main effect of day, but there was a significant interaction between sex and day ($F_{(1,35)} = 5.494$, $P < 0.05$). * $P < 0.05$ relative to no preference at 50%.

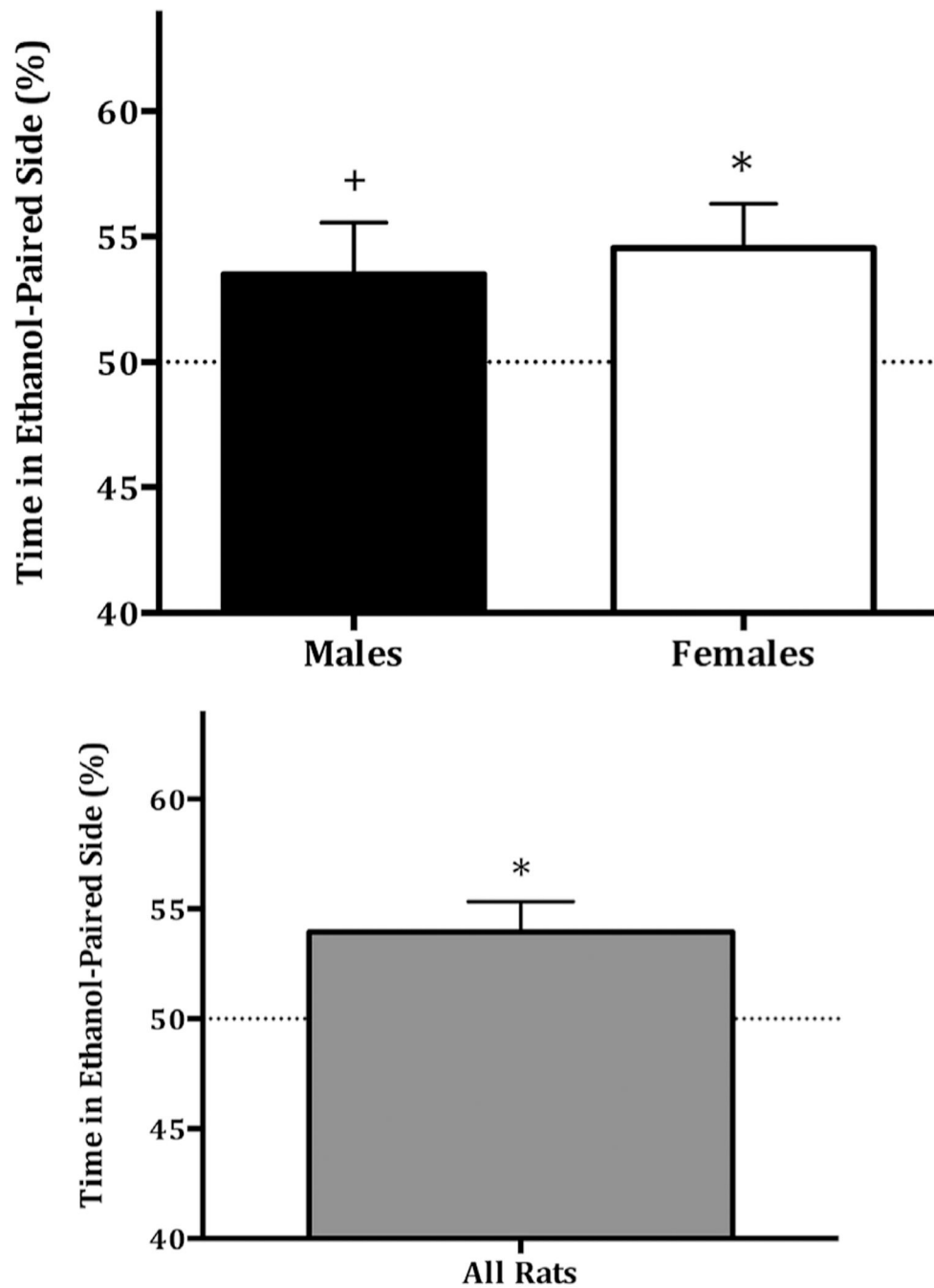


Fig. 2. Mean \pm SEM % place preference to 1.0 g/kg ethanol expressed as [time in ethanol context/ (time in ethanol context + time in Saline Context)].

Upper panel shows % preference for male ($n = 19$) and female ($n = 17$) rats, respectively.

Lower panel shows % preference for all rats ($N = 36$). * $P < 0.05$ relative to no preference at 50% as determined by one-tailed t-tests. + $P = 0.054$.