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Oxytocin reduces alcohol consumption in prairie voles

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ABSTRACT

Alcohol use disorder (AUD) negatively affects millions of people every year in the United States, and effective treatments for AUD are still needed. The neuropeptide oxytocin has shown promise for reducing alcohol drinking in mice and rats. Because oxytocin also plays a key role in complex prosocial behaviors like bonding and attachment, we tested the effect of oxytocin on alcohol drinking in prairie voles, a species that both consumes high amounts of alcohol and forms oxytocin dependent social bonds in a manner similar to humans. Oxytocin treatment (1.0, 3.0, and 10.0 mg/kg, i.p.) reduced alcohol consumption in male and female prairie voles in animals that had access to 15% ethanol vs water every other day for 12 alcohol drinking sessions. In animals with continuous access to 15% alcohol and water, oxytocin (3.0 mg/kg) reduced alcohol consumption only in the first hour of access after treatment, with no significant effects on consumption over the 24-hr period. In an open field locomotor test, oxytocin (1.0, 3.0, and 10.0 mg/kg, i.p.) did not affect overall locomotor activity; however, ethanol (2 g/kg, i.p.) increased locomotor activity in males and females, and produced anxiolytic effects (increased time in the center of an open field) in females only. Because prairie voles have been shown to match the alcohol consumption of their cage mate, we evaluated the relationship between cage mates' alcohol drinking. There was an overall pattern of social facilitation (consumption by one cage mate predicted consumption by the other cage mate); however, we found significant individual differences across cages in which many cages did not show significant matching, and, in some cases one cage mate's consumption negatively predicted the other cage mate's consumption. Overall, our data provide support for the potential of oxytocin as a treatment to reduce alcohol consumption.

1. Introduction

Oxytocin is a neuropeptide known to be involved in maternal, reproductive, and other types of social behavior. Recently oxytocin has been shown to have diverse and generally protective effects in multiple aspects of drug abuse, including alcohol abuse [7,28,31]. Alcohol use disorder (AUD) remains a major public health concern, with approximately 17 million adults suffering with AUD (niaaa.nih.gov), and yet the available treatments for AUD have offered only limited success [18]. Oxytocin has shown some promise in animal models of alcohol consumption and responses to alcohol.

Oxytocin has been shown to be generally effective at reducing alcohol consumption, as well as physiological and behavioral responses to alcohol. Oxytocin administration, both peripheral and ICV inhibited the development of tolerance to the hypothermic effects of high doses of ethanol in mice [43,44]. Additionally, ICV oxytocin administration reduced ethanol-induced sedation and ataxia in the wire-hanging, righting reflex, and open-field tests in rats [6]. Oxytocin administration reduced alcohol consumption in several studies. Low dose peripheral oxytocin administration reduced self-administration of an ethanol solution and ethanol gelatin in rats [30]. McGregor and Bowen [31] found an impressive 6-week decrease in consumption of a low alcohol sweet vodka drink compared to a sucrose solution following a single peripheral oxytocin administration in rats. Additionally, they found that 10 days of oxytocin administration prior to drinking reduced preference for the sweet alcohol drink. ICV oxytocin administration reduced alcohol consumption in rats with long-term chronic intermittent alcohol access [37]. Oxytocin administration also reduced two-bottle ethanol drinking in mice but with restrictions: oxytocin reduced alcohol drinking only when administered peripherally at a relatively high dose (10 mg/kg) in singly housed mice, not in mice subjected to chronic subordinate housing, and not when administered ICV [36]. Most

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recently, oxytocin administration reduced operant alcohol self-administration and breakpoints in a progressive ratio test, in addition to reducing alcohol consumption in the drinking in the dark method in mice [26]. Recent studies have illuminated aspects of the mechanism by which oxytocin can inhibit ethanol responses and reward, acting to block the accumbens dopamine response to both acute and chronic ethanol injections [37]. Oxytocin also acts directly on GABA receptors to reduce ethanol-induced GABA receptor activity [6]. Especially given data from a clinical trial in which oxytocin treatment reduced withdrawal severity and requests for Lorazepam in detoxing alcoholics [34], the literature to date suggests significant potential for treatments with oxytocin or drugs that can act via similar mechanisms.

Though studies of oxytocin and alcohol support a generally inhibitory role for oxytocin in alcohol consumption and responses, some inconsistencies are apparent. The duration and robustness of the effects of oxytocin seem to differ according to species, dose, route of administration, and method of alcohol administration [30,31,36,37]. Further studies of the effects of oxytocin on alcohol consumption and alcohol responses will benefit our understanding of oxytocin's potential for ameliorating AUD. Given the importance of oxytocin for social behavior, the use of an animal model that shares important social characteristics with humans is useful. Prairie voles and humans have in common complex social structures, social monogamy, biparental and alloparental behavior, and show devastating responses to isolation [12–15,32] and much is known about the function of oxytocin in these social behaviors in prairie voles. Furthermore, prairie voles are useful for alcohol studies because they voluntarily consume high levels of unsweetened alcohol without training [1,41]). Notable work has shown their alcohol consumption, like humans, is sensitive to complex social factors [1–4,23]. Finally, it is valuable to expand studies of potential AUD treatments to species beyond mice and rats, as these two species are closely related, are members of genetically closed populations, and have lost many of the behaviors of mice and rats in the wild [40]. Prairie voles, in contrast, are typically only a small number of generations removed from wild-caught animals, allowing for an understanding of the effects of oxytocin in a genetically diverse population. Finally, drug reward and social reward pathways are thought to overlap and include dopamine pathways that can be modulated by oxytocin [5,29,31,37,39]; therefore, understanding how oxytocin impacts alcohol reward in a species that, unlike mice and rats, displays lasting social bonds indicative of profound social reward in a manner similar to humans is valuable.

The purpose of this study was to determine the effects of oxytocin treatment on alcohol consumption in two different alcohol access methods (chronic intermittent and continuous alcohol access) in prairie voles, to assess the locomotor and anxiolytic effects of oxytocin and ethanol in prairie voles, and to investigate if oxytocin treatment could alter social facilitation of alcohol drinking in prairie voles. Sex differences in alcohol drinking, locomotor, and anxiolytic effects of oxytocin and ethanol were characterized.

2. Materials & methods

2.1. Subjects

Adult male and female prairie voles were bred and housed at Bucknell University. Animals were F3 generation descendants of prairie voles caught near Champagne-Urbana, Illinois, USA. Animals were pair-housed (except where specified) with a same-sex sibling in polycarbonate cages (19 cm × 29.2 cm × 12.7 cm, Ancare, Bellmore, NY, USA) with Harlan Teklad aspen sani-chip bedding (Envigo, Somerset, NJ, USA), and given ad libitum access to water and high fiber rabbit chow (Purina Mills, Inc., Gray Summit, MO, USA). Colony rooms were maintained on a 14:10 light cycle (lights on at 06:00) at approximately 70 °F. Animals were ages 8–27 weeks, and ages were counterbalanced across studies and treatment groups. All experiments and procedures were approved by the Bucknell University IACUC and conducted in accordance with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research [46].

2.2. Drugs

Oxytocin acetate salt (Bachem, Torrence, CA, USA) dissolved in 0.9% saline was injected (i.p.; 1.0, 3.0, and 10.0 mg/kg). These doses were chosen based on their efficacy in other studies of alcohol consumption [30,31,36]. In the case of rat studies, the equivalent surface area dosage conversion [10] was used to estimate a dose range for prairie voles. Injection volumes for oxytocin and saline were 10 mL/kg. 95% ethyl alcohol was diluted to 15% alcohol in water for alcohol drinking experiments. For injection in locomotor studies, 95% ethyl alcohol was diluted to 20% in saline, then administered at a dose of 2 g/kg, i.p. The 2.0 g/kg dose of ethanol was chosen because it has previously been shown to induce acute locomotor activation in mice [42].

2.3. Procedure

2.3.1. Effect of oxytocin on chronic intermittent access alcohol drinking in male and female prairie voles

Adult male (n = 34) and female (n = 34) prairie voles were given access to 15% ethanol and water according to a chronic intermittent access two-bottle method [33]. A mesh divider made of galvanized steel hardware cloth with 0.5 in. square openings was inserted down the middle of the length of the cage, bisecting it such that each animal had access to half of the cage (approximately 8.9 cm × 29.2 cm). This allowed for measurement of individual fluid consumption, while still providing animals with visual, olfactory, and tactile contact with their cage mate. Dividers were only used on the first habituation day and on alcohol drinking days. On water days, dividers were removed when alcohol bottles were replaced with water bottles in order to limit any possible stress associated with having the divider in the cage, although no signs of stress were observed. Specifically, on day 1, dividers were inserted and each animal had access to two bottles of water to habituate them to two-bottle access. On day 2, dividers were removed and animals had access to all four water bottles that were in place on day 1. Day 3 was the first day of ethanol access: dividers were inserted and each animal had access to one bottle of 15% ethanol and one bottle of water. On day 4 dividers were removed, and animals had access to 4 bottles of water. The conditions for days 3 and 4 were repeated, such that animals had access to alcohol for 24 h every other day until they had completed 12 alcohol drinking days (25 days total). On all alcohol access days, the position (left-right) of the water and alcohol bottles was alternated relative to their last drinking session. Thirty minutes before alcohol drinking sessions 8, 9, 10, animals were injected with saline (i.p.) to habituate them to injection procedures. Vehicle (saline) or oxytocin (OT 1.0, 3.0, or 10.0 mg/kg, i.p.) treatment was administered 30-min before the start of sessions 11 and 12 (injections at 08:00, bottles on at 08:30). Animals in the same cage were given the same treatment. Each animal received a vehicle treatment and one dose of oxytocin, with the order counterbalanced across sessions 11 and 12. On vehicle and oxytocin treatment days, ethanol and water consumption were measured 1, 6, 12, and 24 h after alcohol and water bottles were put on each cage. Animals were weighed once per week at the start of a water drinking day.

2.3.2. Effect of oxytocin on continuous access alcohol drinking in male and female prairie voles

To determine if oxytocin would reduce drinking when animals had continuous access to alcohol, and to utilize the drinking method that has been used frequently in prairie voles, we tested the effect of oxytocin on alcohol consumption in 12 male and 14 female prairie voles with continuous access to 15% ethanol vs water for 13 days. The position of water and alcohol bottles was alternated each day. Divided

cages (described above) allowed for measurement of individual alcohol and water consumption. Saline habituation injections were administered on days 8, 9, and 10. Then vehicle (saline) and oxytocin treatment (OT 3 mg/kg) were administered on days 11 and 13, with treatments counterbalanced across days. Both animals in a cage received the same treatment. Saline injections were administered on day 12, but this data was not included in the analysis. The 3 mg/kg oxytocin dose was used because it was the most effective dose at which we had not observed evidence from any individual animals of non-specific locomotor reductions; whereas at 10 mg/kg, we observed that some animals experienced decreased locomotion, although no group difference in locomotion was evident at any dose (see below). On days when injections were administered, alcohol and water bottles were removed, animals were injected and placed back in their cage, and then alcohol and water bottles were returned 30 min later (bottles removed and injections given at 08:00, bottles returned at 08:30). This ensured the same 30 min time period between treatment and alcohol access as in the intermittent access study. On vehicle and oxytocin treatment days, ethanol and water consumption were measured 1, 6, 12, and 24 h after alcohol and water bottles were placed back on each cage.

Because prairie voles are known to show social facilitation of drinking [22], we wanted to determine if reducing alcohol consumption by one animal in the cage with oxytocin treatment would alter alcohol consumption by the cage mate. On day 15 (after a day without treatment), one animal per cage was treated with oxytocin (10 mg/kg). Here we used 10 mg/kg oxytocin because it produced the most robust decrease in alcohol consumption in previous experiments, and, because our goal was to understand the effects on alcohol consumption in the untreated cage mate, we did not have any concerns about possible non-specific locomotor effects of this dose. Half the animals that received oxytocin on this day were the higher drinker in the cage, and half were the lower drinker in the cage. The other animal in the cage received a saline injection. 30 min after the injection, animals were given access to ethanol and water. Results from this part of the continuous access study are described below under *Social facilitation of alcohol drinking in prairie voles*.

2.3.3. Effects of oxytocin and ethanol on locomotor activity in male and female prairie voles

Male and female prairie voles (n = 64 each) were assessed for the effects of oxytocin, ethanol, or oxytocin plus ethanol on locomotor activity. On days 1 and 2, animals were injected i.p. with saline, then injected with saline again 30 min later before being placed immediately into a locomotor chamber (50.8 cm × 27.94 cm × 33 cm) where their activity was recorded with a digital video camera for 15 min. On day 3 animals were injected with either saline followed 30 min later by saline (Sal-Sal, n = 8 males and 8 females), saline followed by 2 g/kg ethanol (Sal-EtOH, n = 8 males and 8 females), oxytocin (1.0, 3.0, or 10.0 mg/kg) followed by saline (OT (dose)-Sal, n = 8 males and 8 females per dose of OT), or oxytocin (1.0, 3.0, or 10.0 mg/kg) followed by 2 g/kg ethanol (OT (dose)-EtOH, n = 8 males and 8 females per dose of OT). Locomotor activity was analyzed using ANY-maze video tracking system (Stoelting Co., Wood Dale, IL, USA) to determine distance traveled, as well as time spent in the center vs perimeter (center was defined as the central 12 × 6 cm of the chamber) during the 15 min activity sessions.

2.3.4. Statistical analyses

We analyzed the data in three ways. First, we used mixed model ANOVAs (SPSS, IBM Corp., Released 2015, IBM SPSS Statistics for Macintosh, Version 23.0., Armonk, NY: IBM Corp.) to compare dose and sex effects at specific time points (1-hr and cumulative 24-hr consumption) directly. In these analyses, dose and sex were between-subject factors, and treatment (vehicle vs. oxytocin) was the within-subject factor. Then linear mixed effects models (JMP Pro 11, SAS Institute Inc., Cary, NC, USA) were used to explore the strength of the relationships between variables, for example if and how much the dose of oxytocin linearly decreased alcohol consumption. It also allowed us to characterize each factor that affected drinking simultaneously, controlling for each other (e.g. controlling for water consumption while testing the effect of dose). In most mixed effects analyses, subject was a random effect (allowing the intercept to vary per subject). In the social facilitation results, the intercepts were allowed to vary per cage (not subject). We report the standardized coefficients. The degrees of freedom for the mixed effects models were approximated in JMP by the Kenward-Roger correction [25]. Lastly, two-way repeated measures ANOVAs of difference scores (g/kg under vehicle conditions – g/kg under oxytocin conditions) were performed to determine how effective oxytocin was at reducing hourly alcohol consumption at each time point (GraphPad Prism 6 for Mac OS X, GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1. Chronic intermittent alcohol access

Oxytocin reduced alcohol consumption in the first hour and cumulatively over the 24-hour period following oxytocin treatment. Mean alcohol and water consumption and preference ratio for the first hour and for the 24-hour period under vehicle and oxytocin conditions is shown in Tables 1–3. A mixed model (repeated measures) ANOVA in which treatment (oxytocin vs. vehicle) was a within subjects factor, and the dose of oxytocin and sex were between subjects factors was used to directly compare the effects of each dose of oxytocin on the first hour of alcohol consumption (g/kg). There was a significant main effect of treatment. Oxytocin significantly reduced drinking in the first hour of alcohol access following oxytocin treatment ($F(1,56) = 29.593$, $p < 0.0001$) (Fig. 1A). There was no significant interaction between dose and treatment ($F(2,56) = 0.480$, $p = 0.622$) indicating all doses produced similar reductions in alcohol consumption in the first hour. Sex did not significantly affect alcohol consumption in the first hour ($F(1,56) = 0.188$, $p = 0.667$). Difference scores at 1 h for alcohol consumption (OT-vehicle) are shown in Fig. 1C.

There was also a main effect of treatment on cumulative alcohol consumption over the 24-hr test period. Oxytocin reduced cumulative 24-hr alcohol consumption ($F(1,56) = 32.180$, $p < 0.0001$) (Fig. 1B). There was no significant interaction between treatment and dose ($F(2,56) = 0.826$, $p = 0.443$). There was a significant main effect of sex on alcohol consumption over the 24 h test period ($F(1,56) = 9.297$, $p = 0.004$), demonstrating that females consumed more alcohol than males over the 24-hour period. There was also a significant interaction between treatment and sex ($F(2,56) = 4.471$, $p = 0.039$), and a post hoc comparison showed a greater effect of oxytocin treatment in males than females, $p < 0.05$, Cumulative 24-hour difference scores for alcohol consumption at each dose are shown in Fig. 1D. A *t*-test showed no significant effect of order of treatment ($t(60) = 1.346$, $p = 0.1833$).

To better understand when oxytocin was most effective, we performed a two-way repeated measures ANOVA on difference scores (for each animal, alcohol consumption under oxytocin conditions was

Table 1
Intermittent access mean (SEM) alcohol consumption (g/kg).

	Oxytocin treatment (mg/kg)	1 Hour		Cumulative 24 h	
		Vehicle	Oxytocin	Vehicle	Oxytocin
Males	1	2.2 (0.2)	1.8 (0.3)	16.6 (2.0)	13.2 (2.2)
	3	2.0 (0.3)	1.1 (0.2)	11.1 (1.1)	14.3 (1.8)
	10	1.9 (0.4)	0.8 (0.2)	15.7 (2.3)	11.0 (2.1)
Females	1	2.1 (0.4)	1.1 (0.3)	19.6 (1.3)	17.8 (1.2)
	3	1.8 (0.2)	1.2 (0.3)	20.4 (1.5)	19.1 (1.9)
	10	2.0 (0.3)	1.1 (0.2)	19.4 (3.6)	17.6 (1.3)

Table 2
Intermittent access mean (SEM) water consumption (mL).

	Oxytocin treatment (mg/kg)	1 h		Cumulative 24 h	
		Vehicle	Oxytocin	Vehicle	Oxytocin
Males	1	0.4 (0.1)	0.2 (0.1)	9.6 (1.4)	11.3 (1.7)
	3	0.4 (0.2)	0.6 (0.3)	11.1 (1.1)	12.2 (1.8)
	10	0.4 (0.1)	0.1 (0.1)	9.5 (1.6)	11.8 (1.5)
Females	1	0.3 (0.1)	0.2 (0.1)	8.3 (1.2)	9.4 (1.2)
	3	0.3 (0.1)	0.3 (0.1)	8.0 (0.8)	9.4 (1.0)
	10	0.4 (0.1)	0.2 (0.1)	9.2 (1.6)	9.2 (1.0)

Table 3
Intermittent access mean (SEM) preference ratio.

	Oxytocin treatment (mg/kg)	1 h		Cumulative 24 h	
		Vehicle	Oxytocin	Vehicle	Oxytocin
Males	1	0.7 (0.1)	0.8 (0.1)	0.4 (0.0)	0.3 (0.1)
	3	0.7 (0.1)	0.6 (0.1)	0.4 (0.0)	0.3 (0.0)
	10	0.7 (0.1)	0.8 (0.1)	0.4 (0.1)	0.3 (0.1)
Females	1	0.7 (0.1)	0.8 (0.1)	0.4 (0.0)	0.4 (0.0)
	3	0.7 (0.1)	0.7 (0.1)	0.4 (0.0)	0.4 (0.0)
	10	0.7 (0.1)	0.8 (0.1)	0.4 (0.1)	0.4 (0.0)

subtracted from consumption under vehicle conditions) at each time point (1, 6, 12, and 24 h). Doses of oxytocin were combined in this analysis. There was a main effect of time point ($F(3180) = 9.393$, $p < 0.0001$) and sex ($F(1,60) = 4.703$, $p = 0.0341$), as well as a significant interaction ($F(3180) = 11.67$, $p < 0.0001$). Tukey's multiple comparisons revealed that the difference scores in males at hour 6 were significantly different from hours 1, 12, and 24, $p < 0.01$; while in females difference scores at hour 1 were significantly greater than hours 6, $p < 0.01$, and hours 12 and 24, $p < 0.0001$. Also for females hour 6 was significantly different from hour 24, $p < 0.05$, see Fig. 1E.

In order to better quantify how much time and level of treatment affected drinking, and to control for water consumption and age of the animal, we ran a mixed effects regression (allowing for random intercepts by subject) predicting alcohol consumption from oxytocin dose, sex, time with alcohol (1, 2, 6, 12, 24 h), water consumption, and age. We included water consumption as a predictor to control for a possible effect of oxytocin on fluid consumption in general. Water consumption did not predict alcohol consumption, ($\beta = -0.0464 \pm 0.0420$, $t(459.3) = -1.10$, $p = 0.2700$). Controlling for water consumption allowed us to estimate the effects of our measures of interest (dose, sex, time) more precisely. Oxytocin significantly reduced alcohol consumption (g/kg/h), and, unlike the ANOVA, the regression model revealed a linear relationship between doses of oxytocin and drinking: higher doses of oxytocin reduced drinking more ($\beta = -0.2053 \pm 0.0397$, $t(488.2) = -5.17$, $p < 0.0001$). The regression showed that voles drank less alcohol as time went on over the 24-hour period ($\beta = -0.4048 \pm 0.0358$, $t(430.4) = -11.30$, $p < 0.0001$). Further, there was a significant interaction between treatment and time point demonstrating that higher doses of oxytocin decreased drinking more over time than lower doses ($\beta = 0.1779 \pm 0.0359$, $t(431.4) = 4.95$, $p < 0.0001$). Females tended to drink more alcohol than males ($\beta = 0.1271 \pm 0.0584$, $t(58.9) = 2.17$, $p = 0.0337$). There was no significant effect of age on alcohol consumption in animals with intermittent access ($\beta = 0.0257 \pm 0.0581$, $t(58.4) = 0.44$, $p = 0.6599$). These results are displayed in Table 4.

Linear mixed effects regression results (random intercepts by subject) are shown. Oxytocin reduced alcohol consumption, animals drank less over time, and there was a significant interaction between oxytocin treatment and time point. Females drank significantly more alcohol than males. Age had no effect on drinking.

A mixed effects linear regression showed no significant effect of oxytocin on water consumption. Neither oxytocin dose ($\beta = 0.0160 \pm 0.0421$, $t(481.8) = 0.38$, $p = 0.7042$) nor time with alcohol ($\beta = 0.0440 \pm 0.0369$, $t(431.1) = 1.19$, $p = 0.2334$) predicted water consumption. There was, however, a weak interaction between oxytocin treatment and time point ($\beta = 0.0763 \pm 0.0369$, $t(431.1) = 2.07$, $p = 0.0395$), indicating that increasing treatment level was associated with a slight increase (rather than decrease) in water drinking over time. Sex did not significantly predict water consumption ($\beta = 0.1079 \pm 0.0814$, $t(60) = -1.32$, $p = 0.1903$).

As expected given that oxytocin reduced alcohol consumption but did not change water consumption, oxytocin significantly reduced alcohol preference (alcohol consumption/total fluid consumption). A mixed model regression testing the effects of oxytocin on alcohol preference showed a significant effect of oxytocin ($\beta = -0.026 \pm 0.01$, $t(487) = -2.53$, $p = 0.012$), and time ($\beta = -0.095 \pm 0.009$, $t(487) = -10.64$, $p < 0.0012$), but no interaction ($p = 0.949$). Sex was also not a significant predictor of alcohol preference ($p = 0.110$).

3.2. Continuous alcohol access

Oxytocin treatment reduced alcohol consumption in the first hour, but did not significantly reduce drinking over the 24-hour test period in animals with continuous access. Mean alcohol and water consumption and preference ratio under vehicle and oxytocin treatment conditions are shown in Tables 5–7. A mixed model (repeated measures) ANOVA in which treatment (vehicle vs. oxytocin) was a within subjects factor and sex was a between subject factor showed that oxytocin significantly reduced alcohol consumption in the first hour ($F(1,24) = 8.667$, $p = 0.007$) (Fig. 2A). There was also a main effect of sex ($F(1,24) = 6.548$, $p = 0.017$), in which males drank more alcohol than females in the first hour, but no significant interaction between treatment and sex ($F(1,24) = 1.262$, $p = 0.272$). In contrast with the effects after 1-hr, oxytocin did not significantly reduce cumulative alcohol consumption over the 24-hr test period ($F(1,24) = 0.764$, $p = 0.391$) (Fig. 2B). Sex did not significantly affect cumulative alcohol consumption ($F(1,24) = 0.046$, $p = 0.832$), and there was no significant interaction between treatment and sex ($F(1,24) = 2.784$, $p = 0.108$). Difference scores are shown in Fig. 2C and D. A *t*-test showed no effect of order of treatment ($t(24) = 1.497$, $p = 0.1475$).

Again, to understand when oxytocin was most effective, a two-way repeated measures.

ANOVA of difference scores at each time point was performed. There was a main effect of time point ($F(3,72) = 6.291$, $p = 0.0007$), but no significant effect of sex ($F(1,24) = 0.0006$, $p = 0.9810$), and no significant interaction ($F(3,72) = 1.600$, $p = 0.1968$). Although there was not a significant interaction between sex and treatment, the significant interaction in the intermittent access animals offered a priori justification for follow up multiple comparisons. Tukey's multiple comparisons showed that in males the difference scores at 1 h were significantly larger than at hours 6, 12, and 24, $p < 0.01$, while differences scores were not significantly different for females at any time point, see Fig. 2E.

In a mixed effects linear regression model using the same factors as above, water consumption did not significantly predict alcohol consumption ($\beta = 0.0615 \pm 0.0665$, $t(200.0) = 0.92$, $p = 0.3561$). Sex did not significantly predict alcohol consumption ($\beta = -0.1809 \pm 0.1208$, $t(22.3) = -1.50$, $p = 0.1485$). There was an effect of time point, such that drinking decreased over the 24-hr measurement period ($\beta = -0.1114 \pm 0.0541$, $t(176.7) = -2.06$, $p = 0.0410$). Importantly, when animals had continuous access to alcohol, oxytocin did not significantly reduce consumption ($\beta = -0.2125 \pm 0.2228$, $t(176.4) = -0.95$, $p = 0.3415$), and there was no significant interaction between oxytocin treatment and time point ($\beta = -0.1020 \pm 0.0541$, $t(176.8) = 1.88$, $p = 0.0623$). Age did significantly affect alcohol consumption per hour in animals with

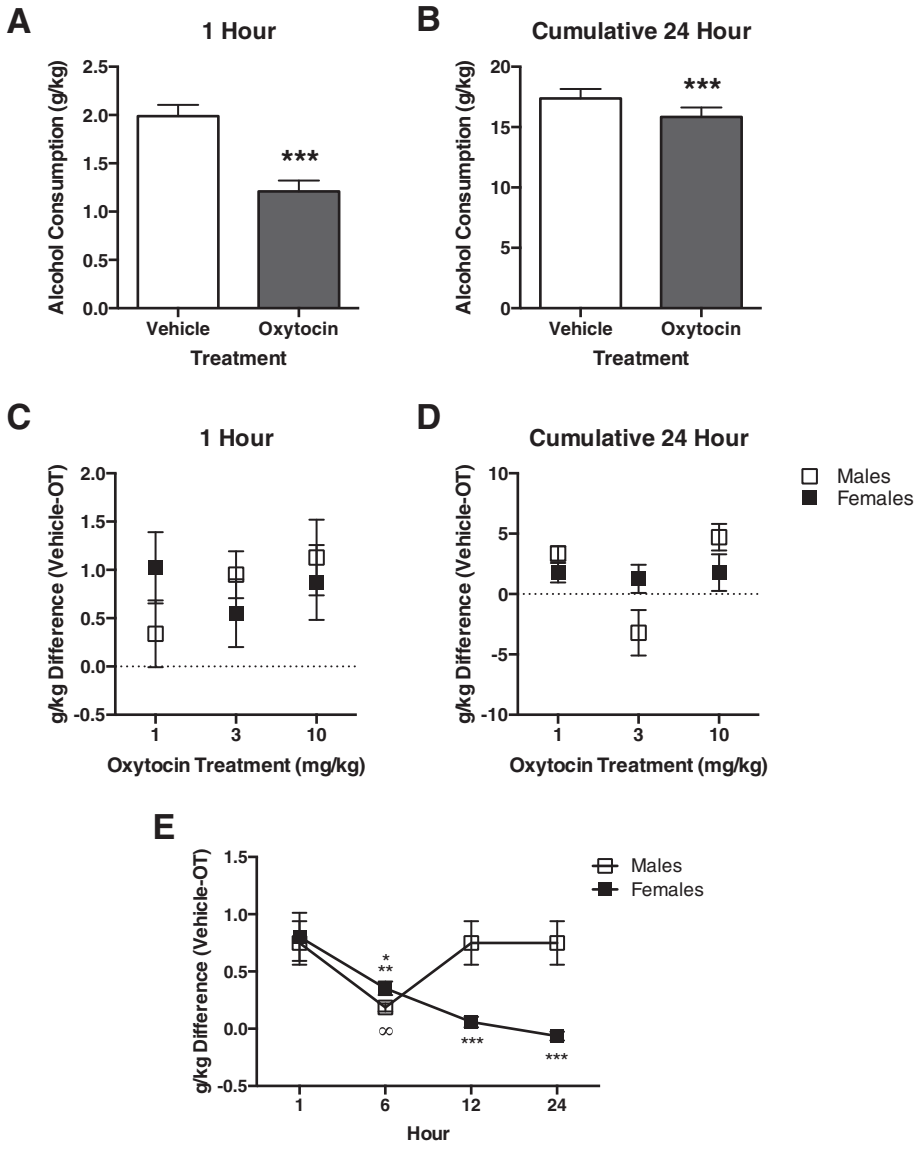

Fig. 1. Chronic Intermittent Access. A and B show the significant main effect (doses and sexes combined) of oxytocin on mean ± SEM alcohol consumption (g/kg) after 1 h (A) and after 24 h (B). Open bars show consumption after vehicle injections, and gray bars show consumption after oxytocin injections. ***$p < 0.0001$. C and D. Mean ± SEM alcohol consumption (g/kg) difference scores (Vehicle g/kg − OT g/kg) for each dose of oxytocin in males and females after 1 h (C) and after 24 h (D) are shown. Open squares represent male difference scores and black squares represent female difference scores. Larger difference scores indicate a larger effect of oxytocin. Difference scores are within subject, and each animal received only one dose of oxytocin. E. Mean ± SEM difference scores (Vehicle g/kg − OT g/kg) for males and females at each time point (doses combined) are shown. In females the effect of oxytocin was significantly stronger at hour 1 compared to all other time points, showing that oxytocin's effects diminished after the first hour. In males, difference scores at hour 6 were significantly different from all other time points, but the time course of oxytocin's effects was unclear in males due to variability among individuals. **$p < 0.01$ females hr. 6 compared to females hr. 1, *$p < 0.05$ females hr. 6 compared to females hr. 24, ***$p < 0.0001$ females hrs 12 and 24 compared to females hr. 1, ∞$p < 0.01$ male hr. 6 compared to all other time points for males.

Table 4
Effect on chronic intermittent access alcohol consumption (g/kg).

Factor	Standardized β (SEM)	t score	*p*-Value
Oxytocin	− 0.21 (0.04)	− 5.18	< 0.0001*
Time point	− 0.40 (0.04)	− 11.30	< 0.0001*
Oxytocin * time point	0.18 (0.04)	4.95	< 0.0001*
Water consumption	− 0.05 (0.04)	− 1.10	0.2738
Sex	0.13 (0.06)	2.17	0.0337*
Age	0.03 (0.06)	0.44	0.6599

* $p < 0.0001$

Table 5
Continuous access mean (SEM) alcohol consumption (g/kg).

	1 h		Cumulative 24 h	
	Vehicle	Oxytocin	Vehicle	Oxytocin
Males	1.9 (0.4)	1.2 (0.3)	18.6 (2.9)	19.5 (3.1)
Females	0.7 (0.2)	0.4 (0.2)	19.7 (2.7)	16.8 (2.5)

Table 6
Continuous access mean (SEM) water consumption (mL).

	1 h		Cumulative 24 h	
	Vehicle	Oxytocin	Vehicle	Oxytocin
Males	0.5 (0.1)	0.4 (0.1)	10.4 (1.7)	10.5 (1.2)
Females	0.4 (0.1)	0.1 (0.1)	10.8 (1.6)	9.7 (1.2)

Table 7
Continuous access mean (SEM) preference ratio.

	1 h		Cumulative 24 h	
	Vehicle	Oxytocin	Vehicle	Oxytocin
Males	0.5 (0.1)	0.5 (0.1)	0.4 (0.1)	0.4 (0.1)
Females	0.4 (0.1)	0.6 (0.1)	0.4 (0.1)	0.3 (0.1)

continuous access ($\beta = -0.3251 \pm 0.1206$, $t(22.3) = -2.69$, $p = 0.0131$), such that older animals drank less alcohol. Because age significantly predicted consumption, we also included an interaction between age and oxytocin treatment as a possible predictor, but this

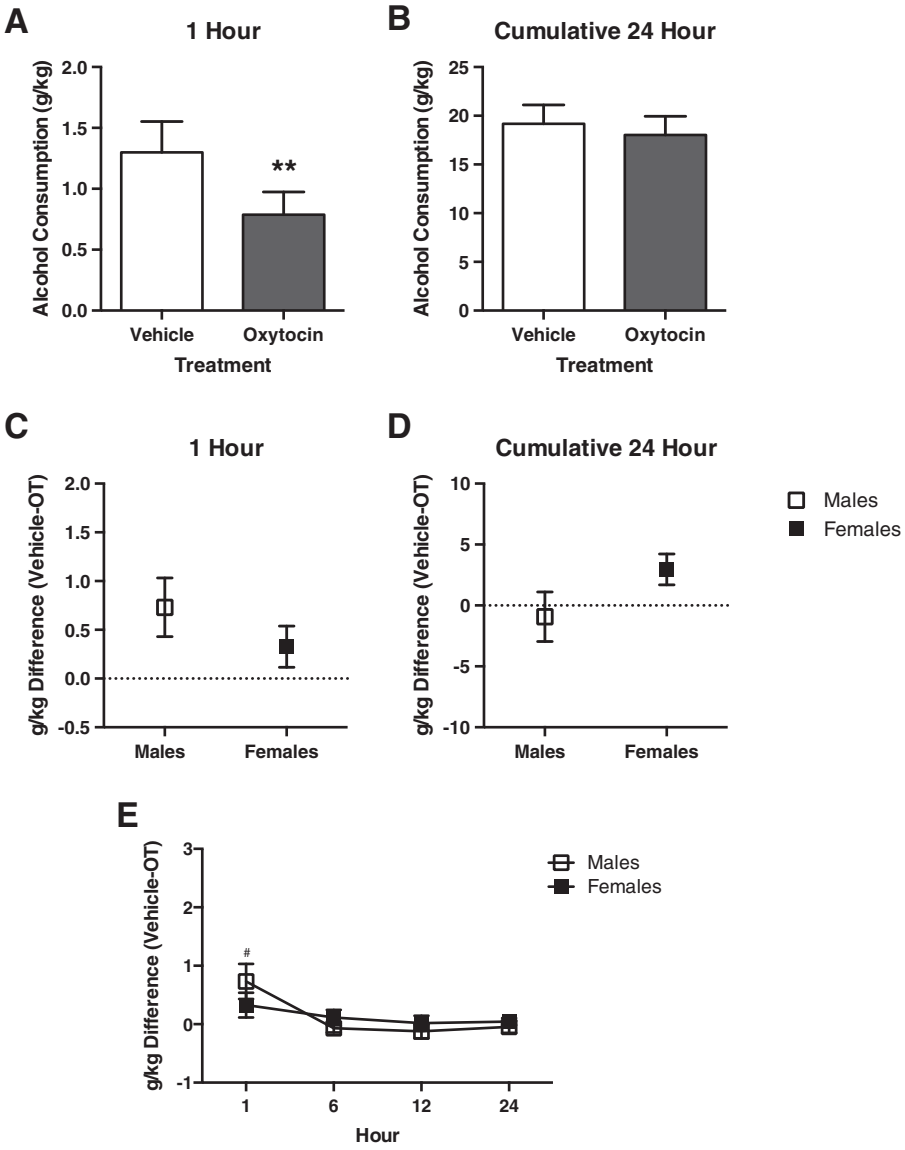

Fig. 2. Continuous Access. A and B show a significant main effect of oxytocin (sexes combined) on mean ± SEM alcohol consumption (g/kg) after 1 h (A) but no overall effect on cumulative alcohol consumption over 24-hours (B). Open bars show consumption after vehicle injections, and gray bars show consumption after oxytocin injections. $^{**}p < 0.01$. C and D. Mean ± SEM difference scores (Vehicle g/kg − OT g/kg) for males and females after 1 h (C) and after 24 h (D). Open squares represent male difference scores and black squares represent female difference scores. Larger difference scores indicate a larger effect of oxytocin. Difference scores are within subject. E. Mean ± SEM difference scores (Vehicle g/kg − OT g/kg) for males and females at each time point are shown. In males the effect of oxytocin was stronger at hour 1 compared to all other time points, $\#p < 0.05$ males hr. 1 compared to all other hrs in males. In females, there were no significant differences between any time points.

Table 8
Effect on continuous access alcohol consumption (g/kg).

Factor	Standardized β (SEM)	t score	*p*-Value
Oxytocin	− 0.21 (0.22)	− 0.95	0.3415
Time point	− 0.11 (0.05)	− 2.06	0.0410*
Oxytocin * time point	− 0.10 (0.05)	1.88	0.0623
Water consumption	0.06 (0.07)	0.92	0.3516
Sex	− 0.18 (0.12)	− 1.50	0.1485
Age	− 0.33 (0.12)	− 2.69	0.0131*
Age * oxytocin	0.01 (0.02)	0.60	0.5474

* $p < 0.0001$

interaction was not significant, indicating that the effects of oxytocin did not depend on age (β = 0.0123 ± 0.0203, t(176.4) = 0.60, $p = 0.5474$), see Table 8.

Linear mixed effects regression results (random intercepts by subject) are shown. Time point significantly predicted alcohol consumption (animals drank less over time). Older animals drank less alcohol, but there was no interaction between age and the effects of oxytocin.

None of the factors in the regression significantly predicted water consumption in animals with continuous access to alcohol (oxytocin, β = − 0.1069 ± 0.0578, t(179) = − 1.85, $p = 0.0660$; time point β = 0.0669 ± 0.0578, t(179) = 1.16, $p = 0.2485$; oxytocin x time point interaction β = 0.0836 ± 0.0579, t(179) = 1.44, $p = 0.1504$; and sex β = − 0.0998 ± 0.1242, t(24) = − 0.80, $p = 0.4295$). Oxytocin did not significantly predict alcohol preference, but time did (oxytocin β = 0.0218 ± 0.0499, t(171.8) = 0.44, $p = 0.6621$; time point β = − 0.107 ± 0.0503, t(172.1) = − 2.13, $p = 0.0347$; oxytocin x time point interaction β = − 0.0383 ± 0.0503, t(171.8) = − 0.76, $p = 0.4467$; sex β = − 0.0359 ± 0.1555, t(23.7) = − 0.23, $p = 0.8195$).

3.3. Social facilitation of alcohol drinking in prairie voles

Others have previously shown that when paired, prairie voles tend to drink similar amounts of alcohol as their cage mate in a way that suggests that they actively match their consumption patterns [1–3,22]. We tested this in our experiment with a mixed effects regression trying to predict one vole's alcohol consumption (in g/kg) from its partner's consumption. We controlled for day (1 through 11), sex, and whether they had intermittent or continuous access to alcohol. Treatment days were not included in the analyses. We allowed random intercepts for each cage. Animals consumed more alcohol over days (β = 0.104 ± 0.03001, t(445) = 3.47, $p = 0.0006$). Sex did not significantly predict alcohol consumption (β = 0.1327 ± 0.1088, t

Table 9
Matched alcohol consumption (g/kg) by cage mates.

Factor	Standardized β (SEM)	t score	*p*-Value
Day	0.10 (0.03)	3.47	0.0006*
Animal A consumption (g/kg)	0.11 (0.05)	2.52	0.0121*
Sex	0.13 (0.12)	1.22	0.2289
Animal A consumption * day	− 0.01 (0.03)	− 0.22	0.8827
Intermittent vs continuous	− 0.01 (0.25)	0.05	0.9582

* $p < 0.0001$

(45.4) = 1.22, $p = 0.2289$), and neither did the alcohol access method (β = − 0.0130 ± 0.2458, t(45.1) = 0.05, $p = 0.9582$), but the alcohol consumption patterns of one prairie vole did significantly predict the consumption patterns of the other, suggesting some degree of socially facilitated drinking (β = 0.1135 ± 0.0451, t(487.2) = 2.52, $p = 0.0121$). There was no significant effect of the interaction between cage mate and day (β = − 0.0068 ± 0.0312, t(451.9) = − 0.22, $p = 0.8827$), indicating that the degree of matching was not dependent on the day of alcohol access, see Table 9. Cage mates did not significantly match their water consumption (β = − 0.0326 ± 0.0838, t(304.0) = − 0.39, $p = 0.1322$) or their alcohol preference (β = 0.0830 ± 0.1025, t(17.5) = 0.81, $p = 0.2847$).

Linear mixed effects regression results (random intercepts by cage) are shown. Alcohol consumption by one cage mate significantly predicted alcohol consumption by the other cage mate. Animals drank more over days.

The same pattern of social facilitation is evident when consumption is measured in mL instead of g/kg (day β = 0.0707 ± 0.0294, t(443.4) = 2.40, $p = 0.0169$; sex β = − 0.1272 ± 0.1075, t(44.6) = − 1.18, $p = 0.2428$; cage mate β = − 0.107 ± 0.0503, t(487.1) = − 2.13, $p = 0.0347$; cage mate x day interaction β = − 0.0221 ± 0.0312, t(451.9) = − 0.71, $p = 0.4786$).

To determine if reducing one animal's alcohol consumption with oxytocin would also reduce alcohol consumption in the cage mate, one animal in each cage was treated with the highest dose of oxytocin (10.0 mg/kg). First, a mixed effects regression analysis was performed to determine if 10.0 mg/kg oxytocin reduced alcohol consumption only in the animals that received the treatment, controlling for water consumption and sex, neither of which significantly predicted alcohol consumption, (water β = − 0.0597 ± 0.2557, t(16.8) = − 0.23, $p = 0.8182$; sex β = − 0.1671 ± 0.3142, t(9.9) = − 0.53, $p = 0.6066$). Oxytocin did significantly reduce alcohol consumption (β = − 0.5798 ± 0.2618, t(13.1) = − 2.22, $p = 0.0415$). Oxytocin also significantly reduced alcohol preference (oxytocin β = − 0.7490 ± 0.1749, t(10.0) = − 4.28, $p = 0.0016$; sex t(9.0) = 0.04, $p = 0.9660$). Notably, water consumption significantly increased with oxytocin (β = 0.7504 ± 0.1947, t(10.0) = 3.85, $p = 0.0032$). Sex had no significant effect on water consumption (β = − 0.4140 ± 0.2480, t(9.0) = − 1.67, $p = 0.1293$).

Because oxytocin reduced alcohol consumption in these voles, we used a mixed effects regression to determine if an oxytocin-induced reduction in alcohol consumption in one animal would cause its cage mate to similarly reduce its alcohol consumption; however, we found no evidence of this (β = 0.1613 ± 0.3289, t(9) = 0.49, $p = 0.6356$, controlling for the same factors as above). That said, these particular animals in the continuous access experiment did not match alcohol intake levels, even on days when animals all received vehicle treatment (β = 0.3554 ± 0.3116, t(9) = 0.49, $p = 0.2834$).

Therefore, to test the possibility that social facilitation is present overall, but that there are large individual differences among cages, we first re-ran the above regression on each cage individually across days, giving us the degree of social facilitation per cage. Six of the 49 animals showed strong social facilitation (smallest effect $t = 2.36$, $p = 0.045$), with the average slope β = 1.19 ± 0.13 (min = 0.76, max = 1.71). A slope near one indicates almost perfect social facilitation. Three of the 49 animals showed the exact opposite; when one prairie vole drank more, the other drank less (smallest effect $t = -3.097$, $p = 0.012$), with an average slope of β = − 0.875 ± 0.075 (min = − 0.79, max = − 1.02). But in 39 of the 49 cages (81%), there is no evidence of social facilitation (minimum $p = 0.083$, maximum $p = 0.98$, mean = 0.51 ± 0.043). That being said, 30 of the 49 cages (62%) had positive slopes, with the average slope β = 0.18 ± 0.09. This may be why there is, overall, evidence for social facilitation, but the results of any given pair may differ, Fig. 3.

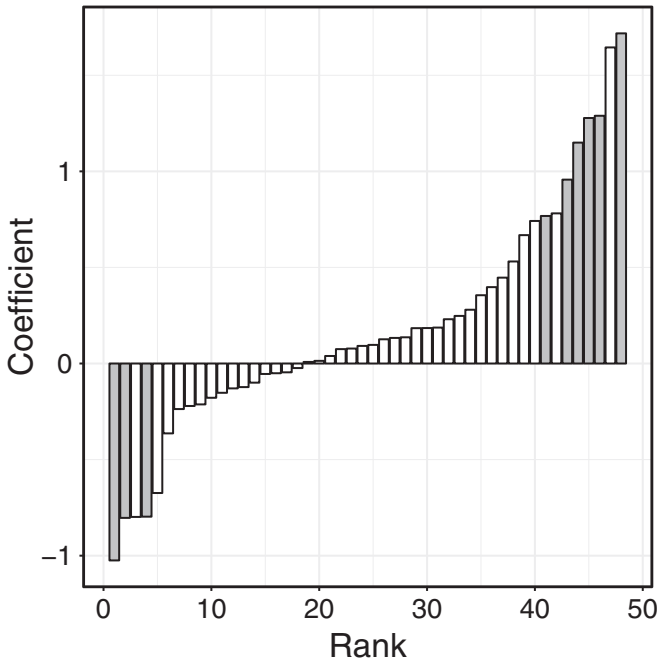

Fig. 3. Social Facilitation of Drinking. Cages were ranked along the x-axis according to the relationship of alcohol consumption (g/kg) between cage mates. The y-axis shows the correlation coefficient. The cages that matched their drinking more were ranked higher. The gray bars show cages in which the consumption by one animal in the cage significantly predicted consumption by the other animal. Notice that 9 cages showed consumption by one cage mate significantly predicted consumption by the other cage mate. Also note that of those 9 cages, 6 showed a positive correlation, while the other 3 showed a negative correlation. However, 30 of the 48 cages showed a positive relationship slope for consumption by cage mates.

Further, this may give insight into why the prairie voles treated with oxytocin did not show any social facilitation, even when given saline. To test this more rigorously, we obtained bootstrapped estimates of the regression run on a random subset of 12 subjects at a time (the same number of animals used in the oxytocin treatment). That is, we reran the overall regression 10,000 times, each time only using a random 12 animals. This gives us a reasonable null hypothesis for the degree to which any 12 animals in our study will show social facilitation. In the 10,000 bootstrapped samples, the mean β estimate for social facilitation was β = 0.10 ± 0.001, but the average p value was 0.29 ± 0.003, with only 32% of the samples significant at the 0.05 level. As a point of comparison, the 12 animals given oxytocin as an attempt to experimentally induce socially facilitated drinking had β = 0.3554 ± 0.3116, t(9) = 0.49, $p = 0.2834$.

We also performed this bootstrap test using the simpler correlation coefficient on the mean consumption of the two cage mates to more closely match analysis of social facilitation in previous studies (see [1–3,22]). In 10,000 bootstrapped samples, the mean correlation coefficient was 0.56 ± 0.003, but the average p value was 0.13 ± 0.002, with only 56% of the samples significant at the 0.05 level. As a point of comparison, the 12 animals given oxytocin as an attempt to experimentally induce socially facilitated drinking had as its

correlation coefficient 0.51, t(12) = 2.002, $p = 0.07$. Together, these results suggest that there are large individual differences in socially facilitated drinking, and that our failure to experimentally induce social facilitation is likely the result of sampling differences: our sample treated with oxytocin was not among the social facilitated drinkers in the first place.

3.4. Effects of alcohol and oxytocin on locomotor activity and anxiety-like behavior

We used a mixed effects model (again allowing random intercepts per subject) to predict locomotor activity (in total distance traveled) from oxytocin dose (0.0, 1.0, 3.0, and 10.0 mg/kg), ethanol treatment (0.0 and 2.0 g/kg), sex, age, and locomotor activity of each vole on the previous day (as a control). As expected, ethanol significantly increased the distance traveled (β = 0.3216 ± 0.1416, t(119.0) = 2.27, $p = 0.0249$), but oxytocin did not significantly alter distance traveled (β = 0.0750 ± 0.1009, t(119.0) = − 0.74, $p = 0.4591$), and there was no significant interaction between oxytocin and ethanol (β = − 0.0857 ± 0.14028, t(119.0) = − 0.60, $p = 0.5499$). Day 2 significantly predicted distance traveled on day 3 (the treatment day) (β = 0.5458 ± 0.0725, t(119.0) = 7.52, $p < 0.0001$), suggesting a consistent amount of locomotor activity within a prairie vole. Males traveled significantly greater distances than females (β = − 0.1732 ± 0.0810, t(119.0) = − 2.14, $p = 0.0347$). Age did not significantly predict distance traveled (β = 0.0792 ± 0.1080, t(119.0) = 0.73, $p = 0.4647$), the interaction term (ethanol * age) was not significant, indicating that the effects of ethanol did not depend on age (β = − 0.0782 ± 0.1441, t(119.0) = − 0.54, $p = 0.5880$), Table 10 and Fig. 4A and B.

The time spent in the center of the open field showed the same general pattern of results: ethanol significantly increased time spent in the center (β = 0.5517 ± 0.1512, t(118.0) = 3.65, $p = 0.0004$), but oxytocin had no significant effect (β = − 0.0432 ± 0.1081, t(118.0) = − 0.40, $p = 0.6904$). There was no significant interaction between oxytocin and ethanol (β = 0.1129 ± 0.1550, t(118.0) = 0.73, $p = 0.4679$). However there was a significant interaction between ethanol and sex, such that ethanol increased time in center for females to a greater extent than males (β = 0.4048 ± 0.1706, t(118.0) = 2.37, $p = 0.0193$). Although age did not significantly predict time in center (β = 0.0131 ± 0.1233, t(118.0) = 0.11, $p = 0.9149$), there was a significant interaction between the effects of age and ethanol (β = 0.4452 ± 0.1728, t(118.0) = 2.58, $p = 0.0112$), in which older animals showed a greater effect of ethanol on time in center. There was no significant effect of sex overall (β = − 0.0192 ± 0.1241, t(118.0) = − 0.15, $p = 0.8773$), and time spent in the center on the previous day did not significantly `1predict time spent in the center on Day 3 (β = 0.0744 ± 0.0799, t(118.0) = 0.93, $p = 0.3535$) (Table 10, Fig. 4C and D).

Table 10
Ethanol increased distance traveled and time in center.

	Factor	Standardized β (SEM)	t score	*p*-Value
Distance traveled	Oxytocin	0.07 (0.10)	− 0.74	0.4591
	Ethanol	0.32 (0.14)	2.27	0.0259*
	Oxytocin * ethanol	− 0.09 (0.14)	− 0.60	0.5499
	Day 2 distance	0.55 (0.07)	7.52	< 0.0001*
	Sex	− 0.17 (0.08)	− 2.14	0.0347*
	Age	0.08 (0.11)	0.73	0.4647
	Age * ethanol	0.08 (0.14)	0.54	0.5880
Time in center	Oxytocin	− 0.04 (0.11)	− 0.40	0.6904
	Ethanol	0.55 (0.15)	3.65	0.0004*
	Oxytocin * ethanol	0.11 (0.16)	0.73	0.4679
	Ethanol * sex	0.40 (0.17)	2.37	0.0193*
	Day 2 time in center	0.07 (0.08)	0.93	0.3535
	Sex	0.02 (0.12)	− 0.15	0.8773
	Age	0.01 (0.12)	0.11	0.9149
	Age * ethanol	0.44 (0.17)	2.58	0.0112*

* $p < 0.0001$

Linear mixed effects regression results (random intercepts by subject) are shown. Ethanol significantly increased the distance traveled. Day 2 Distance significantly predicted Day 3 Distance traveled; animals that were more active on Day 2 were also more active on Day 3. Males showed more locomotor activity than females overall. Ethanol increased time in the center, and there was a strong interaction between ethanol and sex, such that ethanol increased time in the center much more in females. There was also an age by ethanol interaction; older animals showed more time in the center in response to ethanol. For both distance traveled and time in center, there was no significant interaction between oxytocin and ethanol.

4. Discussion

The data presented here show that oxytocin is effective at reducing voluntary alcohol consumption in prairie voles, supporting studies in other animal models and drinking methods. We found, however, that the effects of oxytocin are not long lasting in this species, producing the largest effect on alcohol drinking in the first hour of alcohol access. We also demonstrate that ethanol produces acute locomotor activation and, in females in particular, anxiolytic effects in prairie voles at a dose of 2 g/kg, but that oxytocin itself does not have effects on general locomotor activity or anxiolytic effects. Finally, our data support a role of social facilitation in alcohol consumption in prairie voles, but identify that not all pairs show social facilitation. Together these data broaden our understanding of the effects of oxytocin on alcohol consumption, and expand the characterization of alcohol consumption and responses to alcohol in this high alcohol consuming species.

Oxytocin reduced alcohol consumption at all doses in animals with chronic intermittent access to alcohol. The reduction in alcohol consumption was not due to a general decrease in fluid intake, as water consumption did not predict alcohol consumption, and a mixed model ANOVA showed a slight increase in water consumption. Furthermore, the effects of oxytocin on alcohol drinking are not associated with an overall decrease in locomotor activity, as the distance traveled in the locomotor test was not significantly affected by oxytocin treatment at any dose. In this high alcohol consuming species, oxytocin reduced chronic intermittent consumption by about 40% in the first hour, but only reduced 24-hour consumption by a small amount that was probably not biologically meaningful. The first hour effects are comparable to reductions observed following peripheral oxytocin administration in rats [30] and mice [26,36] at similar doses of oxytocin in limited access models (note that MacFadyen et al. and King et al. also found similar reductions with lower doses of oxytocin). At the highest dose, oxytocin produced a more significant reduction in alcohol consumption over 24-hours in males, which was similar to the reduction observed by Peters et al. [36] in male mice at this dose. Even with this larger reduction at the highest dose of oxytocin, prairie voles still consumed a relatively high amount of alcohol over the 24-hour period. Our diminished effects over the 24-hour period are in contrast to McGregor and Bowen [31] who observed a 6-week decrease in alcohol consumption in rats. This difference could be due to the species used or their alcohol solution, which contained sugar and less alcohol than the 15% ethanol solution used here. Because all doses produced similar effects on alcohol drinking, future studies should investigate a wider range of doses to detect the threshold for oxytocin's effects on alcohol drinking in prairie voles. Overall, our data support the findings from studies in mice and rats showing that oxytocin can reduce drinking in several models of free access, limited access, and operant self-administration of alcohol.

In animals with continuous access to alcohol, oxytocin reduced alcohol consumption by 40% in the first hour, but had no significant effect on alcohol consumption beyond the first hour. Because these

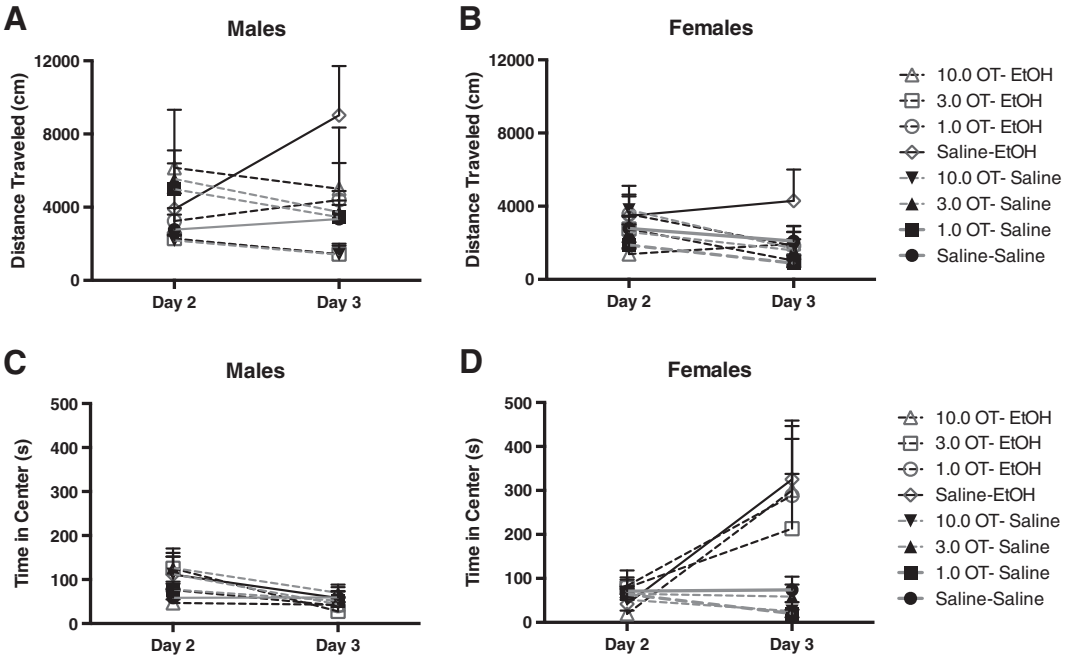

Fig. 4. Locomotor Activity. Mean ± SEM distance traveled (cm) (A and B) and mean ± SEM time in Center (s) (C and D) are shown. Day 2 is the second habituation day, Day 3 is the treatment day on which animals received oxytocin (1.0, 3.0, or 10.0 mg/kg) or saline, then 30-min later received ethanol (2 g/kg) or saline before being placed immediately in the locomotor chamber for 15 min. Solid gray lines are used for the Saline-Saline group, dashed gray lines are used for groups that received oxytocin then saline, solid black lines are used for groups that received saline then ethanol, and dashed black lines are used for groups that received oxytocin then ethanol. Males (A and C) and Females (B and D) are graphed separately for clarity. A and B. Ethanol significantly increased the distance traveled, but oxytocin did not alter distance traveled, and there was no interaction between ethanol and oxytocin. Females traveled significantly shorter distances than males overall. C and D. Ethanol significantly increased time in the center, while oxytocin had no significant effect. There was a significant interaction between ethanol and sex, producing clear anxiolytic-like effects in females, but not in males.

animals were run separately from animals with chronic intermittent access to alcohol, and because only one dose of oxytocin was tested, we cannot directly compare the effects of oxytocin across these two studies. However, these data suggest that the method of alcohol access (continuous versus chronic intermittent) may affect the ability of oxytocin to reduce alcohol consumption. Although the effects of oxytocin differed, baseline alcohol consumption was similar in both methods of access in our study. Chronic intermittent alcohol consumption matched what has previously been reported in mice; however, those previous studies reported significantly higher consumption with chronic intermittent access compared to continuous access [9,24,33]. In prairie voles, intermittent access did not enhance 24-hour intake in our animals, although first hour consumption was higher with chronic intermittent access. In both drinking experiments alcohol consumption diminished over the 24-hour test period. In the chronic intermittent access experiment, treatments were given in the morning 30 min before bottles were put on the cage; in the continuous access experiment all bottles were removed just before treatments were given and then returned 30 min after in order to ensure that the pre-treatment window was equivalent in both experiments. Therefore, even though access to alcohol was mostly continuous, the 30 min without alcohol may have elevated consumption at the beginning of the 24-hour period. Because of this, the strongest effects of oxytocin were paired with the highest alcohol consumption in both experiments, and enhanced consumption at the start of the 24-hour period could be partially responsible for the augmented effect of oxytocin in the first hour.

Ethanol administration produced acute locomotor activation and, in females, an anxiolytic response as indexed by increased time spent in the center of an open field. This indicates that prairie voles show similar responses to other commonly used rodent species to an acute injection of ethanol at a non-sedative dose [11,38]. Interestingly, oxytocin did not produce locomotor deficits at any dose alone or in combination with ethanol. This is important because locomotor effects were tested 30 min after oxytocin administration (the same time frame for oxytocin administration and alcohol exposure in the drinking studies); therefore oxytocin alone or in combination with consumed ethanol did not produce significant non-specific locomotor deficits. Previous studies have shown reductions in locomotor activity in rats and mice following oxytocin administration [20,21,26,45]. It is possible that we would have observed a decrease in locomotor activity if we had assessed locomotor behavior for a longer duration, similar to previous studies; however, the duration of our test was chosen to match the literature that describes the acute locomotor activating effects of ethanol [16,17,42]. Because locomotor activity was relatively low overall, it is also possible that a floor effect prevented us from observing effects of oxytocin on locomotor activity. Although we observed that some individuals appeared to have decreased locomotor activity after administration of the highest dose of oxytocin, the fact that there was a slight but statistically significant increase in water drinking in oxytocin treated animals with intermittent access to alcohol suggests that any subtle effects on locomotor activity likely did not impede fluid consumption. In general, we observed that there were large individual differences in the locomotor responses to ethanol and oxytocin.

Multiple studies demonstrate that prairie voles tend to match their alcohol consumption to their cage mate's [1–3,22]. Here we show overall support for this phenomenon. Animals across chronic intermittent and continuous alcohol drinking experiments showed that consumption by one animal predicted consumption by the other animal in the cage. Water consumption was not matched, suggesting this matching behavior was alcohol specific in our study. In contrast to an earlier study of social facilitation of drinking in prairie voles, cage mates did not match their alcohol preference [2], but the lack of preference matching is probably driven by the lack of matching with water consumption. However, we failed to show that reducing consumption by one animal via treatment with oxytocin could reduce drinking in its cage mate. We also demonstrate here that, while averaging consumption across cages and days yields support for social facilitation of drinking in prairie voles, a closer look at individual days and cages

reveals a more nuanced story. There is significant variability across cages, with some cages showing matched consumption by cage mates, some cages showing no relationship between the consumption by cage mates, and some cages showing an inverse relationship between the consumption by cage mates. Individual differences in matching of alcohol consumption are supported by a study by Anacker and Ryabinin [3] in which housing conditions were manipulated such that high drinkers were paired with low drinkers. In response to this manipulation, some high drinkers lowered their alcohol consumption, however others did not alter their consumption. Our data on social influences of drinking in prairie voles provide additional rationale for a deeper investigation of individual differences in social facilitation of alcohol consumption.

This study was the first to investigate sex differences in the effects of oxytocin on alcohol drinking. Small but significant sex differences were evident in both alcohol consumption and locomotor activity. Females tended to drink more than males overall in the chronic intermittent access method, similar to findings from C57BL/6J mice [24]. Interestingly, females did not drink more than males in the continuous access method, and a mixed model ANOVA showed that males drank more than females in the first hour. In contrast, female C57BL/6J mice drank more than males with two-bottle continuous access alcohol consumption [47]. A previous study comparing consumption of 3, 6, and 10% alcohol did not find any sex differences in alcohol consumption by prairie voles [1], while our experiments measured consumption of 15% ethanol, suggesting that sex differences in alcohol consumption by prairie voles may depend on the method of alcohol access and the concentration of alcohol being consumed. Our difference score analyses also indicate sex differences in the effects of oxytocin on drinking. Chronic intermittent access females show a clear reduction in the effectiveness of oxytocin over the 24-hour test period, while the pattern in males is less clear. In continuous access females we see almost no effect of oxytocin, while in males there is an effect at one hour, but not after. We also found that females showed less overall locomotor activity than males. Interestingly, ethanol significantly increased time in the center of an open field in females to a much greater extent than males, suggesting the anxiolytic effects of ethanol are much stronger in female prairie voles. They were not differentially sensitive to oxytocin effects on locomotion or time in center, however. Because prairie voles are induced ovulators (they only ovulate after exposure to a reproductive male and have no spontaneous estrous cycle), sex differences were not influenced by an estrous cycle in females. Our data demonstrate sex differences in baseline alcohol drinking, the effects of oxytocin on alcohol drinking, locomotor behavior, and the anxiolytic effects of ethanol.

It remains unclear how oxytocin reduces alcohol consumption. Although interactions between oxytocin and the mesolimbic dopamine system as well as direct effects of oxytocin on GABA receptors have been identified [6,37], much work remains to understand how oxytocin produces its effects on alcohol consumption. It is likely that oxytocin acts at oxytocin receptors to affect alcohol consumption, and oxytocin receptors have been identified on ventral tegmental neurons that project to the nucleus accumbens [35]. It is also possible that oxytocin produces its effects at the vasopressin 1a receptor (V1a), as multiple effects of peripheral oxytocin administration are blocked by a V1a receptor antagonist [21]. While future studies will need to investigate the mechanism of oxytocin's effects on alcohol drinking, our data add to a body of literature that supports the potential for oxytocin or oxytocin analogues as AUD treatments.

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