HHS Public Access

Author manuscript

*Horm Behav*. Author manuscript; available in PMC 2017 May 01.

Published in final edited form as:

*Horm Behav*. 2016 May ; 81: 68–73. doi:10.1016/j.yhbeh.2016.04.001.

# Trichostatin A (TSA) facilitates formation of partner preference in male prairie voles (*Microtus ochrogaster*)

**F. Duclot**[1,2], **H. Wang**[1,3], **C. Youssef**[1], **Y. Liu**[2,3], **Z. Wang**[2,3], and **M. Kabbaj**[1,2]

[1]Department of Biomedical Sciences, Florida State University, Tallahassee, FL-32306

[2]Program in Neuroscience, Florida State University, Tallahassee, FL-32306

[3]Department of Psychology, Florida State University, Tallahassee, FL-32306

## Abstract

In the socially monogamous prairie voles (*Microtus ochrogaster*), the development of a social bonding is indicated by the formation of partner preference, which involves a variety of environmental and neurochemical factors and brain structures. In a most recent study in female prairie voles, we found that treatment with the histone deacetylase inhibitor Trichostatin A (TSA) facilitates the formation of partner preference through up-regulation of oxytocin receptor (OTR) and vasopressin V1a receptor (V1aR) genes expression in the nucleus accumbens (NAcc). In the present study, we tested the hypothesis that TSA treatment also facilitates partner preference formation and alters OTR and V1aR genes expression in the NAcc in male prairie voles. We thus observed that central injection of TSA dose-dependently promoted the formation of partner preference in the absence of mating in male prairie voles. Interestingly, TSA treatment up-regulated OTR, but not V1aR, gene expression in the NAcc similarly as they were affected by mating – an essential process for naturally occurring partner preference. These data, together with others, not only indicate the involvement of epigenetic events but also the potential role of NAcc oxytocin in the regulation of partner preference in both male and female prairie voles.

### Keywords

Partner preference; Trichostatin A; Nucleus accumbens; Oxytocin; Vasopressin; Prairie voles

## Introduction

The socially monogamous prairie vole (*Microtus ochrogaster*) has emerged as a highly valuable animal model for the study of the neurobiology of social behaviors and social attachment in particular, due to its ability to form enduring pair-bonds. The formation of a

Corresponding author: Mohamed Kabbaj, College of Medicine, Florida State University, 1115 W Call Street, Tallahassee, FL 32306 - phone: 850-644-4930 - Fax: 1-850-644-5781 – ; Email: mohamed.kabbaj@med.fsu.edu

**Conflicts of interest**

The authors declare no conflict of interest.

long-term social bond in prairie voles is characterized by selective affiliation with the established partner over a conspecific stranger (partner preference) (Williams et al., 1992). This behavior can be studied in a laboratory environment and has provided detailed insight into the neurobiology of social bonding (Young et al., 2011, 2008).

Among the neurochemicals examined, the expression profiles of neuropeptides oxytocin receptor (OTR) and vasopressin (AVP) V1a receptor (V1aR) have been highlighted as important drivers of social monogamy in voles. Socially monogamous vole species display higher expression of OTR in the nucleus accumbens (NAcc) and prefrontal cortex, and V1aR in the lateral septum (LS) and ventral pallidum (VP) when compared to non-monogamous species (Insel et al., 1994; Insel and Shapiro, 1992; Wang et al., 1997), suggesting their involvement in the neuroadaptations underlying formation of partner preference. It is now clearly established that oxytocin and AVP are critical in mediating the formation of partner preference in prairie voles. In female prairie voles, for example, oxytocin is released in the NAcc during cohabitation and mating with a male (H. E. Ross et al., 2009). In the brain, and particularly in the NAcc, oxytocin injections facilitate and OTR antagonism impaired partner preference formation (Cho et al., 1999; Liu and Wang, 2003; Williams et al., 1994). Further, virally-mediated alterations of OTR levels in the female NAcc impair mating-induced partner preference (Keebaugh et al., 2015; Keebaugh and Young, 2011; Heather E. Ross et al., 2009). It is worth to mention that in male prairie voles, oxytocin manipulation in the brain also affects partner preference (Cho et al., 1999) and higher OTR expression in the NAcc is associated with male's monogamous mating strategy (Ophir et al., 2012). Similarly, central injection of AVP facilitates the formation of partner preference, whereas V1aR antagonism impairs mating-induced partner preference formation in both male and female prairie voles (Cho et al., 1999; Insel and Hulihan, 1995; Winslow et al., 1993).

In a most recent study in female prairie voles, we found that cohabitation and mating with a male up-regulates OTR and V1aR levels in the female NAcc through epigenetic mechanisms. Notably, potentiating histone acetylation by treatment with an histone deacetylase (HDAC) inhibitor, trichostatin A (TSA), facilitated the formation of partner preference in the absence of mating – a condition that does not lead to pair-bond formation in control animals (Wang et al., 2013). Although these observations clearly demonstrated the existence of an epigenetic regulation of OTR and V1aR in the NAcc in mediating the formation of partner preference in female prairie voles, it remains unclear whether these mechanisms also apply in males.

In this study, we assessed the effects of HDAC inhibition by TSA on the formation of partner preference in male prairie voles, and examined the regulation of OTR and V1aR in the male NAcc. Finally, we tested whether the molecular neuroadaptations observed following TSA treatment were also detected following cohabitation and mating with a female that normally induces partner preference formation.

## Material and methods

### Subjects

Sexually naive male prairie voles (*Microtus ochrogaster*) from a laboratory breeding colony were weaned at 21 days of age and housed in same-sex sibling pairs in plastic cages (12 × 28 × 16 cm) with water and food provided *ad libitum*. All cages were maintained under a 14:10 h light-dark cycle, and the temperature was approximately 20°C. All animals were randomly assigned into experimental groups when they reached 70 – 90 days of age. Experimental procedures were approved by the Institutional Animal Care and Use Committee at Florida State University.

### Stereotaxic cannulation and microinjection

Subjects were anesthetized with sodium pentobarbital (1 mg/10 g body weight) and a 26-gauge stainless steel cannula aimed at the lateral ventricle was stereotaxically implanted unilaterally as previously described (Wang et al., 2013). Following 3 days of recovery, subjects received microinjections (500 nL over 1 min) of either artificial cerebrospinal fluid (CSF, BioFluids, Rockville, MD), or CSF containing different concentrations of Trichostatin A (TSA, 0.08 ng, 0.4 ng, and 4 ng; Sigma-Aldrich, St Louis, MO). The doses of TSA were chosen based on their ability to facilitate partner preference formation in female prairie voles under similar conditions (Wang et al., 2013). At the end of the experiments, all subjects were sacrificed by rapid decapitation and the brains extracted to verify cannulae placement by an observer blind to experimental conditions. Subjects with misplaced cannulae (a total of seven distributed among all groups) were thus excluded from data analysis.

### Cohabitation and partner preference test

In prairie voles, the formation of partner preference can be observed following a prolonged cohabitation with an opposite-sex conspecific under specific conditions. As such, while 24 hours of cohabitation with mating reliably induces partner preference in both males and females, 6 hours of cohabitation in the absence of mating does not (Aragona et al., 2006; Williams et al., 1992). This paradigm has thus been extensively used to investigate the effects of pharmacological manipulation on the induction of partner preference (Young et al., 2011), and all behavioral procedures in this study were performed as previously described (Wang et al., 2013). Briefly, male prairie voles were placed in cohabitation with a female for 6 hours immediately following *i.c.v.* infusion of CSF with or without TSA, and the absence of mating was verified *a posteriori* by examining the videotaped behavior (throughout the entire 6-hr cohabitation period). For the investigation of the neuroadaptations triggered by cohabitation with mating, males were cohabitated for 24 hours with an estrogen-primed female (2 μg per day, *i.p.* for 3 days) (Insel et al., 1995; Liu et al., 2010), and the presence of mating was verified *a posteriori* on videotape.

The partner preference test was performed immediately following the 6-hr cohabitation period, as described previously (Wang et al., 2013). The testing apparatus was constituted of a neutral chamber connected on either side to two parallel identical cages, one housing an unfamiliar female (termed “stranger”), and the other one the familiar female (termed “partner”) used during the cohabitation period. While each stimulus female was tethered

within its respective cage, the male subject was allowed to freely explore the testing apparatus for 3 hours. The entire session was videotaped and the time spent by the male in side-by-side contact with either the stranger or the partner was later quantified by a trained experimenter unaware of the biological groups. A partner preference was defined as subjects spending significantly more time in side-by-side contact with the partner over the stranger, as determined by a paired, two-tailed *t*-test. In addition, we calculated a partner preference index providing a quantitative scoring of each animal's partner preference, defined as: (time spent in contact with the partner - time spent in contact with the stranger)/(time spent in contact with partner + time spent in contact with stranger). Accordingly, an index of 1 or −1 represents a complete preference for the partner or stranger, respectively, while an index of 0 represents an absence of preference for either stimulus female. Furthermore, the three-chamber apparatus is equipped with photobeam-sensors at the entrance of each chamber, allowing the measurement of locomotor activity. This latter measurement allows to control for putative secondary effects of the drugs on the males' behavior, such as general activity, anxiety, or altered exploration of a novel environment, as commonly used by our group and others (Keebaugh and Young, 2011; Wang et al., 2013).

### Total RNA and protein expression analyses

Male prairie voles were sacrificed by rapid decapitation 2 hours or 9 hours (6-hr cohabitation + 3-hr partner preference test) following the microinfusion of CSF or CSF containing TSA, or immediately after 24 hours of cohabitation and mating with a female. Brains were dissected out immediately after decapitation, frozen on dry ice, and stored at −80°C until further processing. Coronal sections (200 μm) were cut on a cryostat and tissue punches (1 mm diameter) were taken bilaterally from the entire NAcc and caudate putamen (CP) before being stored at −80°C until total RNA and protein extraction using the TRI-Reagent protocol according to the manufacturer's instructions (Molecular Research Center, Cincinnati, OH). OTR and V1aR mRNA and protein levels were then measured by semi-quantitative real-time polymerase chain reaction and Western-blotting, respectively, as previously described (Wang et al., 2013).

Briefly, 0.5 μg of total RNA was reversed-transcribed and analyzed by semi-quantitative real-time PCR in triplicates using previously published primers for OTR, V1aR, and nicotinamide adenine dinucleotide dehydrogenase (NADH) mRNA (Wang et al., 2013). OTR and V1aR qPCR signals were each normalized to NADH, which remained unaffected by cohabitation under TSA treatment or 24 hrs of cohabitation with mating in females (data not shown). For Western-blotting experiments, proteins were separated on a 10% sodium dodecyl sulfate polyacrylamide gel, transferred to a nitrocellulose membrane, and incubated with primary antibodies to OTR (sc-8102, 1:1,000), or V1aR (sc-18096, 1:500), purchased from Santa Cruz Biotechnology (Dallas, TX), or actin (A2066, 1:1,000, Sigma Aldrich, St. Louis, MO). Following incubation with a horseradish peroxidase-conjugated secondary antibody, membranes were revealed with ECL (ECL SuperSignal West Dura substrate, Pierce Biotechnologies), and exposed on Fuji XAR film (Fuji Film), before quantification (AIS 6.0 Image software, Imaging Research). In order to analyze the specificity of the commercial antibody for OTR, we performed a peptide competition assay using protein extracts from the NAcc and hippocampus of adult male prairie voles, in which the OTR

antibody (sc-8102) was pre-absorbed by overnight incubation at 4°C with a blocking peptide (sc-8102P) corresponding to a portion of the human OTR in a 30-fold excess. Between 27.6% to 52.9% of reduction in signal was observed in these assays (Supplementary Figure S1), suggesting that this commercially available antibody (sc-8102) can detect OTR protein. Nevertheless, it is important to note that the signal reduction was not complete, which thus denotes the presence of non-specific binding at the same molecular weight as OTR (66 kDa).

All western-blot and semi-quantitative real-time PCR signals were normalized to actin or NADH, respectively, and expressed as percentage of CSF-treated or Naive animals. Notably, in our previous study conducted in female prairie voles, the effects of TSA on mRNA levels were the most pronounced after 2 hours of cohabitation with a partner, whereas regulations at the protein levels were greater at the 9-hr timepoint (Wang et al., 2013). Based on these observations, mRNA levels were measured at 2-hr timepoint, and protein levels at the 9-hr timepoint.

### Statistical analyses and data processing

During the study of the effects of TSA on the formation of partner preference in male prairie voles, animals with misplaced cannulae (a total of seven distributed among all groups) were excluded from the analyses. The time spent in side-by-side contact with either the partner or the stranger during the partner preference test was analyzed using a paired, two-tailed *t*-test, while the locomotion score and total time spent interacting were analyzed using a one-way ANOVA. While the effect of TSA treatment on the partner preference index was tested with a non-parametric ANOVA (Kruskall-Wallis) across all groups, the presence of partner or stranger preference was analyzed by testing the partner preference index of each treatment group against an index of 0 (no preference for either stimulus female) with a Wilcoxon signed rank test. After verification of normality and test for homo- or heteroskedasticity, all molecular data were analyzed using an unpaired two-tailed *t*-test. All statistical analyses were conducted using GraphPad Prism 6.05 (La Jolla, CA), with a significance threshold of $p < 0.05$, and performed on raw data when standardized to their respective controls (% of CSF or Naive groups). For all pairwise comparisons, the Cohen's d effect size was estimated from each group's mean, standard deviation, and sample size, while the effect size for ANOVA was estimated from the eta squared ($\eta^2$).

## Results

### TSA facilitates partner preference formation in male prairie voles

While CSF-treated males did not exhibit selective side-by-side contact with the partner or the stranger ($t_6 = 0.01$, $p = 0.989$, d = 0.01), male voles treated with 0.08 ng TSA preferentially spent more time in contact with the partner than with the stranger ($t_8 = 3.85$, $p = 0.005$, d = 2.00, Fig. 1A), indicating partner preference. Higher doses of TSA, however, did not result in a selective preference for either stimulus animal (0.4 ng TSA: $t_{11} = 0.99$, $p = 0.344$, d = 0.50; 4 ng TSA: $t_7 = 1.23$, $p = 0.257$, d = 0.83). Accordingly, treatment with TSA affected the partner preference index in male prairie voles (H = 9.37, p = 0.025) in a dose-dependent manner. Indeed, only males treated with TSA at the 0.08 ng dose exhibited an index significantly positive (p = 0.008) and higher than CSF-treated animals (p = 0.008),

revealing that only at this dose of TSA did males exhibit partner preference. Notably, locomotor activity and total time spent interacting with either stimulus animal remained unaffected by TSA at any dose (locomotor activity: $F_{3,32} = 2.62$, $p = 0.067$, $\eta^2 = 0.20$, Fig. 1C; time interacting: $F_{3,32} = 1.24$, $p = 0.31$, $\eta^2 = 0.10$, Fig. 1D), suggesting that the increased time spent in side-by-side contact with the partner female following the lowest dose of TSA results from a true social preference rather than a nonspecific effect on locomotion or global social interaction behavior.

### TSA upregulates OTR expression in the NAcc following cohabitation with a female

In order to investigate the molecular correlates of the facilitation of partner preference by TSA in males, we measured mRNA and protein levels of OTR and V1aR in the NAcc following the 6-hr cohabitation without mating and under TSA treatment at the effective dose (0.08 ng, *i.c.v.*). We thus observed an up-regulation of OTR mRNA and protein levels after 2 and 9 hours, respectively, of cohabitation without mating in TSA-treated male prairie voles when compared to CSF-treated controls (OTR mRNA: $t_{10} = 2.32$, $p = 0.043$, d = 1.34, Fig. 2A; OTR protein: $t_9 = 2.96$, $p = 0.016$, d = 1.79, Fig. 2B). In contrast, V1aR mRNA or protein levels in the NAcc remain unaffected by TSA treatment following cohabitation with a female in the absence of mating (V1aR mRNA: $t_{10} = 0.97$, $p = 0.354$, d = 0.56, Fig. 2A; V1aR protein: $t_{10} = 0.20$, $p = 0.844$, d = −0.12, Fig. 2B). In order to assess the brain area specificity of the neuroadaptations induced by cohabitation under TSA treatment, OTR and V1aR protein levels were also measured in the caudate putamen (CP) as a negative control, because OTR and V1aR expression or neurotransmission in the CP are not involved in formation of partner preference following cohabitation with mating or under TSA treatment (Wang et al., 2013; Young et al., 2001). Accordingly, neither OTR nor V1aR protein levels in the CP were affected by TSA treatment following 9 hours of cohabitation without mating (OTR protein: $t_8 = 1.09$, $p = 0.308$, d = −0.70; V1aR protein: $t_{10} = 0.61$, $p = 0.558$, d = 0.35; Fig. 2C). Altogether, these observations show that, at the dose facilitating partner preference, TSA treatment up-regulates OTR but not V1aR expression in male prairie vole's NAcc in a brain region-specific manner following cohabitation with a female.

### Cohabitation with mating induces similar neuroadaptations as reported with TSA

To investigate whether the OTR plasticity observed following cohabitation under TSA treatment were relevant to processes underlying natural social bonding, a separate cohort of males were cohabitated with a sexually-naive female for 24 hours with mating *ad libitum*, which reliably induces partner preference in males (Aragona et al., 2006; Winslow et al., 1993). In line with our previous observations, we found that 24 hours of cohabitation with a female with mating induced an up-regulation of OTR protein levels in the NAcc, but not CP, in male prairie voles when compared to sexually naive controls ($t_9 = 2.64$, $p = 0.027$, d = 1.60 in the NAcc; $t_{10} = 1.83$, $p = 0.097$, d = −1.06 in the CP; Fig. 3).

## Discussion

In the socially monogamous prairie voles, the formation of partner preference is a behavioral index of social bonding, and involves a variety of neurotransmitters and neuroadaptations in specific brain regions. In this study, we report that, similar to what we observed in females

(Wang et al., 2013), treatment with the HDAC inhibitor TSA facilitates the formation of partner preference in males, even in the absence of mating. This behavioral effect is associated with an up-regulation of OTR mRNA and protein levels in the NAcc, whereas V1aR gene expression remained unaffected. Interestingly, 24 hours of cohabitation and mating with a female, which reliably induces formation of partner preference, triggered a similar OTR up-regulation in the NAcc, demonstrating that the presence of TSA during cohabitation with a female promotes similar neuroadaptations as the natural formation of partner preference.

The formation of partner preference in prairie voles requires a complex integration of multiple socio-environmental stimuli through a neural circuit in which the NAcc is proposed to encode the positive reinforcement from the social cues related to the partner (Numan and Young, 2015; Young et al., 2011). In this process, oxytocin and its receptor OTR play a central role, well-described in females. Indeed, following mating and sustained social interaction with a male, oxytocin is released and OTR expression is increased in the female NAcc (H. E. Ross et al., 2009; Wang et al., 2013). Furthermore, central oxytocin injection facilitates the formation of partner preference, whereas OTR antagonism either centrally or in the NAcc prevents mating-induced partner preference (Cho et al., 1999; Liu and Wang, 2003; Williams et al., 1994). While OTR antagonists also prevent formation of partner preference in males when injected centrally (Cho et al., 1999), the structure specificities of OTR regulation and oxytocin neurotransmission in males remain unclear. In our study, we provide additional evidence for the potential involvement of OTR-mediated neurotransmission in the NAcc in the formation of partner preference in male prairie voles. Indeed, we observed a specific up-regulation of OTR, but not V1aR, in the NAcc of males following TSA-facilitation of partner preference, which was also observed following natural initiation of the social bond by cohabitation with a female in the presence of mating. It is important to note, however, that we observed a limited specificity from the OTR antibody used in our study. Indeed, following verification of the antibody's specificity for OTR in the male vole brain in a peptide competition assay, we observed only a partial reduction of the signal, thereby highlighting the presence of non-specific binding at the molecular weight of OTR (Supplementary Figure 1C, Supplementary Note). This partial specificity could impede the accuracy of our OTR proteins measurements. Nonetheless, it is important to note that the changes in OTR protein expression levels we observed following cohabitation under TSA treatment are also supported by a parallel increase in corresponding mRNA levels in our current (Fig. 2A) and previous study in female prairie voles (Wang et al., 2013). Furthermore, although the functional link between OTR up-regulation in the NAcc and facilitation of partner preference in males remains to be clearly established, some evidence points towards a successful impairment of mating-induced partner preference by OTR antagonism in the NAcc (Numan and Young, 2015). Altogether, these findings support the mediation of partner preference formation by the OTR up-regulation observed in the male NAcc following TSA treatment or prolonged cohabitation with mating.

Such up-regulation of OTR in the NAcc following cohabitation with a female under TSA treatment or following mating in male prairie voles brings new evidence for a critical role played by oxytocin neurotransmission in the NAcc in the formation of partner preference. Indeed, it is interesting to note that the absence of V1aR up-regulation we observed in the

male NAcc could seem in contradiction with the known involvement of AVP neurotransmission in mediating the formation of partner preference in male prairie voles (Cho et al., 1999; Winslow et al., 1993). Nevertheless, this observation could be explained by a structure-specificity of V1aR-mediated neurotransmission as V1aR regulation is critical for the formation of partner preference in other structures such as the ventral pallidum (VP) and the lateral septum (LS) (Barrett et al., 2013; Lim and Young, 2004; Liu et al., 2001). Indeed, while V1aR density in these structures differs between the socially monogamous prairie voles and the non-monogamous meadow voles, no difference are detectable between monogamous and non-monogamous species in V1aR density in the NAcc (Insel et al., 1994; Young et al., 2011). Furthermore, V1aR antagonism or knockdown in the VP or LS but not in other regions prevents formation of partner preference in male prairie voles (Barrett et al., 2013; Lim and Young, 2004; Liu et al., 2001), suggesting that in males, V1aR-mediated neurotransmission in the VP and LS, rather than the NAcc, is required for partner preference formation. Notably, it is interesting to note that TSA does not up-regulate OTR by itself in the NAcc but rather potentiates the neuroadaptations induced by cohabitation with a partner (Wang et al., 2013). Therefore, the absence of V1aR up-regulation in the male NAcc following cohabitation under TSA treatment suggests that, in the NAcc of male prairie voles, V1aR-mediated neurotransmission is not critically activated during cohabitation with a female. Accordingly, our findings thus support a limited involvement, in the NAcc, of V1aR-mediated neurotransmission in favor of OTR-mediated neurotransmission during formation of partner preference in male prairie voles.

Interestingly, such preponderance of OTR-mediated neurotransmission in the male NAcc can also be extended between sexes. Indeed, while OTR but not V1aR is up-regulated in males following cohabitation with an opposite-sex partner under TSA treatment, both OTR and V1aR are up-regulated in females (Wang et al., 2013). Although this sex difference could simply be explained by the use of a different dose of TSA between males (0.08 ng, *i.c.v.*) and females (0.4 ng, *i.c.v.*), it is important to note that only the lowest dose (0.08 ng, *i.c.v.*) facilitated the formation of partner preference in males, whereas all three doses tested were effective in females (Wang et al., 2013). In line with the preponderance of OTR-mediated neurotransmission in the NAcc of males, it is interesting to note that a higher dose of oxytocin (*i.c.v.*) is required in females than in males to facilitate the formation of partner preference in the absence of mating (Cho et al., 1999), suggesting that male prairie voles exhibit a higher sensitivity to stimulation of the oxytocinergic system. Therefore, the ineffectiveness of the two highest doses of TSA in facilitating partner preference formation in males could result from eventual side effects that were not detected in females.

It is important to note that the latter hypothesis is reinforced by the fact that TSA was injected centrally, thereby potentially affecting other structures implicated in the formation of partner preference in male prairie voles, such as the LS or VP (Insel et al., 1994; Insel and Shapiro, 1992; Wang et al., 1997). In this context, it is particularly interesting to consider the effects of TSA on the partner preference index (Fig. 1B). Indeed, following treatment with the lowest dose of TSA (0.08 ng), we observed a larger proportion of voles developing a partner preference than CSF-treated animals, which suggests that, as in females (Wang et al., 2013), TSA truly potentiates the impact of social interactions during the cohabitation period and thus lowers the threshold for the formation of partner preference. Combined to the

central mode of TSA injection, this observation further supports the possibility for TSA at the two highest doses for potentiating other consequences of the social cohabitation, including some potentially interfering with the formation of partner preference.

## Conclusions

In the socially monogamous prairie voles, the development of a pair-bond is indicated by the formation of partner preference, which involves a variety of neurotransmitters, neuropeptides, and brain regions. In this study, we showed that treatment with the HDAC inhibitor TSA facilitates the formation of partner preference in male prairie voles even in the absence of mating. Notably, this finding was associated with an up-regulation of OTR in the NAcc that was also observed following natural formation of partner preference by sustained cohabitation with a female in the presence of mating. Although the context-specificity of the TSA effects remain to be confirmed in males, these findings support a potentiation of the impact of the social interactions occurring with the partner during the cohabitation period that ultimately lead to the development of partner preference, as observed in females (Wang et al., 2013). Through the identification of common mechanisms and dose at which TSA facilitates the formation of partner preference in both males and females, our findings refine new promising opportunities to influence social bonding.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

This work was supported by the National Institute of Mental Health (NIMH) grants MHR21-083128 to M.K. and Z.W., MHR01-087583 and MHR01-099085 to M.K., and MHR01-058616 to Z.W.

## References

Aragona BJ, Liu Y, Yu YJ, Curtis JT, Detwiler JM, Insel TR, Wang Z. Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. Nat Neurosci. 2006; 9:133–139. [PubMed: 16327783]

Barrett CE, Keebaugh AC, Ahern TH, Bass CE, Terwilliger EF, Young LJ. Variation in vasopressin receptor (Avpr1a) expression creates diversity in behaviors related to monogamy in prairie voles. Horm Behav. 2013; 63:518–526. [PubMed: 23370363]

Cho MM, DeVries AC, Williams JR, Carter CS. The effects of oxytocin and vasopressin on partner preferences in male and female prairie voles (Microtus ochrogaster). Behav Neurosci. 1999; 113:1071–1079. [PubMed: 10571489]

Insel TR, Hulihan TJ. A gender-specific mechanism for pair bonding: oxytocin and partner preference formation in monogamous voles. Behav Neurosci. 1995; 109:782–789. [PubMed: 7576222]

Insel TR, Preston S, Winslow JT. Mating in the monogamous male: behavioral consequences. Physiol Behav. 1995; 57:615–627. [PubMed: 7777594]

Insel TR, Shapiro LE. Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. Proc Natl Acad Sci U S A. 1992; 89:5981–5985. [PubMed: 1321430]

Insel TR, Wang ZX, Ferris CF. Patterns of brain vasopressin receptor distribution associated with social organization in microtine rodents. J Neurosci. 1994; 14:5381–5392. [PubMed: 8083743]

Keebaugh AC, Barrett CE, Laprairie JL, Jenkins JJ, Young LJ. RNAi knockdown of oxytocin receptor in the nucleus accumbens inhibits social attachment and parental care in monogamous female prairie voles. Soc Neurosci. 2015; 10:561–570. [PubMed: 25874849]

Keebaugh AC, Young LJ. Increasing oxytocin receptor expression in the nucleus accumbens of pre-pubertal female prairie voles enhances alloparental responsiveness and partner preference formation as adults. Horm Behav. 2011; 60:498–504. [PubMed: 21851821]

Lim MM, Young LJ. Vasopressin-dependent neural circuits underlying pair bond formation in the monogamous prairie vole. Neuroscience. 2004; 125:35–45. [PubMed: 15051143]

Liu Y, Aragona BJ, Young KA, Dietz DM, Kabbaj M, Mazei-Robison M, Nestler EJ, Wang Z. Nucleus accumbens dopamine mediates amphetamine-induced impairment of social bonding in a monogamous rodent species. Proc Natl Acad Sci U S A. 2010; 107:1217–1222. [PubMed: 20080553]

Liu Y, Curtis JT, Wang Z. Vasopressin in the lateral septum regulates pair bond formation in male prairie voles (Microtus ochrogaster). Behav Neurosci. 2001; 115:910–919. [PubMed: 11508730]

Liu Y, Wang ZX. Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female prairie voles. Neuroscience. 2003; 121:537–544. [PubMed: 14568015]

Numan M, Young LJ. Neural mechanisms of mother-infant bonding and pair bonding: Similarities, differences, and broader implications. Horm Behav. 2015; doi: 10.1016/j.yhbeh.2015.05.015

Ophir AG, Gessel A, Zheng DJ, Phelps SM. Oxytocin receptor density is associated with male mating tactics and social monogamy. Horm Behav. 2012; 61:445–453. [PubMed: 22285648]

Ross HE, Cole CD, Smith Y, Neumann ID, Landgraf R, Murphy AZ, Young LJ. Characterization of the oxytocin system regulating affiliative behavior in female prairie voles. Neuroscience. 2009; 162:892–903. [PubMed: 19482070]

Ross HE, Freeman SM, Spiegel LL, Ren X, Terwilliger EF, Young LJ. Variation in oxytocin receptor density in the nucleus accumbens has differential effects on affiliative behaviors in monogamous and polygamous voles. J Neurosci. 2009; 29:1312–1318. [PubMed: 19193878]

Wang H, Duclot F, Liu Y, Wang Z, Kabbaj M. Histone deacetylase inhibitors facilitate partner preference formation in female prairie voles. Nat Neurosci. 2013; 16:919–924. [PubMed: 23727821]

Wang Z, Young LJ, Liu Y, Insel TR. Species differences in vasopressin receptor binding are evident early in development: comparative anatomic studies in prairie and montane voles. J Comp Neurol. 1997; 378:535–546. [PubMed: 9034909]

Williams JR, Catania KC, Carter CS. Development of partner preferences in female prairie voles (Microtus ochrogaster): the role of social and sexual experience. Horm Behav. 1992; 26:339–349. [PubMed: 1398553]

Williams JR, Insel TR, Harbaugh CR, Carter CS. Oxytocin administered centrally facilitates formation of a partner preference in female prairie voles (Microtus ochrogaster). J Neuroendocrinol. 1994; 6:247–250. [PubMed: 7920590]

Winslow JT, Hastings N, Carter CS, Harbaugh CR, Insel TR. A role for central vasopressin in pair bonding in monogamous prairie voles. Nature. 1993; 365:545–548. [PubMed: 8413608]

Young KA, Gobrogge KL, Liu Y, Wang Z. The neurobiology of pair bonding: insights from a socially monogamous rodent. Front Neuroendocrinol. 2011; 32:53–69. [PubMed: 20688099]

Young KA, Liu Y, Wang Z. The neurobiology of social attachment: A comparative approach to behavioral, neuroanatomical, and neurochemical studies. Comp Biochem Physiol C Toxicol Pharmacol. 2008; 148:401–410. [PubMed: 18417423]

Young LJ, Lim MM, Gingrich B, Insel TR. Cellular mechanisms of social attachment. Horm Behav. 2001; 40:133–138. [PubMed: 11534973]

## Highlights

- Trichostatin A facilitates partner preference formation in male prairie voles.
- Cohabitation with trichostatin A up-regulates OTR, but not V1aR, in the NAcc.
- Cohabitation and mating also up-regulates OTR expression in the NAcc.

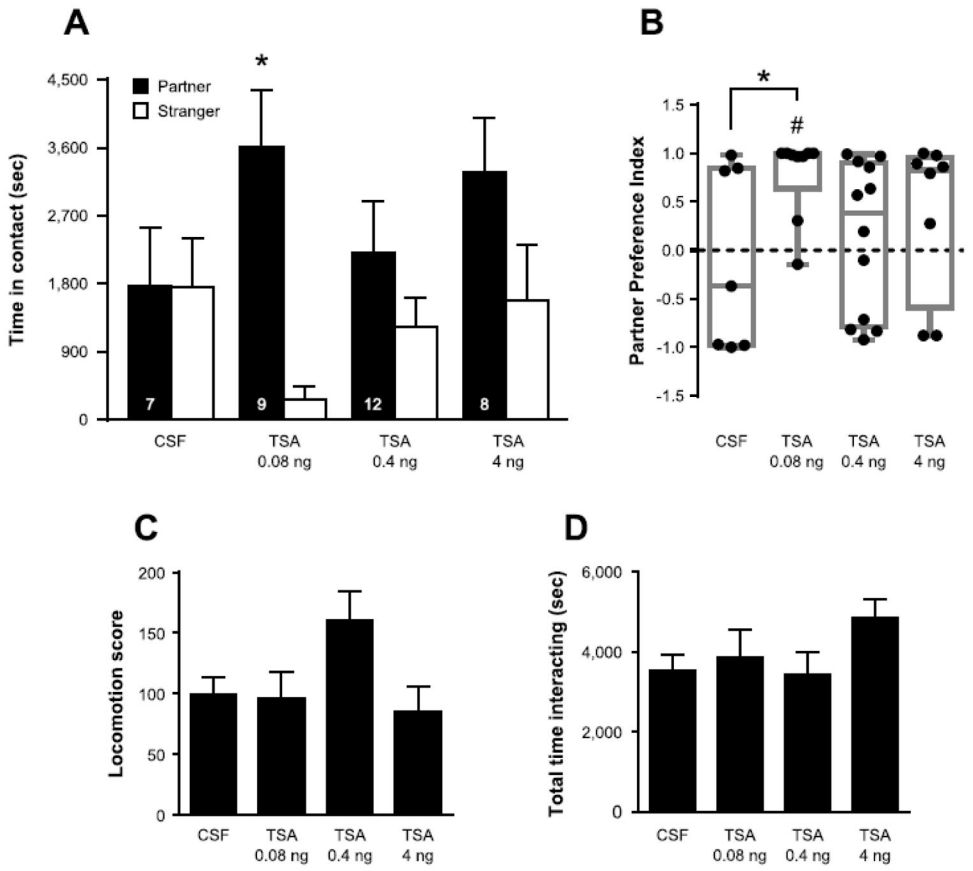


**Figure 1.**

Trichostatin A (TSA) facilitates partner preference formation in male prairie voles in the absence of mating. (A) Males injected with artificial cerebrospinal fluid (CSF) spent a similar amount of time in side-by-side contact with the partner and stranger female, while males receiving an acute injection of 0.08 ng TSA, but not higher doses, spent preferentially more time with the partner than with the stranger during the partner preference test. Similarly, only males treated with 0.08 ng TSA exhibited a partner preference index (B) positive and higher than CSF-treated voles. Neither locomotor activity (C), nor total time spent interacting with either stimulus female (D) was affected by TSA treatment at any dose. In (A,C,D), the number of animals per group is represented within each column, and data are presented as mean ± SEM. *$p < 0.05$ vs "Stranger" of the same biological group, paired two-tailed *t* test. In (B), data are represented as whisker-plot where the horizontal bar corresponds to the median, and each animal is depicted as individual black dot. $^{\#}p < 0.05$ vs. an index of "0", Wilcoxon signed ranked test.

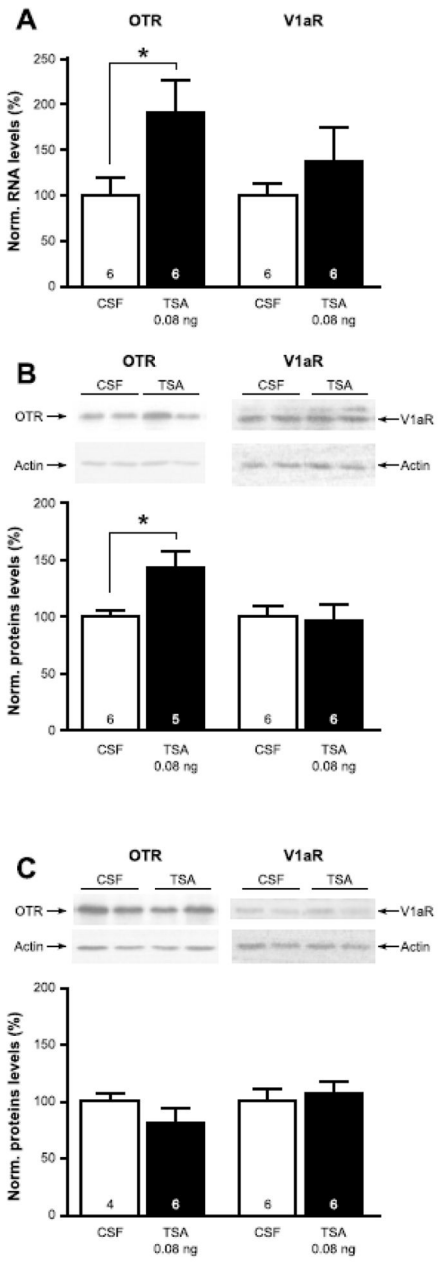


**Figure 2.**

Trichostatin A (TSA, 0.08 ng) up-regulates oxytocin receptor (OTR) but not vasopressin V1a receptor (V1aR) expression in the nucleus accumbens (NAcc) but not caudate putamen (CP) in male prairie voles during cohabitation with a female in the absence of mating. OTR but not V1aR mRNA levels (A) are up-regulated in the NAcc following two hours of cohabitation with a female in the absence of mating in TSA-treated males. Similarly, OTR but not V1aR protein levels are up-regulated in the NAcc (B) of TSA-treated males following 9 hours of cohabitation with a female in the absence of mating. In the CP, however, OTR and V1aR protein levels remained unaffected by TSA treatment (C). In (B,C), representative blots for each target protein (top line) and actin (bottom line) are shown above

their respective column. Data are presented as mean ± SEM, and the number of animals per group is detailed within each column. *$p < 0.05$ vs CSF-treated voles, unpaired two-tailed $t$ test.

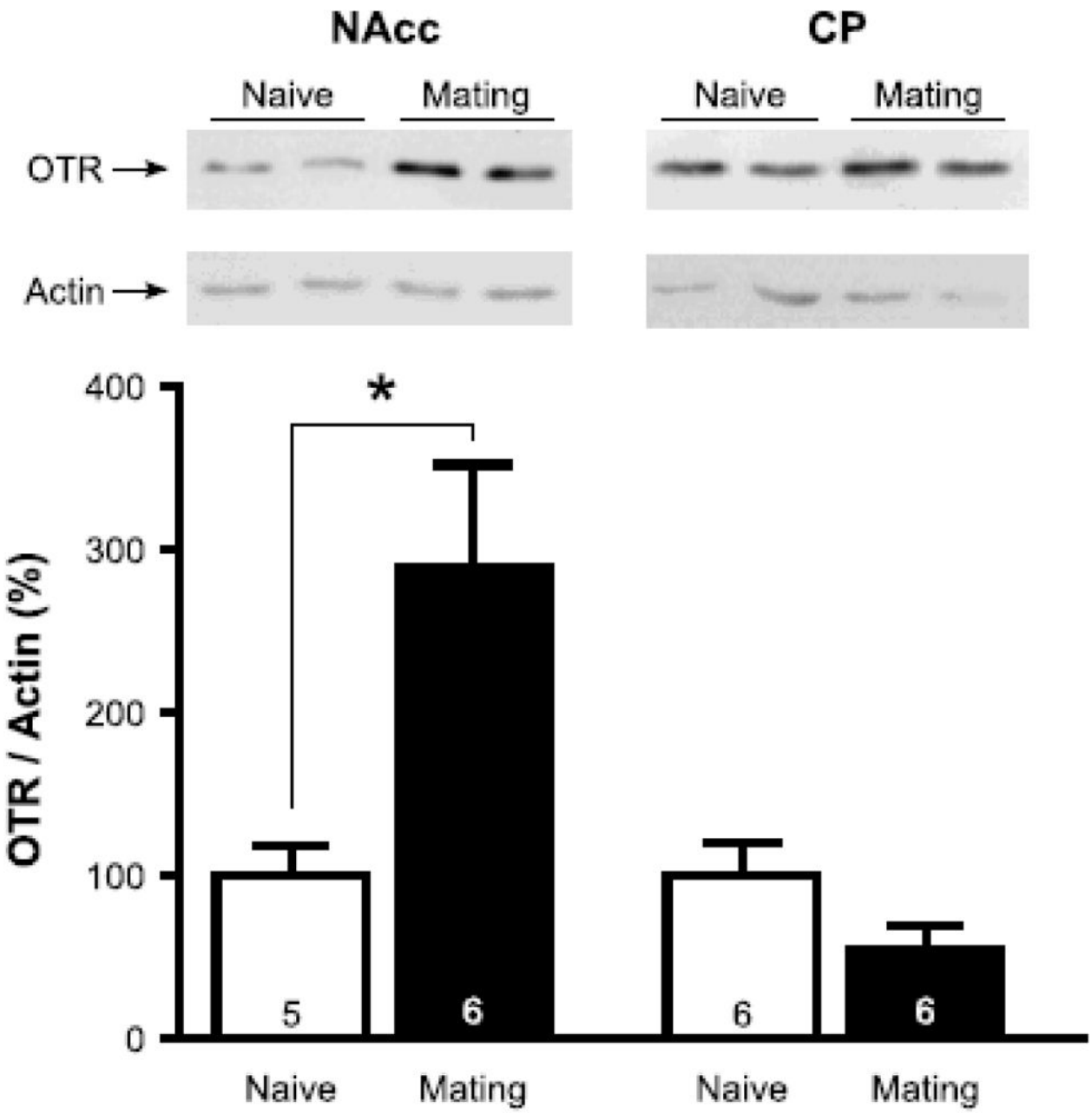


**Figure 3.**

Cohabitation with mating for 24 hours with a female induces an up-regulation of oxytocin receptor (OTR) protein levels in the nucleus accumbens (NAcc) but not in the caudate putamen (CP) of male prairie voles. Representative blots for OTR (top line) and actin (bottom line) are shown above their respective column. Data are presented as mean ± SEM, and the number of animals per group is represented within each column. *$p < 0.05$ vs sexually-naive voles, unpaired two-tailed *t* test.