Physiology & Behavior 151 (2015) 469–477

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Social housing conditions and oxytocin and vasopressin receptors contribute to ethanol conditioned social preference in female mice

Ruth I. Wood [a,*], Allison T. Knoll [c], Pat Levitt [b,c]

[a] Department of Cell and Neurobiology, Keck School of Medicine of the University of Southern California, Los Angeles, CA 90033, United States
[b] Department of Pediatrics, Keck School of Medicine of the University of Southern California, Los Angeles, CA 90033, United States
[c] Children's Hospital Los Angeles, Los Angeles, CA 90027, United States

HIGHLIGHTS

- Wild-type female mice show ethanol-induced conditioned social preference (CSP).
- Oxytocin and vasopressin 1a receptors are required for ethanol-induced CSP.
- Wild-type sisters pair-housed with knock-out females also lack ethanol-induced CSP.
- Aberrant behavior of siblings and cage-mates can disrupt behavior of normal mice.

ARTICLE INFO

Article history:
Received 4 June 2015
Received in revised form 23 July 2015
Accepted 14 August 2015
Available online 15 August 2015

Keywords:
Alcoholic intoxication
Animal behavior
Social behavior
Oxytocin receptors
Vasopressin receptors

ABSTRACT

Social behavior modulates response to alcohol. Because oxytocin (OXT) and vasopressin (AVP) contribute to rewarding social behavior, the present study utilized a genetic strategy to determine whether OXT and AVP receptors (OXTR, AVPR1a) are essential for female mice to demonstrate a conditioned social preference for ethanol. The study compared wild-type (WT) and knock-out (KO) females lacking either *Oxtr* or *Avpr1a* in a conditioned social preference (CSP) test. KO females and WT females from Het–Het crosses were pair-housed: KO and WT (ko). WT females from Het–WT crosses were pair-housed: WT(wt). Test mice received 2 g/kg ethanol or saline ip, and were paired four times each with one stimulus female (CS−) after saline, and with another female (CS+) following ethanol. After pairing, the time spent with CS+ and CS− females was measured. WT(wt) females showed conditioned preference for the CS+ female paired with ethanol, demonstrated by greater interaction time ($p < 0.05$). In both KO lines, ethanol significantly reduced interaction with the CS+ female ($p < 0.05$), and there was no change in interaction for WT(ko) females. Response to odors by habituation–dishabituation was unaffected in both KO lines, and the response to a hypnotic dose of ethanol also was the same as in WT mice. However, anxiety, measured as time on the open arms of the elevated plus maze, was reduced in KO^{Oxtr} females compared with WT(wt). The results suggest that *Oxtr* and *Avpr1a* are required for conditioned effects of an ethanol-associated social stimulus. The lack of CSP in WT(ko) females suggests that the quality of social interactions during postnatal and postweaning life may modulate development and expression of normal social responses.

1. Introduction

Oxytocin (OXT) and vasopressin (AVP) mediate complex endocrine and behavioral functions. The hormones regulate parturition, lactation and blood pressure peripherally, and act in the brain as neuromodulators to promote social behavior and social bonding (reviewed in [1,2]). OXT and AVP facilitate individual recognition, but also enhance the rewarding aspects of social interaction. It is unknown how these neuropeptides might contribute to regulating behaviors that are enhanced in social settings. For example, social behavior is an important contributor to the use of drugs and alcohol. There is, therefore, the potential for a convergence of OXT/AVP and ethanol reward in a social context.

OXT and AVP promote both social recognition and reward. Social recognition is absent in mice lacking OXT, and is facilitated in rats by exogenous OXT [3]. AVP neurons are present in central olfactory pathways [4] that mediate social recognition [5]. However, OXT and AVP also promote rewarding social interactions. In humans, exogenous OXT has calming effects similar to those of social support in stressful settings,

* Corresponding author at: Department of Cell and Neurobiology, Keck School of Medicine at the University of Southern California, 1333 San Pablo St., BMT 408a, Los Angeles, CA 90033, United States.
E-mail address: riw@usc.edu (R.I. Wood).

http://dx.doi.org/10.1016/j.physbeh.2015.08.018
0031-9384/

reduces threat-related amygdala activity and increases both trust and generosity [6]. Chronic social stress reduces AVP mRNA in the hypothalamic paraventricular nucleus of male mice [7]. Among monogamous prairie voles, OXT and AVP are essential for pair-bonding [1], and these effects are mediated by central OXT receptors (*Oxtr*; [8]) and AVP 1a receptors (*Avpr1a*; [9,10]).

In humans, peer interactions often promote ethanol consumption [11,12]. In addition, moderate consumption of ethanol promotes social interactions in humans [13]. Similar relationships exist in animals. Adolescent rats paired with an intoxicated cage-mate will voluntarily consume more ethanol than rats paired with a sober companion [14]. Furthermore, meadow and prairie voles drink more ethanol in a social condition than in a non-social setting [15]. Our laboratory has used a conditioned social preference (CSP) model to explore conditioned reinforcing effects of an ethanol-associated stimulus [16,17]. CSP is derived from conditioned place preference (CPP), a well-established model to study the motivational effects of drugs and other unconditioned stimuli (US) [18]. In CPP, the US is paired repeatedly with a unique environment (conditioned stimulus, CS+) while the control is paired with a separate environment (CS−). In CSP, the CS+ and CS− are unfamiliar stimulus mice. Using this model, male [16] and female mice [17] show a conditioned preference for the CS+ stimulus female with whom they have been intoxicated previously. Specifically, female mice show CPP [19] and CSP [17] in response to 2 g/kg ethanol, the dose used in the present study. CSP has also been used to demonstrate sexual reward in quail [20] and rats [21,22], as well rewarding effects of OXT in mice [23].

Recent evidence demonstrates that OXT and AVP modify responses to ethanol. OXT reduces ethanol consumption in wild-type (WT) rats [24] and mice [25]. Reports of ethanol consumption in mice lacking *Avpr1a* (KO^{Avpr1a}) are conflicting: one study of males and females found no effect [26], while another reported increased ethanol consumption and preference in males, with limited effects in KO^{Avpr1a} females [27]. Because OXT and AVP enhance social behavior [28] but reduce drug [29,30] and ethanol consumption [24,25], we hypothesized that *Oxtr* and *Avpr1a* are necessary for conditioned effects of an ethanol-associated social stimulus. The present study explored this hypothesis using ethanol-induced CSP in female mice lacking either *Oxtr* or *Avpr1a*. In particular, the CSP model uses social partners as the CS+ and CS−, while ethanol is the US. Responses in both knock-out (KO) lines were compared with those of WT littermates. We also evaluated ethanol-induced CSP in pair-house WT females [WT(wt)] from the same experimental line.

2. Materials and methods

2.1. Animals

Female mice (n = 9–10/group) were offspring of C57BL/6J-backcrossed stock from Jackson Laboratories (Bar Harbor, ME). The C57Bl/6 strain shows high voluntary ethanol intake in a 2-bottle preference test [31], and relatively low aversion to ethanol [32]. The Oxtr and Avpr1a lines are fully backcrossed onto the C57BL/6J background. In order to generate mice for the experiments, WT and KO females were obtained from crosses of heterozygous mating pairs obtained from Dr. Larry Young (Emory University). WT and KO female mice from the *Oxtr* line used in the experiment were obtained from 10 litters; females from the *Avpr1a* line used in the studies were from 7 litters. After weaning at postnatal day 21, WT females pair-housed with a KO female from the same line were designated as WT(ko). Based on our previous finding that ethanol induces CSP in C57Bl/6 female mice [17], the lack of ethanol-induced CSP in WT(ko) females in the initial phase of the study was unexpected. Therefore, a follow-up experiment was conducted at a later time, using additional pair-housed WT females designated as WT(wt). These females were obtained from heterozygous males crossed with WT females (4 litters from *Oxtr* line, 3 litters from *Avpr1a* line; Fig. 1A). Mouse genotypes were determined as previously described [33,34] with purified DNA collected from tail biopsy at P25. All stimulus females were C57Bl/6N mice purchased from Charles River Laboratories (Wilmington, MA), of similar age and weight as test females. The C57Bl/6J and C57Bl/6N strains show similar patterns of ethanol intake [35], and there are no consistent substrain differences in behavior of female C57Bl/6J and C57Bl/6N mice [36]. Experimental procedures were approved by the USC Institutional Animal Care and Use Committee and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, 8th Ed (National Research Council, National Academies Press, Washington DC; 2011).

To control for fluctuations in endogenous estradiol which can modify affiliative behavior, OXT and AVP [9,37–39], we delivered estradiol at constant levels by ovariectomy and systemic estrogen replacement (OVX + E). All test and stimulus females were ovariectomized as adults via bilateral dorsal flank incision under 2,2,2-tribromoethanol anesthesia (250 mg/kg), and received chronic estradiol replacement via Silastic implant sc (o.d.: 2.16 mm, i.d.: 1.02 mm, Dow Corning, Midland, MI). The 5-mm implant was filled with a 1:1 mixture of crystalline 17b-estradiol and cholesterol, and the ends were sealed with silicone adhesive. As determined by uterine weights, this regimen provides physiologic levels of estrogen [40]. Females were allowed to recover from surgery for 2 weeks before testing and pairing.

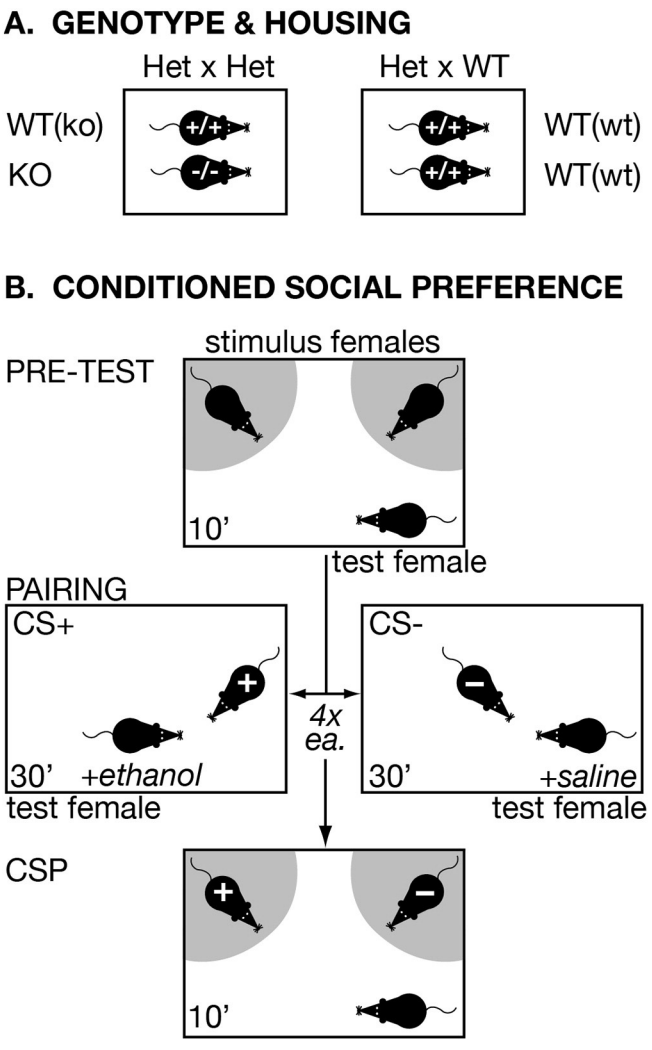

Fig. 1. A. Genotype and post-weaning housing of female test mice. Abbreviations: Het, heterozygote; WT, wild-type; KO: null pair-housed with wild-type; WT(ko): wild-type pair-housed with null; and WT(wt): wild-type pair-housed with wild-type. B: Testing conditioned social preference (CSP) for a stimulus female mouse (CS+) paired with ethanol vs another stimulus female (CS−) paired with saline. See Materials and methods for details.

2.2. Ethanol-induced CSP

KO, WT(ko) and WT(wt) females from the *Oxtr* and *Avpr1a* lines were tested for CSP in response to ethanol ip at 2 g/kg. This represents a moderate dose of ethanol [41], which is sufficient to demonstrate ethanol-induced CSP [17] and CPP [42] in mice.

CSP was conducted with minor modification of our previous study [17]. Briefly, pairing and testing were conducted in identical empty plastic arenas (40 × 50 × 40 cm), which were cleaned after each test. During the pre-test, 2 unfamiliar stimulus females were tethered on opposite sides of the area (see Fig. 1B). Stimulus females were lightly restrained by taping the end of the tail to a flexible metal chain. A test female was introduced, and the amount of time spent in proximity to each stimulus female was recorded for 10 min by an observer blind to the treatment groups. Unlike our previous study [17], stimulus females were not scented with topical odors, as test females do not develop a generalized preference for stimulus females with the same artificial odor stimuli. Proximity was defined when all 4 paws of the test female were within reach of the stimulus female (shaded area, Fig. 1B). This measure reflects contact with the stimulus female, as well as exposure to urine and scent gland secretions.

For the next 8 days, test females were paired individually for 30 min with each stimulus female in the testing arena on alternate days (4 pairings each). During pairing, stimulus females were unrestrained to facilitate interaction with the test female. However, behavior during pairing was not recorded. Thirty minutes before pairing, each test female received an injection ip of ethanol or an equivalent volume of saline, and was temporarily housed individually in a clean cage. Ethanol for injection was prepared daily from a commercially-available neutral grain spirit (Everclear, Luxco, St. Louis, MO; [43]).

Following a saline injection, the test female was paired with one stimulus female, designated as the CS−. On alternate days, the test female received an ethanol injection, and was paired with the opposite (CS+) stimulus female. The order of pairing (beginning with ethanol or saline) was balanced. During the pre-test, half of the test females (30 of 59) showed a positive preference score (time with CS+ minus CS−, in seconds) for the CS+ stimulus female. The day after the final pairing, CSP was measured under conditions identical to the pre-test.

2.3. Habituation–dishabituation

To ensure that mutant mice from both lines exhibited the capacity to detect and differentiate different social and non-social odors, all test mice were evaluated for investigation and discrimination of odor cues according to Yang and Crawley [44]. The day after the CSP test, each test female was introduced into an empty cage (17.5 × 28 × 12 cm). After a 2-min acclimation, the mouse was presented with a series of 5 odorants in aqueous solution delivered via cotton swabs. During the 30-min test, each odor was delivered on a fresh swab at the top of the cage for three consecutive 2-min trials. Water, coconut extract (diluted 1:100; McCormick baking extracts, Sparks MD) and lemon extract (1:100; McCormick) were presented first, followed by urinary odors acquired from two unfamiliar OVX + E females: urine A (1:10) and urine B (1:10). An observer blind to treatment groups recorded investigation time. Habituation was measured as the decrease in investigation time in seconds from the first to the third presentation of the same odor. Dishabituation was measured as the increase in investigation from the third odor presentation to the first in the next odor series (e.g. from the third water presentation to the first presentation of coconut).

2.4. Elevated plus maze

Because oxytocin has been reported to be anxiolytic in mice [45,46], it is possible that increased anxiety due to lack of *Oxtr* may might impair ethanol-induced CSP in the present study. Twenty-four hours after habituation/dishabituation testing, we measured anxiety on the elevated plus maze, according to File et al. [47]. The apparatus consisted of a plus-shaped maze with two arms (5 × 30 cm) closed by 15 cm clear Plexiglas sidewalls and two arms without walls. The maze was located 50 cm above the floor and visually isolated by a curtain enclosure. Test females were placed at the center of the maze facing an open arm and allowed to explore freely for 5 min. Exploratory activity was recorded on video, and scored by an observer blind to the treatment groups. The number of entries into open and closed arms was recorded, as well as time spent on the open arms. An entry was recorded when all four paws entered the arm.

2.5. Loss of righting reflex

To determine if deletion of *Oxtr* modifies the duration of ethanol sedation, we compared KO, WT(ko) and WT(wt) test females from the *Oxtr* line on the ethanol-induced loss of righting reflex test [48]. Mice received a hypnotic dose of ethanol (4 g/kg) ip, and were placed on their backs in a V-shaped trough once they were unable to right themselves. 4 g/kg is a high dose of ethanol that causes rapid loss of recumbency [41]. An experimenter blind to the treatment group recorded time until the righting reflex was regained.

2.6. Data analysis

Preference score and total interaction (time with CS+ plus CS−) were determined for each test female during pre-test and CSP tests. Preference scores and total interaction among KO, WT(ko), and WT (wt) females in each experimental line (*Oxtr*, *Avpr1a*) were evaluated by repeated measures ANOVA (RM-ANOVA) with time (pre-test, CSP) as the repeated measure. When significant differences were observed by RM-ANOVA, changes in preference score and total interaction during pre-test and CSP in each experimental group [KO, WT(ko) and WT(wt)] were compared by paired t-test.

For the habituation–dishabituation test, habituation and dishabituation to social (urine) and non-social odors (baking extracts) were evaluated by RM-ANOVA, with time as the repeated measure. When statistically significant differences ($p < 0.05$) were found, post-hoc comparison was conducted against WT(wt) females by Dunnett's test.

For the elevated plus maze, time in the open arms, number of open arm entries and total entries, and percent open arm entries among *Oxtr* KO, WT(ko), and WT(wt) females were evaluated by ANOVA, with post-hoc comparison versus WT(wt) females by Dunnett's test. For the loss of righting reflex test, time to regain ventral recumbency among *Oxtr* KO, WT(ko), and WT(wt) females was evaluated by ANOVA. For all analyses, $p < 0.05$ was considered significant.

3. Results

3.1. Oxtr CSP

Preference scores and total interaction in *Oxtr* KO, WT(ko), and WT (wt) females paired with 2 g/kg ethanol are shown in Fig. 2. By RM-ANOVA, there was a significant effect of time [pre-test vs CSP, ($F_{1,30} = 10.34$; $p < 0.05$)] and a significant interaction of genotype × time ($F_{2,30} = 13.44$; $p < 0.05$) on preference score for the CS+ stimulus female. However, there was no overall effect of genotype (N.S. $p > 0.05$). Pairing with ethanol and saline had no effect on total interaction with the 2 stimulus females (Fig. 2B; N.S. $p > 0.05$).

In WT(wt) females, repeated pairing with 2 g/kg ethanol induced a significant increase in preference for the CS+ stimulus female ($p < 0.05$), as in our previous study of C57Bl/6 female mice [16]. Preference scores for the CS+ stimulus female averaged −12.3 ± 23.1 s during pre-test (mean ± SEM) and 103.1 ± 22.6 s after pairing, a +115.4 ± 22.0 second increase. By contrast, in *Oxtr* KO females, preference for the CS+ stimulus female declined significantly after pairing: from

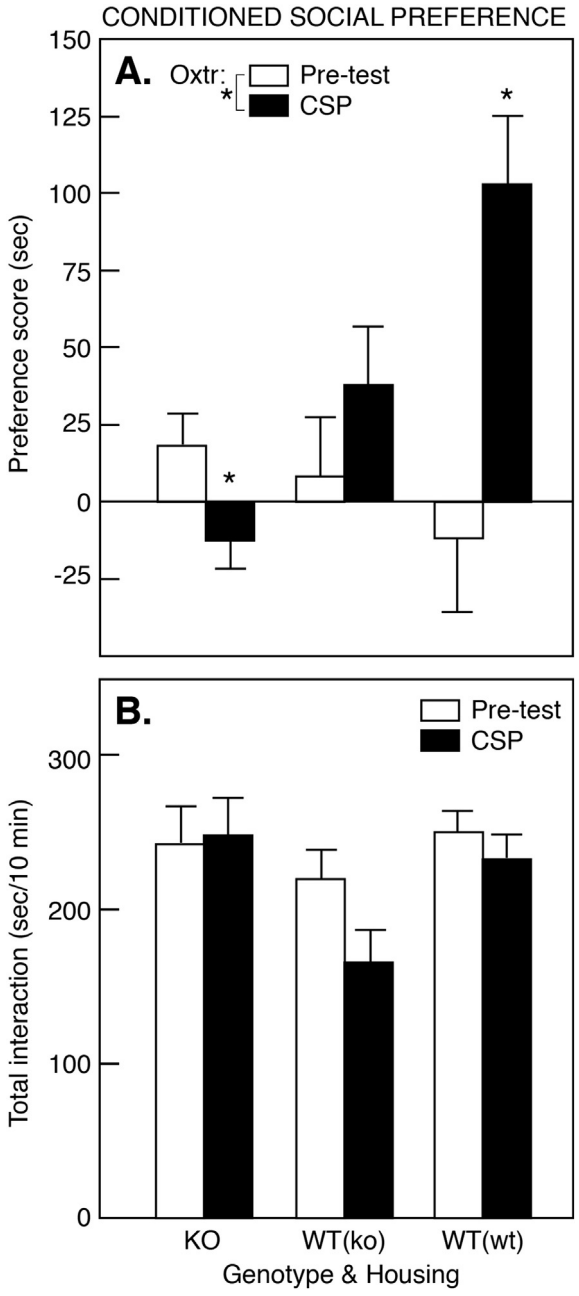

Fig. 2. Conditioned social preference in *Oxtr* mice. A: Preference score (mean ± SEM) for the conditioned stimulus female mouse (CS+) paired with ethanol vs the CS− stimulus female during pre-test (open bars) and conditioned social preference tests (CSP, closed bars). B: Total interaction (seconds/10 min) with both stimulus females during the pre-test and CSP tests. Abbreviations: KO: null pair-housed with wild-type; WT(ko): wild-type pair-housed with null; and WT(wt): wild-type pair-housed with wild-type. Asterisks indicate significant difference by paired t-test vs pre-test values in the same experimental group.

18.7 ± 10.5 s during pre-test to −12.3 ± 9.7 s during CSP testing, a −31.0 ± 12.5 second decrease ($p < 0.05$). Among WT(ko) females, pairing with ethanol failed to alter preference for the CS+ female. Preference averaged 8.2 ± 19.5 s during pre-test, and 37.4 ± 20.0 s during CSP testing (N.S. $p > 0.05$).

3.2. Avpr1a CSP

Fig. 3 reports the preference scores and total interaction during CSP testing in response to 2 g/kg ethanol for females from the *Avpr1a* line. As with *Oxtr* mice, there was a significant genotype × time interaction on preference score for the CS+ female ($F_{2,23} = 10.51$; $p < 0.05$). However, there was no overall effect of either genotype or time (N.S. $p > 0.05$). Total interaction with the two stimulus females decreased significantly over time (Fig. 3B; $F_{1,23} = 4.48$; $p < 0.05$), from 264.0 ± 13.9 s initially to 237.4 ± 11.8 s after pairing.

Similar to the *Oxtr* line, WT(wt) females from the *Avpr1a* line demonstrated CSP for the CS+ stimulus female in response to 2 g/kg ethanol. Pre-test preference scores averaged −11.2 ± 9.7 s, while the preference score during CSP testing was 67.4 ± 19.2 s (+78.7 ± 25.2 s increase, $p < 0.05$). Total interaction remained unchanged (N.S. $p > 0.05$). In *Avpr1a* KO females, preference for the CS+ stimulus female

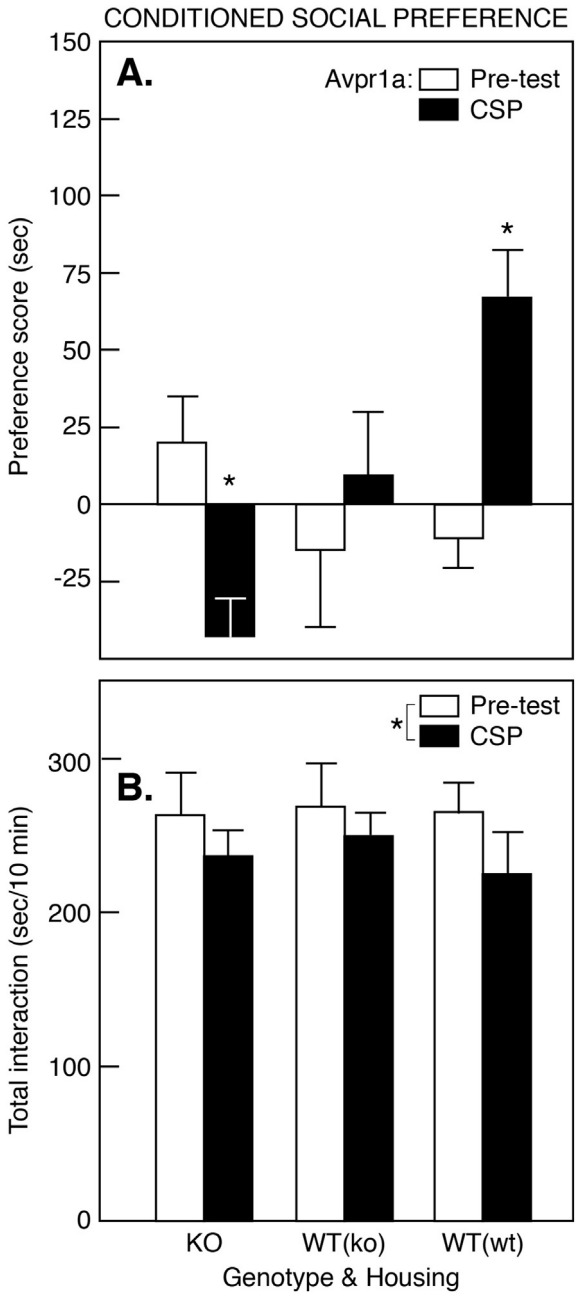

Fig. 3. Conditioned social preference in *Avpr1a* mice. A: Preference score (mean ± SEM) for the conditioned stimulus female mouse (CS+) paired with ethanol vs the CS− stimulus female during pre-test (open bars) and conditioned social preference tests (CSP, closed bars). B: Total interaction (seconds/10 min) with both stimulus females during the pre-test and CSP tests. Abbreviations: KO: null pair-housed with wild-type; WT(ko): wild-type pair-housed with null; and WT(wt): wild-type pair-housed with wild-type. Asterisks indicate significant difference by paired t-test vs pre-test values in the same experimental group.

decreased by −62.9 ± 14.8 s ($p < 0.05$), although total interaction was unchanged (N.S. $p > 0.05$). Among WT(ko) females, there was no effect of ethanol pairing on preference score or total interaction. Pre-test preference averaged −15.3 ± 23.6 s, while preference during CSP testing was 9.0 ± 20.0 s (N.S. $p > 0.05$).

3.3. Habituation–dishabituation

Results of olfactory investigation in response to repeated presentation of social and non-social odor cues are presented in Fig. 4. For dishabituation, there was a significant effect by RM-ANOVA of time (odor source) in both mouse lines (*Oxtr*: $F_{3,22} = 3.51$; $p < 0.05$; *Avpr1a*: $F_{3,22} = 15.78$; $p < 0.05$). However, there was no effect of genotype and no genotype × time interaction. For example, for all genotypes in the *Avpr1a* line, mice spent 5.7 ± 0.7 s of the 2-min test investigating urine A at the first presentation, compared with 1.0 ± 0.3 s for the third presentation of lemon extract, a +4.7 ± 0.6 second increase. By contrast, with the first presentation of urine B, females increased their investigation by only +0.9 ± 0.5 s.

Habituation responses were similar for all genotypes in each line. There was a significant effect of time for both *Oxtr* ($F_{3,22} = 6.30$; $p < 0.05$) and *Avpr1a* ($F_{3,22} = 3.76$; $p < 0.05$) lines, but no effect of genotype. Not surprisingly, the decrease in investigation was particularly marked where the initial dishabituation response was high. Thus, all genotypes in the *Avpr1a* line investigated urine A for only 1.6 ± 0.5 s by the third presentation, a decrease of −4.1 ± 0.7 s from the first presentation. For the *Oxtr* genotype, there was no genotype × time interaction. In the *Avpr1a* line, there was a significant genotype × time interaction ($F_{6,44} = 3.30$; $p < 0.05$). Comparing habituation responses to each odor stimulus in WT(ko) and KO females vs WT(wt) females by Dunnett's post-hoc test, the WT(ko) females failed to habituate to repeated presentation of urine B (+0.2 ± 0.4 s vs initial presentation, $p < 0.05$), unlike responses in WT(wt) females (−3.9 ± 1.1 s) and KO females (−1.6 ± 0.8 s). However, WT(ko) females showed little initial investigation of urine B, thereby reducing the opportunity for habituation. There were no other significant differences in habituation responses.

3.4. Oxtr elevated plus maze

Behavior on the elevated plus maze in *Oxtr* females is shown in Fig. 5. By ANOVA, the numbers of open-arm entries, total entries, and time in

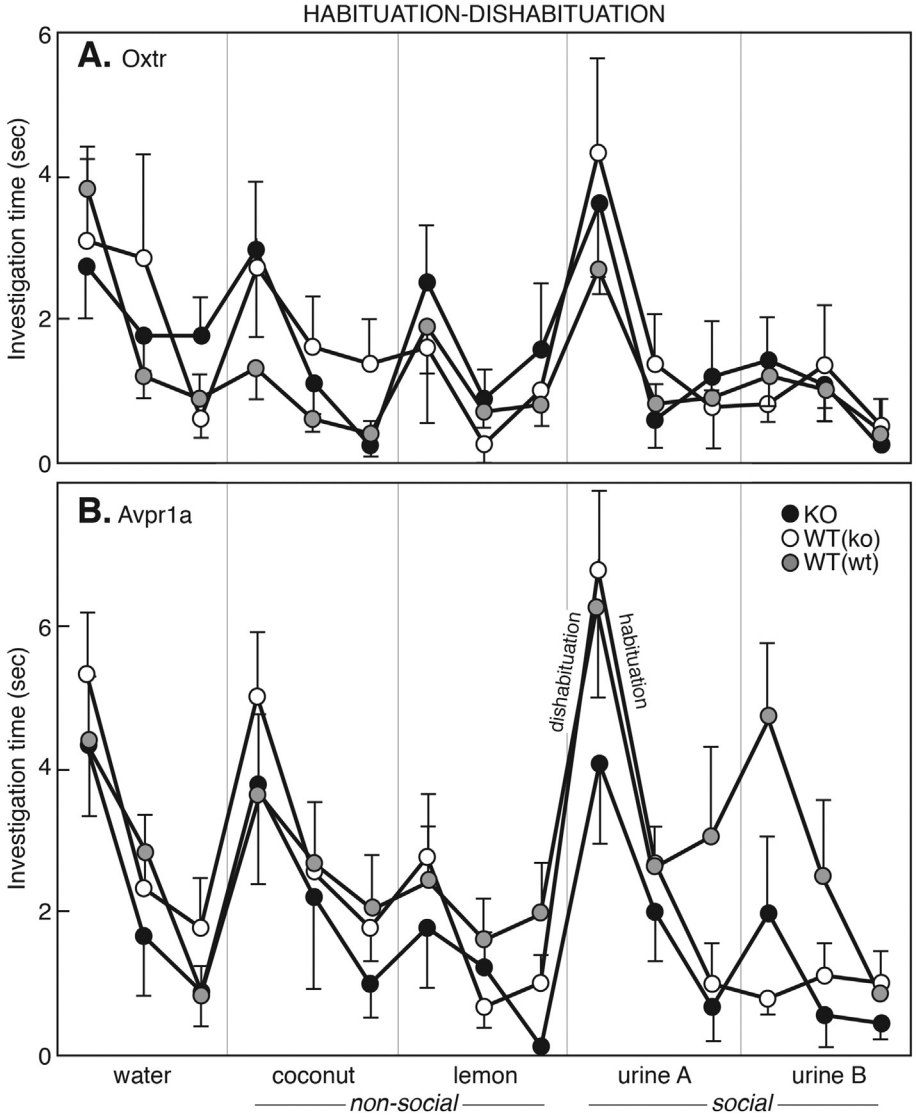

Fig. 4. Habituation–dishabituation to repeated presentation of olfactory stimuli in female *Oxtr* (A) and *Avpr1a* mice (B). Investigation in seconds (mean ± SEM) in response to three 2-min exposures each to a cotton swab scented with water, baking extracts (coconut, lemon) or urine from 2 unfamiliar C57Bl/6 females (urine A and urine B). For each line, KO: null pair-housed with wild-type (closed symbols); WT(ko): wild-type pair-housed with null (open symbols); and WT(wt): wild-type pair-housed with wild-type (shaded symbols).

the open arms were significantly different between genotypes. WT(wt) females averaged 20.5 ± 1.4 arm entries during the 5-min test, including 6.5 ± 0.9 open-arm entries (31.0 ± 3.4% open arm entries). They spent 50.9 ± 10.3 s on the open arms. By contrast, KO females made significantly more entries into the open arms (9.8 ± 1.1, $p < 0.05$), resulting in significantly more total entries (25.9 ± 1.8, $p < 0.05$). They also spent more time on the open arms (83.9 ± 7.3 s, $p < 0.05$). Responses of WT(ko) females were not different from WT(wt) controls (N.S. $p > 0.05$).

3.5. Oxtr loss of righting reflex

In response to a hypnotic dose of ethanol (4 g/kg), mice rapidly lost the ability to maintain ventral recumbency, and this effect persisted for 43.2 ± 5.3 min in WT(wt) females, 52.4 ± 5.3 min in WT(ko) females, and for 53.2 ± 7.0 min in KO females. There was no difference between groups in the duration of loss of righting reflex (N.S. $p > 0.05$).

4. Discussion

The present study examined ethanol-induced CSP in female mice with a goal of determining the role of OXTR and AVPR1a signaling in the conditioned effects of an ethanol-associated social stimulus. Utilizing genetic lines in which one or the other of the genes was deleted (*Oxtr* and *Avpr1a*), the findings show that each neuropeptide contributes to the impact of social context on the response to ethanol. Whereas WT(wt) females developed a conditioned preference for the CS+ stimulus female paired with ethanol, both KO^{Oxtr} and KO^{Avpr1a} females decreased their preference for the CS+ stimulus female, reflecting a conditioned social aversion for the CS+ female paired with ethanol. However, total interaction time with both stimulus females was unchanged. Surprisingly, ethanol-induced CSP was unchanged in WT(ko) females. The reduced preference observed in the mutant lines was not due to disruption of olfactory discrimination, because short-term response to repeated presentation of social and non-social odors was unimpaired in KO^{Oxtr} and KO^{Avpr1a} females. Furthermore, the absence of *Oxtr* had no effect on the duration of response to ethanol sedation, as measured in the test for loss of righting reflex. However, KO^{Oxtr} females showed reduced anxiety and increased arm entries in the elevated plus maze. The current results are consistent with OXTR and AVPR1a both being essential for social interactions in different contexts. This includes when a WT social partner serves as a CS+, as in the present study, rather than as a US. It should be emphasized, however, that the present study is unique in focusing on OVX + E females, rather than more standard gonad-intact males. Comparing and contrasting results of this and previous studies, therefore, should be done with this in mind. Finally, the lack of ethanol-induced CSP in WT(ko) females suggests an important role for developmental social interactions within the litter structure, allowing expression of social responses even in genetically-normal individuals.

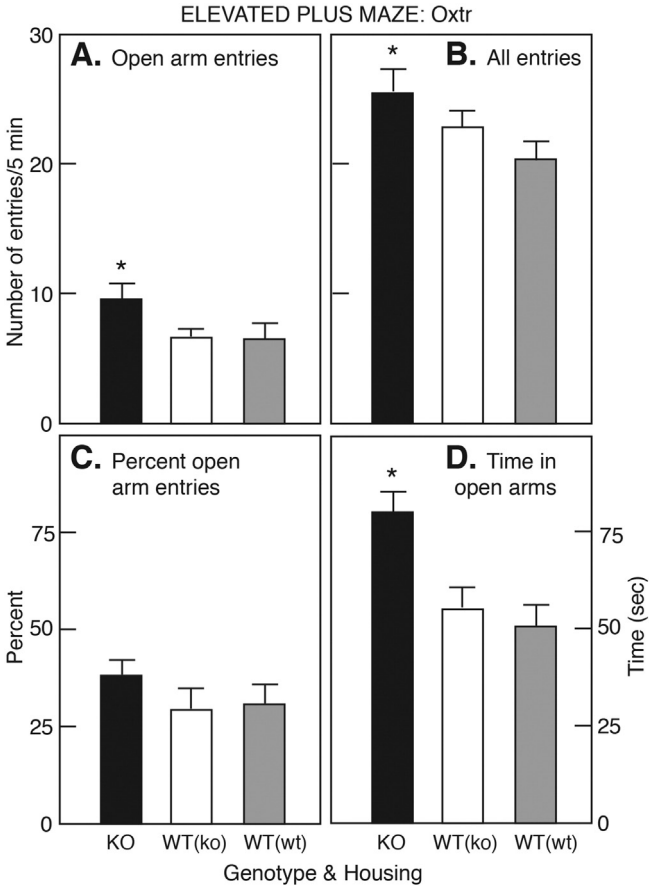

Fig. 5. Elevated plus maze in female *Oxtr* mice. A: Number of entries (mean ± SEM) into the open arms/5 min, B: total number of arm entries (closed + open arms), C: percent of open arm entries, and D: time in open arms in seconds. KO: Null pair-housed with wild-type (closed symbols); WT(ko): wild-type pair-housed with null (open symbols); and WT(wt): wild-type pair-housed with wild-type (shaded symbols). Asterisks indicate significant difference from WT(wt) by ANOVA with Dunnett's post-hoc test.

KO^{Oxtr} and KO^{Avpr1a} mice were first described approximately 10 years ago [34,49,50]. Perhaps what is most striking about these mice is the relative absence of major physiologic disruptions, especially in reproductive function. The lack of *Oxtr* or *Avpr1a* has minimal impact on mating or parturition [50,51], although lactation is impaired in KO^{Oxtr} dams. In part, the lack of effect in KO mice may be due to compensatory mechanisms when either of the OXT or AVP systems individually is disrupted, but not both (reviewed in [52]). The studies reported here used constitutive deletion of Oxtr or Avpr1a, both of which have been studied extensively [50,51]. Dissection of the regional expression responsible for CSP studied here will require the use of multiple conditional mutant lines for each gene.

In the present study, the effects of *Oxtr* on anxiety and arm entries on the elevated plus maze were somewhat unexpected. Because KO^{Oxtr} females made more entries in and remained for a longer time on the open arms of the elevated plus maze, one interpretation is that KO^{Oxtr} females are less anxious than WT(wt) females from the same line. This is counter to the anxiolytic effects of exogenous OXT previously reported [53]. This finding notwithstanding, there is precedent for reduced anxiety when OXT and AVP systems are disrupted. For example, KO^{Otx} males cross-fostered from KO–KO pairs exhibited reductions in anxiety, as in the present study, whereas the KO^{Otx} offspring of Het–Het pairs were unaffected [54]. Likewise, KO^{Avpr1a} males spend more time than WT-^{Avpr1a} males in the open arms of the elevated plus maze ([55,56]; but see [50]). In addition to spending more time on the open arms, KO^{Oxtr} females in the present study showed increased numbers of total arm entries in the elevated plus maze. This is consistent with our informal observations of KO female behavior. Although total interaction with the two stimulus females during pre-test and CSP test was not different in KO and WT females, the pattern of behavior in KO females from both *Oxtr* and *Avpr1a* lines was unusual. KO females circled the test chamber rapidly, thereby making typically brief but frequent contacts with the two stimulus females. This pattern mirrored behavior in the home-cage. Similar effects on locomotor behavior have not been reported previously for KO^{Oxtr} or KO^{Avpr1a} males and females [50,55].

As measured by the loss of righting reflex test, ethanol sedation of KO^{Oxtr} females in the present study was unaffected compared to WT mice. This is consistent with previous studies showing no effect of Avpr1a in mice [26] and or exogenous OXT in rats [57] on acute intoxicating effects of ethanol. In addition, we did not observe increased aggression in KO female mice during pairing, as has been reported for males in the KO^{Oxt} and KO^{Oxtr} lines [51,54]. Our finding is not surprising, given that fighting is relatively uncommon among non-lactating female

mice. Furthermore, the assay used for CSP tests mice in a neutral arena, which is less likely to induce aggression compared with a home cage-intruder model [56].

In the context of ethanol-induced CSP, OXT and AVP have contrasting effects: in male mice, these neuropeptides increase social behavior [1] and modify ethanol consumption [24–27]. Nonetheless, the lack of ethanol-induced CSP in KO^{Oxtr} and KO^{Avpr1a} females in the present study was consistent with our hypothesis. Previous studies of mice lacking *Oxtr* and *Avpr1a* have demonstrated specific deficits in social behavior [33], which would likely interfere with recognition of the CS+ female in CSP. KO^{Oxtr} males fail to show habituation to repeated presentation of the same female [34], although the deficit is limited to intrastrain recognition of individuals, and not discrimination of females from different strains [58]. In a similar manner, KO^{Avpr1a} males show impaired habituation-dishabituation to female odors, but normal response to non-social odors [50]. In the present study, there was no effect in KO^{Oxtr} and KO^{Avpr1a} females compared to their WT littermates on habituation or dishabituation to odor cues, either social or non-social odors. This suggests that investigation and retention of chemosensory information is unimpaired within the duration of this test. We note, however, that overall levels of investigation even in WT(wt) females from the present study were low because females typically show relatively little interest in urine from other females, compared with male attraction to females and their odors. Few previous studies have explored social recognition deficits in females of either KO^{Oxtr} or KO^{Avpr1a} mice, yet deciphering potential sex-based differences in the role of each neuropeptide in social engagement is both biologically and clinically relevant. Importantly, KO^{Oxtr} females show a pregnancy block (Bruce effect) when separated from their mate for 24 h, but not when the mate is continuously present [59]. This suggests that even if KO females can demonstrate normal habituation to repeated presentation of odor cues over a short interval, social recognition is not sustained over a more extended duration. On the other hand, KO females from both genetic lines showed a significant decrease in preference for the CS+ stimulus female paired with ethanol. This argues in favor of social recognition for KO females. What is less clear is why ethanol appears to induce a conditioned social aversion in these mice. In this regard, treatment with exogenous OXT reduces symptoms of alcohol withdrawal in alcohol-dependent patients treated for detoxification [60]. Thus, it may be that aversive consequences of ethanol are enhanced in mice lacking Oxtr or Avpr1a.

Perhaps the most surprising finding from this study was the lack of ethanol-induced CSP in WT(ko) females. We have previously demonstrated ethanol-induced CSP in OVX + E C57Bl/6 females at 2 g/kg ethanol [17], and WT(wt) females in the present study demonstrated robust preference for the CS+ female at the same ethanol dose. The failure of WT(ko) females to respond to the CS+ female paired with ethanol suggests that a disrupted postnatal and/or post-weaning social environment can negatively impact the social behavior of females who are genetically wild-type, but whose siblings and cage-mates are not. It is important to keep in mind that we cannot rule out potential effects of altered maternal care on social behavior, since WT(wt) females and females in our previous study were from WT dams, while WT(ko) females were born to heterozygote dams. Even so, the disruption of social behavior in WT(ko) females adds to a growing literature demonstrating behavioral and physiologic responses in animals to the behavior of their cage-mates. This can include responses to pain, fear, and illness in mice and rats. When cage-mates share a stressful experience, stress responses are reduced [61,62]. Alternatively, responses to painful stimuli are heightened when pairs of mice are tested together [63]. Social transmission of response to pain and fear occurs even when only one member of a pair is affected. In this regard, exposure to a fearful cage-mate increases freezing responses [64–67]. Mice also respond negatively to a cage-mate injected with formalin [68]. They will approach and maintain contact with a cage-mate in pain, but they also develop a conditioned place aversion for the location [69]. Similarly, odor cues from a tumor-infected cage-mate increase anxiety-like behavior in female mice [70,71].

Cage-mates may also modify responses to drugs of abuse. Monkeys self-administer more PCP in the presence of a cage-mate [72], and mice consume more alcohol when their cage-mate is intoxicated [73]. With CPP, simultaneous drug exposure in pairs of mice enhances the reinforcing effects of morphine [74] and methamphetamine [75]. With regard to ethanol-induced CSP in the present study, it may be that impaired social behavior in KO mice interferes with ethanol-induced CSP in WT(ko) mice. Previously, we have shown that the response to ethanol is increased when both the test mouse and the CS+ stimulus mouse are intoxicated [17]. This observation raises the interesting possibility that preference for the CS+ stimulus mouse during CSP testing is shaped by the response of the CS+ stimulus mouse towards an intoxicated partner during pairing. This could be addressed in the future by monitoring the behavior of test and stimulus mice during pairing and testing.

Often, social stress is linked with increased anxiety and ethanol consumption [77–79]. In the present study, performance in the EPM was not different in WT(ko) and WT(wt) females from the *Oxtr* line, suggesting that WT(ko) females do not exhibit generalized anxiety as a result of their KO^{Oxtr} cage-mates. Instead, it appears that the effects on WT(ko) females of having a KO cage-mate are specific to social behavior. Interestingly, this effect may be mediated through OXT and OXTR. Exogenous OXT has anti-nociceptive effects, not only in rats subject to thermal pain, but also in untreated cage-mates mediated via olfactory stimuli [80]. In a similar manner, OXTR is reduced in response to either impaired maternal care and/or an impoverished environment (reviewed in [81]). It would be of interest in future studies to determine if the OXTR is reduced in WT(ko) females compared with WT(wt) controls. Ultimately, these findings offer a cautionary note to genetic studies of behavior in mice, suggesting that the composition of litters with regard to genotype may shape behavior even of WT animals.

Acknowledgments

We thank Ms. Sydney P. Goings, Ms. Jessica Kim and Ms. Kathryn G. Wallin for assistance with animal surgery and behavioral testing. We are grateful to Larry Young, PhD of Emory University for the founder mice. This work supported by a grant from the NIH (R21-AA020575 to RIW).

References

[1] M.M. Lim, L.J. Young, Neuropeptidergic regulation of affiliative behavior and social bonding in animals, Horm. Behav. 50 (4) (2006) 506–517.
[2] E.A. Hammock, Developmental perspectives on oxytocin and vasopressin, Neuropsychopharmacology 40 (1) (2015) 24–42.
[3] C.S. Carter, A.J. Grippo, H. Pournajafi-Nazarloo, M.G. Ruscio, S.W. Porges, Oxytocin, vasopressin and sociality, Prog. Brain Res. 17 (2008) 331–336.
[4] D.W. Wacker, M. Ludwig, Vasopressin, oxytocin, and social odor recognition, Horm. Behav. 61 (3) (2012) 259–265.
[5] J.L. Hurst, C.E. Payne, C.M. Nevison, A.D. Marie, R.E. Humphries, D.H.L. Robertson, et al., Individual recognition in mice mediated by major urinary proteins, Nature 414 (2001) 631–634.
[6] K. Macdonald, T.M. Macdonald, The peptide that binds: a systematic review of oxytocin and its prosocial effects in humans, Harv. Rev. Psychiatry 18 (1) (2010) 1–21.
[7] S.O. Reber, I.D. Neumann, Defensive behavioral strategies and enhanced state anxiety during chronic subordinate colony housing are accompanied by reduced hypothalamic vasopressin, but not oxytocin, expression, Ann. N. Y. Acad. Sci. 1148 (2008) 184–195.
[8] H.E. Ross, S.M. Freeman, L.L. Spiegel, X. Ren, E.F. Terwilliger, L.J. Young, Variation in oxytocin receptor density in the nucleus accumbens has differential effects on affiliative behaviors in monogamous and polygamous voles, J. Neurosci. 29 (5) (2009) 1312–1318.
[9] R. Dantzer, Vasopressin, gonadal steroids and social recognition, Prog. Brain Res. 119 (2008) 409–414.
[10] Z.R. Donaldson, L. Spiegel, L.J. Young, Central vasopressin V1a receptor activation is independently necessary for both partner preference formation and expression in socially monogamous male prairie voles, Behav. Neurosci. 124 (1) (2010) 159–163.
[11] M.M. Ali, D.S. Dwyer, Social network effects in alcohol consumption among adolescents, Addict. Behav. 35 (4) (2010) 337–342.

[12] K.T. Brady, C.L. Randall, Gender differences in substance use disorders, Psychiatr. Clin. North. Am. 22 (2) (1999) 241–252.
[13] Källmén H, Gustafson R. Alcohol and disinhibition. Eur. Addict. Res. 1998; 4(4):150–162.
[14] J.M. Fernandez-Vidal, J.C. Molina, Socially mediated alcohol presences in adolescent rats following interactions with an intoxicated peer, Pharmacol. Biochem. Behav. 79 (2004) 229–241.
[15] A.M.J. Anacker, T.H. Ahern, L.J. Young, A.E. Ryabinin, The role of early life experience and species differences in alcohol intake in microtene rodents, PLoS One 7 (6) (2012) e39753.
[16] K. Kent, K. Butler, R.I. Wood, Ethanol induces conditioned social preference in male mice, Alcohol. Clin. Exp. Res. 38 (4) (2014) 1184–1192.
[17] R.I. Wood, R. Rice, Ethanol-induced conditioned partner preference in female mice, Behav. Brain Res. 243 (2013) 273–277.
[18] M.T. Bardo, R.A. Bevins, Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology 153 (2000) 31–43.
[19] C.L. Cunningham, M.A. Howard, S.J. Gillb, M. Rubinstein, M.J. Lowd, D.K. Grandy, Ethanol-conditioned place preference is reduced in dopamine D2 receptor-deficient mice, Pharmacol. Biochem. Behav. 67 (4) (2000) 693–699.
[20] S. Nash, M. Domjan, Learning to discriminate the sex of conspecifics in male Japanese quail (*Coturnix coturnix japonica*): tests of "biological constraints, J. Exp. Psychol. Anim. Behav. Process 17 (1991) 342–353.
[21] G.A. Coria-Avila, A.J. Ouimet, P. Pacheco, J. Manzo, J.G. Pfaus, Olfactory conditioned partner preference in the female rat, Behav. Neurosci. 119 (3) (2005) 716–725.
[22] T.E. Kippin, J.G. Pfaus, The nature of the conditioned response mediating olfactory conditioned ejaculatory preference in the male rat, Behav. Brain Res. 122 (1) (2001) 11–24.
[23] K. Kent, V. Arientyl, M.M. Khachatryan, R.I. Wood, Oxytocin induces a conditioned social preference in female mice, J. Neuroendocrinol. 25 (9) (2013) 803–810.
[24] M.T. Bowen, D.S. Carson, A. Spiro, J.C. Arnold, I.S. McGregor, Adolescent oxytocin exposure causes persistent reductions in anxiety and alcohol consumption and enhances sociability in rats, PLoS One 6 (11) (2011) e27237.
[25] S. Peters, D.A. Slattery, P.J. Flor, I.D. Neumann, S.O. Reber, Differential effects of baclofen and oxytocin on the increased ethanol consumption following chronic psychosocial stress in mice, Addict. Biol. 18 (1) (2013) 66–77.
[26] H.K. Caldwell, J. Stewart, L.M. Wiedholz, R.A. Millstein, A. Iacangelo, A. Holmes, W.S. Young 3rd, S.R. Wersinger, The acute intoxicating effects of ethanol are not dependent on the vasopressin 1a or 1b receptors, Neuropeptides 40 (5) (2006) 325–337.
[27] A. Sanbe, N. Takagi, Y. Fujiwara, J. Yamauchi, T. Endo, R. Mizutani, et al., Alcohol preference in mice lacking the Avpr1a vasopressin receptor, Am. J. Physiol. Regul. Integr. Comp. Physiol. 294 (5) (2008) R1482–R1490.
[28] J.P. Burkett, L.J. Young, The behavioral, anatomical and pharmacological parallels between social attachment, love and addiction, Psychopharmacology 224 (1) (2012) 1–26.
[29] M.R. Thompson, P.D. Callaghan, G.E. Hunt, J.L. Cornish, I.S. McGregor, A role for oxytocin and 5-HT(1A) receptors in the prosocial effects of 3,4 methylenedioxymethamphetamine ("ecstasy"), Neuroscience 146 (2) (2007) 509–514.
[30] D.S. Carson, J.L. Cornish, A.J. Guastella, G.E. Hunt, I.S. McGregor, Oxytocin decreases methamphetamine self-administration, methamphetamine hyperactivity, and relapse to methamphetamine-seeking behaviour in rats, Neuropharmacology 58 (1) (2010) 38–43.
[31] N. Yoneyama, J.C. Crabbe, M.M. Ford, A. Murilloa, D.A. Finn, Voluntary ethanol consumption in 22 inbred mouse strains, Alcohol 42 (3) (2008) 149–160.
[32] J. Broadbent, K.J. Muccino, C.L. Cunningham, Ethanol-induced conditioned taste aversion in 15 inbred mouse strains, Behav. Neurosci. 116 (2002) 138–148.
[33] I.F. Bielsky, S.-B. Hu, K.L. Szegda, H. Westphal, L.J. Young, Profound impairment in social recognition and reduction in anxiety-like behavior in vasopressin V1a receptor knockout mice, Neuropsychopharmacology 29 (3) (2004) 483–493.
[34] Y. Takayanagi, M. Yoshida, I.F. Bielsky, H.E. Ross, M. Kawamata, T. Onaka, et al., Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 16096–16101.
[35] K. Yoshimoto, S. Komura, Reexamination of the relationship between alcohol preference and brain monoamines in inbred strains of mice including senescence-accelerated mice, Pharmacol. Biochem. Behav. 27 (2) (1987) 317–322.
[36] M.M. Simon, S. Greenaway, J.K. White, H. Fuchs, V. Gailus-Durner, S. Wells, et al., A comparative phenotypic and genomic analysis of C57BL/6J and C57BL/6N mouse strains, Genome Biol. 14 (7) (2013) R82.
[37] E.M. Scordalakes, E.F. Rissman, Aggression and arginine vasopressin immunoreactivity regulation by androgen receptor and estrogen receptor alpha, Genes Brain Behav. 3 (1) (2004) 20–26.
[38] E. Choleris, A.E. Clipperton-Allen, A. Phan, M. Kavaliers, Neuroendocrinology of social information processing in rats and mice, Front. Neuroendocrinol. 30 (4) (2002) 442–459.
[39] G. Murakami, R.G. Hunter, C. Fontaine, A. Ribeiro, D. Pfaff, Relationships among estrogen receptor, oxytocin and vasopressin gene expression and social interaction in male mice, Eur. J. Neuroscience. 34 (3) (2011) 469–477.
[40] D.A. Jacob, J.L. Temple, H.B. Patisaul, L.J. Young, E.F. Rissman, Coumestrol antagonizes neuroendocrine actions of estrogen via the estrogen receptor alpha, Exp. Biol. Med. 226 (2001) 301–306.
[41] R.M. Pautassi, M. Myers, L.P. Spear, J.C. Molina, N.E. Spear, Ethanol induces second-order aversive conditioning in adolescent and adult rats, Alcohol 45 (1) (2011) 45–55.
[42] P.A. Groblewski, L.S. Bax, C.L. Cunningham, Reference–dose place conditioning with ethanol in mice: empirical and theoretical analysis, Psychopharmacology 201 (1) (2008) 97–106.
[43] J.C. Crabbe, A.J. Cameron, E. Munn, M. Bunning, D. Wahlsten, Overview of mouse assays of ethanol intoxication, Curr. Protoc. Neurosci. 42 (2008) 9.26.1–9.2619.
[44] M. Yang, J.N. Crawley, Simple behavioral assessment of mouse olfaction, Curr. Protoc. Neurosci. 8 (8) (2009) 24.
[45] M. Yoshida, Y. Takayanagi, K. Inoue, T. Kimura, L.J. Young, T. Onaka, et al., Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice, J. Neurosci. 29 (7) (2009) 2259–2271.
[46] M.M. McCarthy, C.H. McDonald, P.J. Brooks, D. Goldman, An anxiolytic action of oxytocin is enhanced by estrogen in the mouse, Physiol. Behav. 60 (5) (1996) 1209–1215.
[47] S.E. File, A.S. Lippa, B. Beer, M.T. Lippa, Animal tests of anxiety, Curr. Protoc. Neurosci. 8 (8) (2004) 3.
[48] H.C. Becker, R.F. Anton, C. De Trana, C.L. Randall, Sensitivity to ethanol in female mice: effects of ovariectomy and strain, Life Sci. 37 (14) (1985) 1293–1300.
[49] H.J. Lee, H.K. Caldwell, A.H. Macbeth, S.G. Tolu, W.S. Young III, A conditional knockout mouse line of the oxytocin receptor, Endocrinology 149 (2008) 3256–3263.
[50] S.R. Wersinger, H.K. Caldwell, L. Martinez, P. Gold, S.-B. Hu, W.S. Young, Vasopressin 1a receptor knockout mice have a subtle olfactory deficit but normal aggression, Genes Brain Behav. 6 (2007) 540–551.
[51] K. Nishimori, Y. Takayanagi, M. Yoshida, Y. Kasahara, L.J. Young, M. Kawamata, New aspects of oxytocin receptor function revealed by knockout mice: sociosexual behaviour and control of energy balance, Prog. Brain Res. 170 (2008) 79–90.
[52] H.J. Lee, J. Pagani, W.S. Young III, Using transgenic mouse models to study oxytocin's role in the facilitation of species propagation, Brain Res. 1364 (2010) 216–264.
[53] D.A. Slattery, I.D. Neumann, Chronic icv oxytocin attenuates the pathological high anxiety state of selectively bred Wistar rats, Neuropharmacology 58 (1) (2010) 56–61.
[54] J.T. Winslow, E.F. Hearn, J. Ferguson, L.J. Young, M.M. Matzuk, T.R. Insel, Infant vocalization, adult aggression, and fear behavior of an oxytocin null mutant mouse, Horm. Behav. 37 (2000) 145–155.
[55] N. Egashira, A. Tanoue, T. Matsuda, E. Koushi, S. Harada, Y. Takano, et al., Impaired social interaction and reduced anxiety-related behavior in vasopressin V1a receptor knockout mice, Behav. Brain Res. 178 (1) (2007) 123–127.
[56] P.L. Van Loo, L.F. Van Zutphen, V. Baumans, Male management: coping with aggression problems in male laboratory mice, Lab. Anim. 37 (4) (2003) 300–313.
[57] M.T. Bowen, S.T. Peters, N. Absalom, M. Chebib, I.D. Neumann, I.S. McGregor, Oxytocin prevents ethanol actions at δ subunit-containing GABAA receptors and attenuates ethanol-induced motor impairment in rats, Proc. Natl. Acad. Sci. U. S. A. 112 (10) (2015) 3104–3109.
[58] A.H. Macbeth, H.J. Lee, J. Edds, W.S. Young 3rd., Oxytocin and the oxytocin receptor underlie intrastrain, but not interstrain, social recognition, Genes Brain Behav. 8 (5) (2009) 558–567.
[59] S.R. Wersinger, J.L. Temple, H.K. Caldwell, W.S. Young 3rd., Inactivation of the oxytocin and the vasopressin (Avp) 1b receptor genes, but not the Avp 1a receptor gene, differentially impairs the Bruce effect in laboratory mice (*Mus musculus*), Endocrinology 149 (1) (2008) 116–121.
[60] C.A. Pedersen, K.L. Smedley, J. Leserman, L.F. Jarskog, S.W. Rau, A. Kampov-Polevoi, R.L. Casey, T. Fender, J.C. Garbutt, Intranasal oxytocin blocks alcohol withdrawal in human subjects, Alcohol. Clin. Exp. Res. 37 (3) (2013) 484–489.
[61] M. Leshem, M. Sherman, Troubles shared are troubles halved: stress in rats is reduced in proportion to social propinquity, Physiol. Behav. 89 (3) (2006) 399–401.
[62] L.D. Wright, K.E. Muir, T.S. Perrot, Stress responses of adolescent male and female rats exposed repeatedly to cat odor stimuli, and long-term enhancement of adult defensive behaviors, Dev. Psychobiol. 55 (5) (2013) 551–567.
[63] D.J. Langford, S.E. Crager, Z. Shehzad, S.B. Smith, S.G. Sotocinal, J.S. Levenstadt, et al., Social modulation of pain as evidence for empathy in mice, Science 312 (5782) (2006) 1967–1970.
[64] A.K. Bruchey, C.E. Jones, M.H. Monfils, Fear conditioning by-proxy: social transmission of fear during memory retrieval, Behav. Brain Res. 214 (1) (2010) 80–84.
[65] E. Knapska, M. Mikosz, T. Werka, S. Maren, Social modulation of learning in rats, Learn. Mem. 17 (1) (2010) 35–42.
[66] P. Atsak, M. Orre, P. Bakker, L. Cerliani, B. Roozendaal, V. Gazzola, et al., Experience modulates vicarious freezing in rats: a model for empathy, PLoS One 6 (7) (2011) e21855.
[67] A. Nowak, T. Werka, E. Knapska, Social modulation in extinction of aversive memories, Behav. Brain Res. 238 (2013) 200–205.
[68] L. Gioiosa, F. Chiarotti, E. Alleva, G. Laviola, A trouble shared is a trouble halved: social context and status affect pain in mouse dyads, PLoS One 4 (1) (2009) e4143.
[69] S. Watanabe, Distress of mice induces approach behavior but has an aversive property for conspecifics, Behav. Process. 90 (2) (2012) 167–173.
[70] M.Y. Tomiyoshi, M. Sakai, R.B. Baleeiro, D. Stankevicius, C.O. Massoco, J. Palermo-Neto, et al., Cohabitation with a B16F10 melanoma-bearer cage mate influences behavior and dendritic cell phenotype in mice, Brain Behav. Immun. 23 (4) (2009) 558–567.
[71] G.J. Alves, A. Ribeiro, J. Palermo-Neto, The neuroimmune changes induced by cohabitation with an Ehrlich tumor-bearing cage mate rely on olfactory information, Brain Behav. Immun. 26 (1) (2012) 32–39.
[72] J.L. Newman, J.L. Perry, M.E. Carroll, Social stimuli enhance phencyclidine (PCP) self-administration in rhesus monkeys, Pharmacol. Biochem. Behav. 87 (2) (2003) 280–288.
[73] A.M. Maldonado, L.M. Finkbeiner, C.L. Kirstein, Social interaction and partner familiarity differentially alter voluntary ethanol intake in adolescent male and female rats, Alcohol 42 (8) (2008) 641–648.
[74] S.L. Cole, R.S. Hofford, D.J. Evert, P.J. Wellman, S. Eitan, Social influences on morphine conditioned place preference in adolescent mice, Addict. Biol. 18 (2) (2013) 274–285.

[75] S. Watanabe, Drug–social interactions in the reinforcing property of methamphetamine in mice, Behav. Pharmacol. 22 (3) (2011) 203–206.
[77] T. Advani, J.G. Hensler, W. Koek, Effect of early rearing conditions on alcohol drinking and 5-HT1A receptor function in C57BL/6J mice, Int. J. Neuropsychopharmacol. 10 (5) (2007) 595–607.
[78] H.S. Nagaraja, P.S. Jeganathan, Effect of acute and chronic conditions of overcrowding on free choice ethanol intake in rats, Indian J. Physiol. Pharmacol. 47 (3) (2003) 325–331.
[79] S.I. Knyazeva, N.A. Loginova, E.V. Loseva, Anxiety level and body weight changes in rats living in overpopulated cages, Bull. Exp. Biol. Med. 154 (1) (2012) 3–6.
[80] G. Agren, K. Uvnas-Moberg, T. Lundeberg, Olfactory cues from an oxytocin-injected male rat can induce anti-nociception in its cagemates, Neuroreport 8 (14) (1997) 3073–3076.
[81] K.L. Bales, A.M. Perkeybile, Developmental experiences and the oxytocin receptor system, Horm. Behav. 61 (2012) 313–319.