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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.

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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).

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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.

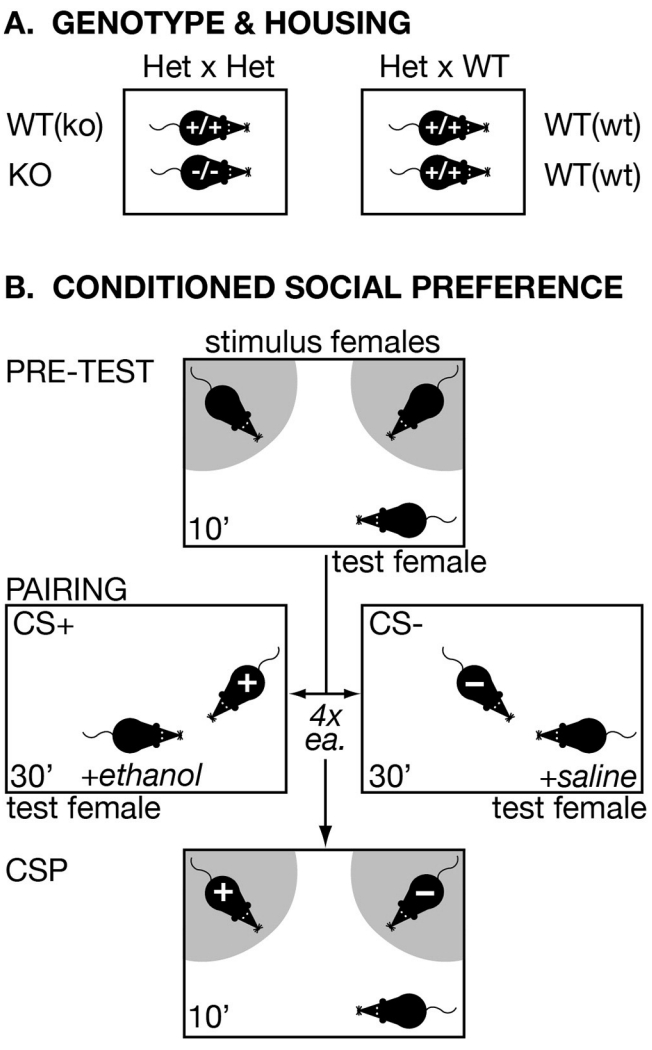

Fig. 1. A. Genotype and post-weaning housing of female test mice. Abbreviations: Het, heterozygote; WT, wild-type; KO: null pair-housed with wild-type; WT(ko): wild-type pair-housed with null; and WT(wt): wild-type pair-housed with wild-type. B: Testing conditioned social preference (CSP) for a stimulus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