nature neuroscience

Histone deacetylase inhibitors facilitate partner preference formation in female prairie voles

Hui Wang[1–4], Florian Duclot[1,2,4], Yan Liu[2,3], Zuoxin Wang[2,3] & Mohamed Kabbaj[1,2]

In the socially monogamous prairie vole (*Microtus ochrogaster*), mating induces enduring pair-bonds that are initiated by partner preference formation and regulated by a variety of neurotransmitters, including oxytocin, vasopressin and dopamine. We examined potential epigenetic mechanisms mediating pair-bond regulation and found that the histone deacetylase inhibitors sodium butyrate and trichostatin A (TSA) facilitated partner preference formation in female prairie voles in the absence of mating. This was associated with a specific upregulation of oxytocin receptor (OTR, *oxtr*) and vasopressin V1a receptor (V1aR, *avpr1a*) in the nucleus accumbens (NAcc), through an increase in histone acetylation at their respective promoters. Furthermore, TSA-facilitated partner preference was prevented by OTR or V1aR blockade in the NAcc. Notably, mating-induced partner preference triggered the same epigenetic regulation of *oxtr* and *avpr1a* gene promoters as TSA. These observations indicate that TSA and mating facilitate partner preference through epigenetic events, providing, to the best of our knowledge, the first direct evidence for epigenetic regulation of pair-bonding.

Social affiliation is an essential characteristic of human social behaviors, and social cognitive deficits are common features in a multitude of neuropsychiatric disorders, including schizophrenia, autism spectrum disorders, addiction and depression[1]. The socially monogamous prairie vole (*M. ochrogaster*) has emerged as a model for the investigation of the neurobiological bases of social attachment, as both laboratory and free-living individuals establish long-term pair-bonds[2–4], which are first initiated by the formation of selective affiliation behaviors toward the partner, known as partner preference[5]. This formation of partner preference involves a variety of neurotransmitters and hormonal systems, including the neuropeptides oxytocin and vasopressin (AVP) and mesolimbic dopamine[5]. In general, partner preference formation is mediated through AVP neurotransmission in the ventral pallidum and lateral septum in males, and oxytocin neurotransmission in the NAcc and prelimbic cortex in females[6–8]. Typically involved in natural reward such as mating, dopamine also acts as a critical mediator of partner preference in prairie voles. Activation of the dopamine D2-type receptors (D2Rs) in the NAcc facilitates, whereas activation of the dopamine D1-type receptors (D1Rs) inhibits, partner preference formation in both male and female prairie voles[9–11]. Notably, variations in gene expression of *oxtr* and *avpr1a* can markedly affect partner preference. In female prairie voles, for instance, the overexpression of OTR in the NAcc facilitates partner preference in the absence of mating[12–14].

Beyond the regulation of pair-bonding, oxytocin and AVP have also been implicated in a broad range of social behaviors, including social recognition, aggression and maternal care[15,16]. Notably, disruptions of the latter behavior in rodents induce long-lasting neuroadaptations through epigenetic mechanisms, including DNA methylation of estrogen receptor alpha[17] and AVP genes[18], as well as histone acetylation of the *glucocorticoid receptor* promoter[19]. Moreover, histone deacetylase (HDAC) inhibitors, which enhance gene expression through increased histone acetylation in the rodent brain[20], can reverse these alterations[19] and directly influence social behaviors such as sexual receptivity[21]. Notably, in a lung cancer cell line, the HDAC inhibitor TSA directly enhances OTR transcription by locally promoting histone acetylation[22].

Thus, an epigenetic basis in partner preference formation in prairie voles is possible. To test this hypothesis, we first assessed the effects of two HDAC inhibitors, sodium butyrate (NaB) and TSA, on partner preference formation in adult female prairie voles. Thereafter, we investigated the molecular mechanisms mediating the effects of TSA in inducing partner preference in female prairie voles. Finally, we sought to determine whether the epigenetic alterations induced by TSA during cohabitation were also triggered by mating.

RESULTS

TSA treatment facilitates partner preference

Sexually naive female prairie voles were injected intracerebroventricularly (icv) with cerebrospinal fluid (CSF) or CSF containing 0.08, 0.4 or 4 ng of TSA immediately before a 6-h cohabitation with a male without mating, and their partner preference was then tested. Partner preference in female prairie voles is not induced by 6 h of cohabitation with a male without mating[4], and this behavioral procedure has been extensively used to assess the effects of various drugs on facilitating partner preference formation[5].

CSF-treated voles showed nonselective side-by-side contact with the partner or the stranger following 6 h of cohabitation without mating ($t_{15} = 0.76$, $P = 0.46$; **Fig. 1a**). However, voles treated with TSA at any of the doses tested spent preferentially more time with the partner

[1]Department of Biomedical Sciences, Florida State University, Tallahassee, Florida, USA. [2]Program in Neuroscience, Florida State University, Tallahassee, Florida, USA. [3]Department of Psychology, Florida State University, Tallahassee, Florida, USA. [4]These authors contributed equally to this work. Correspondence should be addressed to M.K. (mohamed.kabbaj@med.fsu.edu) or Z.W. (zwang@psy.fsu.edu).

Received 14 March; accepted 6 May; published online 2 June 2013; doi:10.1038/nn.3420

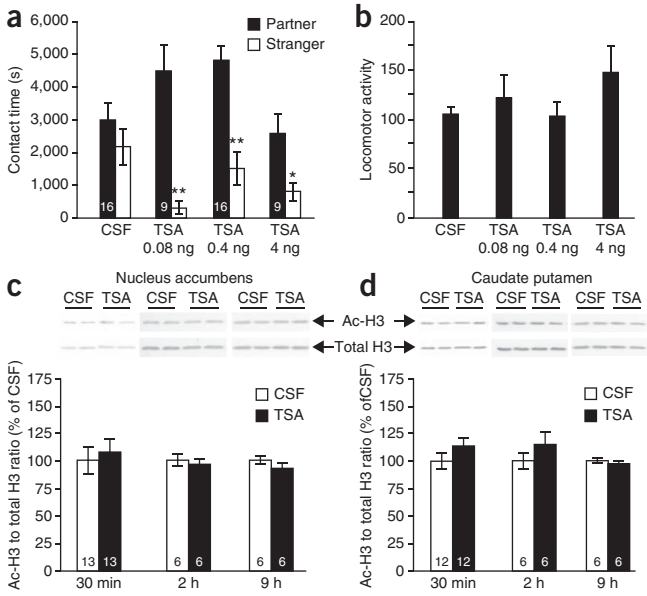

Figure 1 An acute injection of TSA facilitates partner preference formation in female prairie voles in the absence of mating. (**a**) CSF-treated females exposed to a male for 6 h in the absence of mating showed nonselective side-by-side contact during the partner preference test, whereas females injected with 0.08, 0.4 or 4 ng of TSA spent more time with the partner than with the stranger. (**b**) The TSA injection did not influence the locomotion of the voles at any of the doses used. (**c**,**d**) Bottom, at 0.4 ng, TSA did not significantly alter global histone H3 acetylation (Lys14) (Ac-H3) levels in the NAcc or the caudate putamen, as measured 30 min, 2 h and 9 h after the beginning of the cohabitation period. Top, typical blots of CSF- or TSA-treated voles (full-length blots are presented in **Supplementary Fig. 2**). The numbers of voles used are indicated in the columns. $^{*}P < 0.05$, $^{**}P < 0.01$ versus partner (**a**), unpaired two-tailed paired *t*-test. Data are presented as mean ± s.e.m.

than with the stranger (0.08 ng: $t_8 = 4.35$, $P = 0.002$; 0.4 ng: $t_{15} = 3.63$, $P = 0.002$; 4 ng: $t_8 = 2.58$, $P = 0.03$). Notably, we found no group differences in locomotor activity ($F_{3,46} = 1.25$, $P = 0.30$, **Fig. 1b**) and no aggressive behavior by the test female toward either the stranger or the partner, indicating that the effects of TSA were specific to a social preference, rather than being secondary to an alteration of locomotion or social aversion to the stranger.

To investigate whether TSA enhances histone acetylation in brain structures involved in the formation of partner preference, we injected a separate batch of females with the medium dose of TSA (0.4 ng) and cohabited them with a male in the absence of mating for 30 min, 2 h or 9 h. Notably, we detected no significant variation in the global histone H3 acetylation (Lys14) levels at any time point in both the NAcc and caudate putamen ($P > 0.05$ for all groups; **Fig. 1c**,**d**). This suggests that TSA facilitates partner preference formation in the absence of mating, despite having no effect on global histone H3 acetylation in the NAcc or the caudate putamen.

Notably, NaB also facilitated partner preference in female prairie voles following cohabitation with a male for 6 h without mating, and was associated with an increase in global histone H3 acetylation (Lys14) in the NAcc (**Supplementary Figs. 1** and **2**). The effects of TSA on partner preference formation could therefore be reproduced with another HDAC inhibitor, suggesting the involvement of HDAC inhibition, rather than a nonspecific effect of TSA in the facilitation of partner preference. Considering that TSA is a more specific and affine class I and II HDAC inhibitor[23,24], and that the behavioral effects of TSA were more pronounced than NaB, we chose to use TSA over NaB.

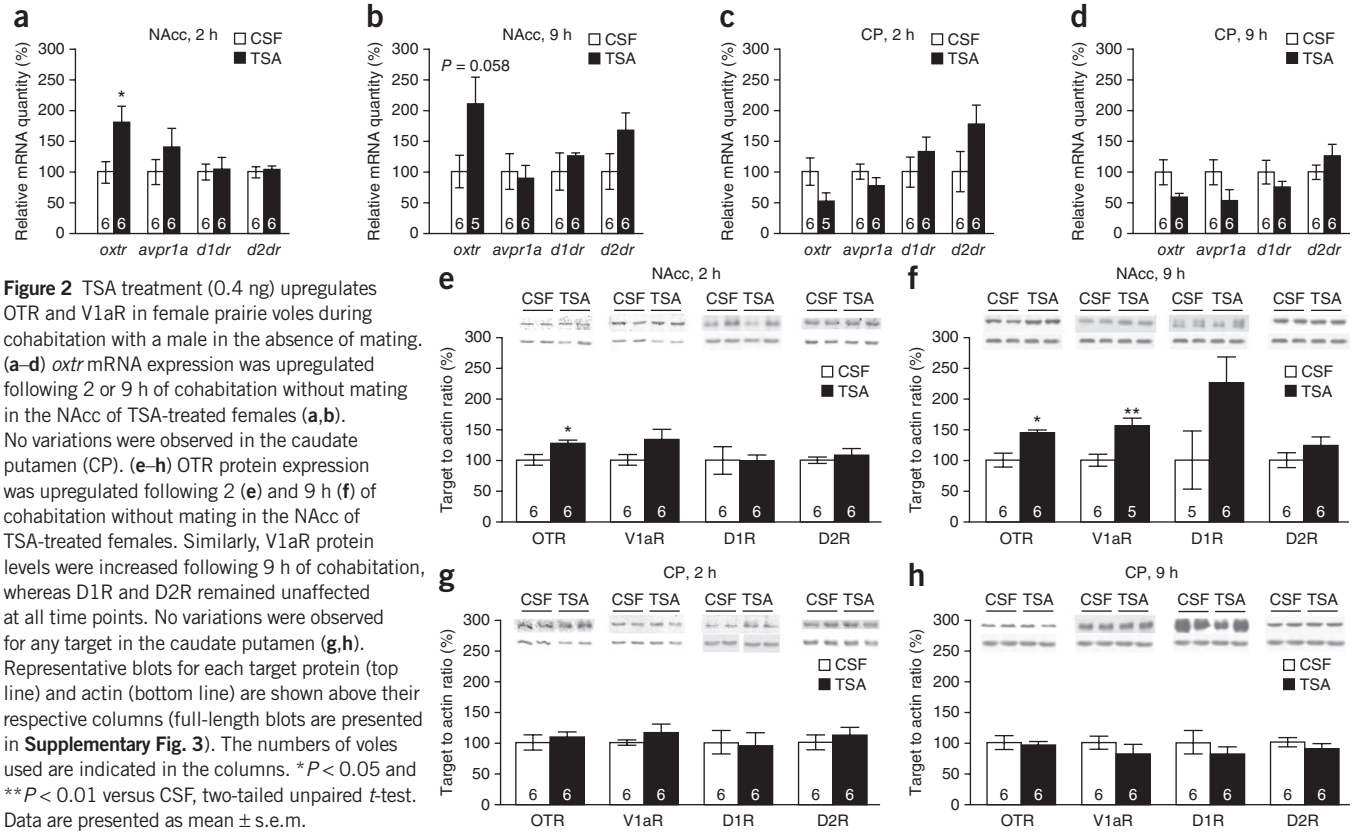

Figure 2 TSA treatment (0.4 ng) upregulates OTR and V1aR in female prairie voles during cohabitation with a male in the absence of mating. (**a**–**d**) *oxtr* mRNA expression was upregulated following 2 or 9 h of cohabitation without mating in the NAcc of TSA-treated females (**a**,**b**). No variations were observed in the caudate putamen (CP). (**e**–**h**) OTR protein expression was upregulated following 2 (**e**) and 9 h (**f**) of cohabitation without mating in the NAcc of TSA-treated females. Similarly, V1aR protein levels were increased following 9 h of cohabitation, whereas D1R and D2R remained unaffected at all time points. No variations were observed for any target in the caudate putamen (**g**,**h**). Representative blots for each target protein (top line) and actin (bottom line) are shown above their respective columns (full-length blots are presented in **Supplementary Fig. 3**). The numbers of voles used are indicated in the columns. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus CSF, two-tailed unpaired *t*-test. Data are presented as mean ± s.e.m.

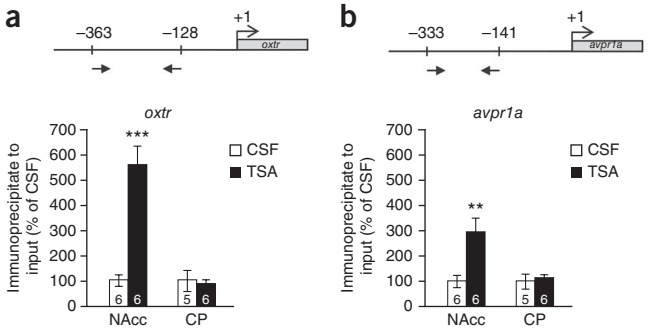

Figure 3 TSA treatment enhances histone acetylation of *oxtr* and *avpr1a* promoters during cohabitation with a male in the absence of mating. (**a**,**b**) Histone H3 acetylation (Lys14) at *oxtr* (**a**) and *avpr1a* (**b**) promoters was increased in the NAcc, but not caudate putamen (CP), of females prairie voles treated with TSA (0.4 ng) after 30 min of cohabitation with a male in the absence of mating. Top, a schematic map of each promoter with the respective primers used (arrows) and their position relative to the transcription start site (+1 site). The numbers of voles used are indicated in the columns. $^{**}P < 0.01$ and $^{***}P < 0.001$ versus CSF, two-tailed unpaired *t* test. Data are presented as mean ± s.e.m.

Molecular correlates of TSA-facilitated partner preference

As variations in gene expression levels in the vole NAcc have been associated with different mating strategies between monogamous and non-monogamous voles, and with alteration of partner preference formation in prairie voles in particular[12,13,25,26], we assessed whether TSA-facilitated partner preference formation was associated with variations of gene expression in the NAcc. TSA treatment (0.4 ng, icv) induced an increase in *oxtr* mRNA levels in the NAcc following 2 h of cohabitation, as compared with CSF-treated controls ($t_{10} = 2.38$, $P = 0.038$; **Fig. 2a**), that tended to be sustained after 9 h of cohabitation ($t_9 = 2.17$, $P = 0.058$; **Fig. 2b**). Although a slight, but not significant, increase in *avpr1a* mRNA could be observed in the NAcc 2 h after the TSA injection, no other group differences were detected at either time point for any of the other mRNAs measured, including *d1dr* or *d2dr* ($P > 0.05$; **Fig. 2a**,**b**). Notably, no group differences were observed in the caudate putamen at any time point and for any mRNA measured ($P > 0.05$ for all groups; **Fig. 2c**,**d**), suggesting that the increase in *oxtr* mRNA observed in TSA-treated voles was specific to the NAcc. Furthermore, such upregulation was present only following cohabitation with a male, as *oxtr* and *avpr1a* mRNA levels in the NAcc remained unchanged 2 h after TSA injection without cohabitation (*oxtr*: CSF, 100.0% ± 11.70; TSA, 86.7% ± 12.11; $t_{12} = 0.79$, $P = 0.444$; *avpr1a*: CSF, 100.0% ± 26.24; TSA, 92.3% ± 13.75; $t_9 = 0.27$, $P = 0.791$).

Consistent with higher *oxtr* mRNA levels, TSA-treated voles also exhibited higher OTR protein levels at both time points in the NAcc (2 h, $t_{10} = 2.34$, $P = 0.041$; 9 h, $t_{10} = 3.16$, $P = 0.01$; **Fig. 2e**,**f**), but not caudate putamen ($t_{10} = 0.41$, $P = 0.69$; **Fig. 2g**,**h**). Although no significant alteration of *avpr1a* mRNA levels could be detected in the NAcc at 2 or 9 h after the TSA injection (**Fig. 2a**,**b**), the V1aR protein levels were significantly increased at 9 h, as compared with CSF-treated voles, in the NAcc ($t_9 = 3.46$, $P = 0.007$; **Fig. 2f**), but not caudate putamen ($t_{10} = 0.98$, $P = 0.35$; **Fig. 2h**). Although with some variation, D1R and D2R protein levels in the NAcc and caudate putamen were not significantly affected by TSA administration ($P > 0.05$; **Fig. 2e–h**).

TSA facilitates histone acetylation of oxtr and avpr1a

The increase in both mRNA and protein levels of OTR following cohabitation after TSA treatment suggests that TSA likely increases the transcription of *oxtr* rather than altering the translation or turnover of the protein. Moreover, V1aR protein levels were higher in the NAcc, associated with a slight, but not significant, increase in mRNA levels following TSA treatment ($P > 0.05$; **Fig. 2**). Considering that TSA is a powerful class I and II HDAC inhibitor[23,24,27], we hypothesized that TSA increases histone acetylation at the *oxtr* and *avpr1a* promoters in the NAcc, thereby enhancing their transcription. A new batch of voles received icv injection of TSA (0.4 ng) and were immediately cohabited with a male without mating for 30 min before being killed. The 30-min time window was chosen on the basis of previous work reporting a maximum increase of histone acetylation after local TSA injection in rats and mice[28,29]. We then analyzed H3K14 acetylation at the *oxtr* and *avpr1a* promoters by chromatin immunoprecipitation.

Consistent with the increase in mRNA and protein levels of OTR that we observed, TSA-treated voles exhibited a very high increase (+460%) in histone H3 acetylation at the *oxtr* promoter, as compared with CSF-treated controls, in the NAcc ($t_{10} = 5.88$, $P = 0.0002$), but not caudate putamen ($t_9 = 0.31$, $P = 0.76$; **Fig. 3a**). Moreover, histone H3 acetylation at the *avpr1a* promoter was significantly elevated 30 min after TSA administration (+196%) in the NAcc ($t_{10} = 3.12$, $P = 0.01$), but not caudate putamen ($t_9 = 0.38$, $P = 0.71$), as compared with CSF-treated controls (**Fig. 3b**). Thus, TSA administration increased histone acetylation site specifically in the NAcc as early as 30 min after the beginning of the cohabitation with a male.

TSA facilitates partner preference via OTR and V1aR

From the previous set of experiments, a molecular model of action emerges in which TSA potentiates histone acetylation at the *oxtr* and *avpr1a* promoters during cohabitation and thereafter enhances their transcription, resulting in higher OTR and V1aR protein levels up to 9 h after the beginning of the cohabitation period. This TSA effect was site specific, as the caudate putamen remained unaffected. We tested whether this TSA-induced increase in OTR and V1aR is related to the facilitation of partner preference formation. We intra-NAcc injected female prairie voles with TSA (0.04 ng per side) with or without prior injection (30 min before TSA injection, 0.5 ng per side) of CSF or CSF containing either one of two different OTR antagonists, OTA(B) and OTA(T), or a V1aR antagonist (V1aRA). Immediately after the TSA injection, the females were cohabited with a male for 6 h without mating, followed by a partner preference test.

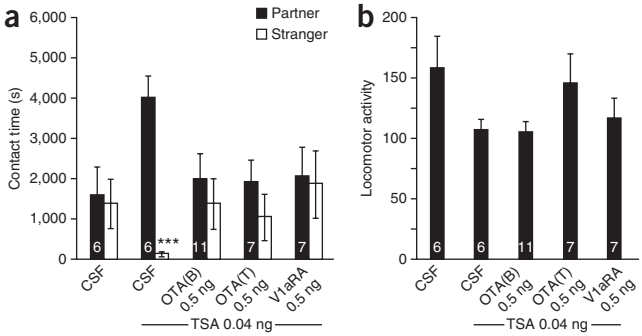

Figure 4 TSA-facilitated partner preference requires OTR- and V1aR-mediated neurotransmission in the female NAcc. (**a**) TSA facilitates partner preference when infused into the NAcc (0.04 ng per side), but its effects are prevented by pre-administration of OTR or V1aR antagonists 30 min prior. (**b**) The locomotion remained unaffected by any of the treatments. The numbers of voles used are indicated in the columns. $^{***}P < 0.001$ versus partner, two-tailed paired *t* test. Data are presented as mean ± s.e.m.

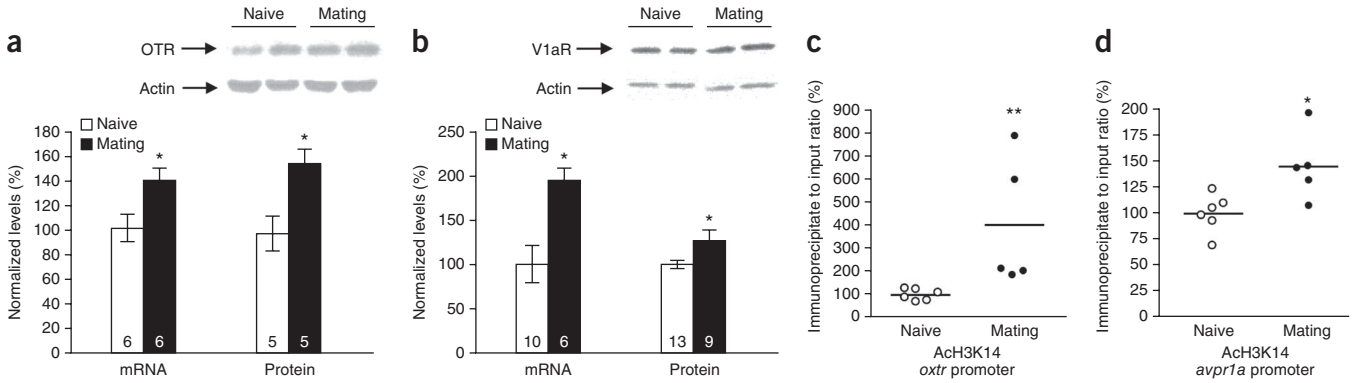

Figure 5 Cohabitation with mating induces an upregulation of OTR and V1aR in the NAcc of female prairie voles. (**a**,**b**) Top, typical blots of naive and mating groups (full-length blots are presented in **Supplementary Fig. 4**). Bottom, 24 h cohabitation with a male with mating upregulated OTR (**a**) and V1aR (**b**) mRNA and protein levels. The numbers of voles used are indicated in the columns. Data are presented as mean ± s.e.m. (**c**,**d**) Accordingly, histone H3 (Lys14) acetylation in the NAcc at the *oxtr* (**c**) and *avpr1a* (**d**) promoters was increased following 6 h of cohabitation with mating. Data are represented as individual data points with mean (black bar). $^{*}P < 0.05$ and $^{**}P < 0.01$ versus naive, two-tailed, unpaired *t* test.

CSF-treated voles did not show a partner preference ($t_5 = 0.17$, $P = 0.87$; **Fig. 4a**). However, TSA-treated voles spent significantly more time in side-by-side contact with the partner than with the stranger ($t_5 = 7.04$, $P = 0.0009$), suggesting that a single TSA injection directly into the NAcc was sufficient to facilitate partner preference formation without mating. Blockade of either OTR or V1aR by pre-treatment with OTA(B), OTA(T) or V1aRA prevented the effects of TSA ($P > 0.05$ for all groups). As no group differences were found in locomotor activity ($F_{4,32} = 1.89$, $P = 0.14$; **Fig. 4b**), these data suggest that TSA in the NAcc facilitates partner preference formation via OTR- and V1aR-mediated mechanisms in a behavior-specific manner.

Mating induces similar neuroadaptations as TSA

Following our previous observations, we established that the epigenetic potentiation of oxytocin and vasopressin neurotransmission in the female NAcc was sufficient to facilitate partner preference formation in the absence of mating. To investigate whether these neuroadaptations also occur during natural formation of partner preference, we cohabited female prairie voles with a male for 24 h in the presence of mating, which induces partner preference[4], and then killed the females. We observed an increase in both *oxtr* and *avpr1a* mRNA and protein levels in the NAcc, as compared with sexually naive females (*oxtr*: +38%, $t_{10} = 2.68$, $P = 0.02$; OTR: +58%, $t_8 = 3.05$, $P = 0.01$; *avpr1a*: +89%, $t_{14} = 2.53$, $P = 0.02$; V1aR: +26%, $t_{20} = 2.23$, $P = 0.037$; **Fig. 5a**,**b**).

As both mRNA and protein levels for OTR and V1aR were increased by cohabitation with mating, we investigated whether this upregulation was associated with an epigenetic enhancement of *oxtr* and *avpr1a* transcription. A new batch of females was cohabited with a male for 6 h with mating, and H3K14 acetylation at the *oxtr* and *avpr1a* promoters was measured by chromatin immunoprecipitation. Consistent with OTR and V1aR mRNA and protein levels, female prairie voles exhibited higher H3K14 acetylation at the *oxtr* and *avpr1a* promoters in the NAcc than sexually naive females (*oxtr*: $t_9 = 2.64$, $P = 0.02$; *avpr1a*: $t_9 = 2.91$, $P = 0.017$; **Fig. 5c**,**d**). These data suggest that cohabitation procedures that reliably induce partner preference in female prairie voles trigger an upregulation of OTR and V1aR expression in the NAcc through epigenetic mechanisms, as observed after TSA treatment.

DISCUSSION

Here we found for the first time, to the best of our knowledge, an epigenetic regulation of partner preference formation. We found that increasing histone acetylation in the NAcc by administration of an HDAC inhibitor facilitated partner preference formation in adult female prairie voles in the absence of mating. Partner preference formation in females was epigenetically driven, as cohabitation and mating with a male increased *oxtr* and *avpr1a* expression through enhanced histone acetylation in the NAcc. TSA administration in the NAcc induced partner preference and led to higher levels of mRNA and protein expression of OTR in the NAcc. Moreover, although global histone H3 acetylation was unaffected in TSA-treated females, a marked enrichment of histone acetylation at the *oxtr* promoter in the NAcc was observed as early as 30 min after TSA administration. Finally, blocking OTR in the NAcc was sufficient to prevent the TSA-facilitated partner preference. Given that similar epigenetically driven modifications were detected following cohabitation with mating, under procedures known to induce partner preference, our data suggest a model for an epigenetic regulation of social behavior. During cohabitation with a male, TSA administration or mating rapidly induces a specific histone H3 acetylation at the *oxtr* promoter in the NAcc that enhances its transcription, resulting in higher OTR mRNA and protein expression, which thereafter facilitates partner preference formation.

In female prairie voles, 6 h of cohabitation with a male without mating does not induce partner preference formation[4], and this behavioral procedure has been used to examine the effects of pharmacological manipulations on the induction of partner preference[5]. Although saline- and CSF-treated controls did not develop partner preference, female prairie voles treated with NaB or TSA did. As neither NaB nor TSA affected the overall locomotion, their effects on partner preference seemed to be behavior specific rather than secondary effects on locomotion. This specific effect of TSA was further confirmed by our molecular observations. Indeed, although administered icv, we were able to detect a specific alteration of gene expression in the NAcc, but not in the caudate putamen. In addition, even in the NAcc, D1R and D2R mRNA and protein levels remained unaffected. Such specificity is surprising for a broad HDAC inhibitor such as TSA, which affects both class I and II HDACs. Nevertheless, TSA has been reported to affect the expression of only a small subset of genes in the mammalian genome[30–32], including in mice[20].

We found that acetylation of histone H3 on Lys14 at the *oxtr* promoter, a modification associated with enhanced gene transcription, including during cerebral plasticity[33,34], reflects higher OTR mRNA and protein levels. In response to TSA, histone acetylation at

the *oxtr* promoter increases and facilitates activation of its transcription in a human cell line[22], supporting our finding that *oxtr* can be regulated epigenetically. Given that a local blockade of OTR in the NAcc was sufficient to prevent the behavioral effects of TSA, our data suggest that the TSA-induced expression of OTR in the NAcc during cohabitation mediates the facilitation of partner preference formation. Moreover, 24 h of cohabitation with mating, a procedure known to reliably induce partner preference in female prairie voles, induced a similar increase in OTR expression in the female NAcc. This is consistent with the known involvement of oxytocin and OTR in the neurobiology of partner preference formation in female voles. Mating induces an increase in extracellular oxytocin levels in the NAcc[25], and local infusion of oxytocin into the NAcc facilitates partner preference formation in the absence of mating[8]. Moreover, OTR antagonists block partner preference formation induced by oxytocin administration or mating[8,35]. Notably, the viral-mediated overexpression of OTR in the female NAcc is sufficient to facilitate partner preference formation[12,13]. In addition to strengthening the role of OTR, our results also provide evidence for an activation of *oxtr* expression through epigenetic mechanisms during cohabitation with a male in the absence of mating. Indeed, although insufficient to induce partner preference, such cohabitation without mating for short periods of time activated the neurobiological processes underlying partner preference formation. For instance, 2 h of free exposure to a male induce slight, but not significant, elevations in oxytocin release in the female NAcc[25]. Thus, we propose that TSA or NaB potentiate the neuroadaptations induced by cohabitation with a male, facilitating the development of partner preference. Notably, such potentiation has already been reported in rodents, with class I and II HDAC inhibitors, including NaB and TSA, facilitating consolidation of a learning event that does not result in long-term memory formation in control animals[36,37]. In support of this notion, longer periods of cohabitation (for example, 48 h) can induce partner preference even in the absence of mating[4]. It is also important to note that cohabitation with mating triggered an upregulation of OTR and V1aR in the female NAcc through the same epigenetic mechanisms as those observed after cohabitation with TSA treatment, which indicates that TSA and mating affect the same pathways to promote partner preference formation. Notably, TSA did not induce an upregulation of OTR and V1aR in the female NAcc in the absence of cohabitation with a male. Altogether, these findings support the hypothesis that TSA facilitates the formation of partner preference through the potentiation of endogenous neuroadaptations naturally triggered by cohabitation with a male, rather than activating these or different neuroadaptations by itself.

Our results also suggest that V1aR in the NAcc is critical for partner preference formation in females, as TSA-treated voles exhibited higher V1aR levels and V1aR blockade prevented TSA-facilitated partner preference formation. Moreover, these effects were associated with higher histone acetylation at the *avpr1a* promoter, despite no significant elevation of *avpr1a* mRNA, likely a result of a non-optimal time point. Although we cannot rule out regulation of protein stability by TSA through acetylation of non-histone proteins[38], this finding suggests that, similar to the *oxtr* promoter, TSA might promote *avpr1a* transcription through local histone acetylation. Although the contribution of AVP in male's pair-bonding has been described[5], its role in the female's behavior remains controversial. On one hand, an icv AVP injection facilitates partner preference formation in both male and female voles, which is prevented by blockade of either V1aR or OTR[39]. On the other hand, an icv injection of the V1aR antagonist blocks the mating-induced partner preference in male, but not in female, prairie voles[40]. However, these studies used icv injections, preventing any further insight into the structures involved. Our results provide, to the best of our knowledge, the first evidence that AVP neurotransmission in the NAcc can be involved in partner preference formation in the female voles, although most of the literature describes its involvement in different areas, such as the ventral pallidum, lateral septum, the bed nucleus of the stria terminalis and amygdala in males[5]. It is interesting to note that the blockade of either OTR or V1aR in the female NAcc was sufficient to prevent partner preference formation following TSA treatment, suggesting that partner preference formation requires the activation of both V1aR and OTR. This finding is consistent with an earlier observation in male prairie voles that concurrent access to both OTR and V1aR in the lateral septum is essential for AVP-induced partner preference[6]. Moreover, the observation of a specific increase in both OTR and V1aR levels in TSA-treated voles further supports the requirement of a simultaneous activation of the AVP and oxytocin neurotransmissions for pair-bonding.

In combination with oxytocin and AVP, dopamine neurotransmission in the NAcc modulates partner preference formation in female voles[9]. Although mating induces dopamine release in the NAcc[9], variations in receptor levels are observed only after an extended period (longer than 24 h) of cohabitation with mating, which is important to the maintenance of pair-bonding[10]. Consistent with these observations, female prairie voles treated with TSA exhibited partner preference without substantial variation in the dopamine D1R and D2R receptors. Thus, this absence of dopamine receptor regulation provides another proof for the specificity of TSA.

Our data describe an epigenetic component in the neurobiology of pair-bonding and suggest that TSA induces a permissive state in female prairie voles, potentiating the natural molecular response to the cohabitation and promoting the formation of stronger social interactions leading to partner preference. It is tempting to hypothesize that TSA-facilitated partner preference could be further strengthened and lead to a persistent bond. Although the specific HDACs involved remain unknown, it would be interesting to investigate the effects of TSA on other behaviors associated with the monogamous life strategy in prairie voles, such as selective aggression and bi-parental care. Considering the relevance of the prairie voles in modeling the neurobiological mechanisms of pair-bonding in humans[5], and the promising HDAC inhibitors already in clinical trials[24,41,42], our data pave the way for new pharmacological possibilities for influencing social behaviors.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary information is available in the online version of the paper.

ACKNOWLEDGMENTS

We also thank M. Manning for the generous gift of the OTR antagonist OTA(T) (University of Toledo). This work was supported by grants from the National Institute of Mental Health (MHR21-083128 to M.K. and Z.W., and MHR01-058616 to Z.W.).

AUTHOR CONTRIBUTIONS

H.W., F.D. and Y.L. performed the experiments. H.W. and F.D. analyzed the data. H.W., F.D., Z.W. and M.K. designed the study. F.D., Z.W. and M.K. wrote the paper. All of the authors discussed the results and commented on the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

1. Bora, E., Yucel, M. & Allen, N.B. Neurobiology of human affiliative behavior: implications for psychiatric disorders. *Curr. Opin. Psychiatry* **22**, 320–325 (2009).
2. Thomas, J.A. & Birney, E.C. Parental care and mating system of the prairie vole (*Microtus ochrogaster*). *Behav. Ecol. Sociobiol.* **5**, 171–186 (1979).
3. Getz, L.L. & Hofmann, J.E. Social organization in free-living prairie voles (*Microtus ochrogaster*). *Behav. Ecol. Sociobiol.* **18**, 275–282 (1986).
4. Williams, J.R., Catania, K.C. & Carter, C.S. Development of partner preferences in female prairie voles *(Microtus ochrogaster*): the role of social and sexual experience. *Horm. Behav.* **26**, 339–349 (1992).
5. Young, K.A., Gobrogge, K.L., Liu, Y. & Wang, Z. The neurobiology of pair bonding: insights from a socially monogamous rodent. *Front. Neuroendocrinol.* **32**, 53–69 (2011).
6. Liu, Y., Curtis, J.T. & Wang, Z. Vasopressin in the lateral septum regulates pair bond formation in male prairie voles (*Microtus ochrogaster*). *Behav. Neurosci.* **115**, 910–919 (2001).
7. Lim, M.M. & Young, L.J. Vasopressin-dependent neural circuits underlying pair bond formation in the monogamous prairie vole. *Neuroscience* **125**, 35–45 (2004).
8. Liu, Y. & Wang, Z.X. Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female prairie voles. *Neuroscience* **121**, 537–544 (2003).
9. Gingrich, B., Liu, Y., Cascio, C., Wang, Z. & Insel, T.R. Dopamine D2 receptors in the nucleus accumbens are important for social attachment in female prairie voles (*Microtus ochrogaster*). *Behav. Neurosci.* **114**, 173–183 (2000).
10. Aragona, B.J. *et al.* Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. *Nat. Neurosci.* **9**, 133–139 (2006).
11. Liu, Y. *et al.* Nucleus accumbens dopamine mediates amphetamine-induced impairment of social bonding in a monogamous rodent species. *Proc. Natl. Acad. Sci. USA* **107**, 1217–1222 (2010).
12. Keebaugh, A.C. & Young, L.J. 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.* **60**, 498–504 (2011).
13. Ross, H.E. *et al.* Variation in oxytocin receptor density in the nucleus accumbens has differential effects on affiliative behaviors in monogamous and polygamous voles. *J. Neurosci.* **29**, 1312–1318 (2009).
14. Pitkow, L.J. *et al.* Facilitation of affiliation and pair-bond formation by vasopressin receptor gene transfer into the ventral forebrain of a monogamous vole. *J. Neurosci.* **21**, 7392–7396 (2001).
15. Veenema, A.H. Toward understanding how early-life social experiences alter oxytocin- and vasopressin-regulated social behaviors. *Horm. Behav.* **61**, 304–312 (2012).
16. Francis, D.D., Young, L.J., Meaney, M.J. & Insel, T.R. Naturally occurring differences in maternal care are associated with the expression of pxytocin and vasopressin (V1a) receptors: gender differences. *J. Neuroendocrinol.* **14**, 349–353 (2002).
17. Champagne, F.A. *et al.* Maternal care associated with methylation of the estrogen receptor-α1b promoter and estrogen receptor-α expression in the medial preoptic area of female offspring. *Endocrinology* **147**, 2909–2915 (2006).
18. Murgatroyd, C. *et al.* Dynamic DNA methylation programs persistent adverse effects of early-life stress. *Nat. Neurosci.* **12**, 1559–1566 (2009).
19. Weaver, I.C.G. *et al.* Epigenetic programming by maternal behavior. *Nat. Neurosci.* **7**, 847–854 (2004).
20. Weaver, I.C.G., Meaney, M.J. & Szyf, M. Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. *Proc. Natl. Acad. Sci. USA* **103**, 3480–3485 (2006).
21. Bonthuis, P.J., Patteson, J.K. & Rissman, E.F. Acquisition of sexual receptivity: roles of chromatin acetylation, estrogen receptor-α, and ovarian hormones. *Endocrinology* **152**, 3172–3181 (2011).
22. Zhong, S., Fields, C.R., Su, N., Pan, Y.X. & Robertson, K.D. Pharmacologic inhibition of epigenetic modifications, coupled with gene expression profiling, reveals novel targets of aberrant DNA methylation and histone deacetylation in lung cancer. *Oncogene* **26**, 2621–2634 (2007).
23. Yoshida, M., Horinouchi, S. & Beppu, T. Trichostatin A and trapoxin: novel chemical probes for the role of histone acetylation in chromatin structure and function. *Bioessays* **17**, 423–430 (1995).
24. Monneret, C. Histone deacetylase inhibitors. *Eur. J. Med. Chem.* **40**, 1–13 (2005).
25. Ross, H.E. *et al.* Characterization of the oxytocin system regulating affiliative behavior in female prairie voles. *Neuroscience* **162**, 892–903 (2009).
26. Young, L.J., Huot, B., Nilsen, R., Wang, Z. & Insel, T.R. Species differences in central oxytocin receptor gene expression: comparative analysis of promoter sequences. *J. Neuroendocrinol.* **8**, 777–783 (1996).
27. Witt, O., Deubzer, H.E., Milde, T. & Oehme, I. HDAC family: What are the cancer relevant targets? *Cancer Lett.* **277**, 8–21 (2009).
28. Vecsey, C.G. *et al.* Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB: CBP-dependent transcriptional activation. *J. Neurosci.* **27**, 6128–6140 (2007).
29. Monsey, M.S., Ota, K.T., Akingbade, I.F., Hong, E.S. & Schafe, G.E. Epigenetic alterations are critical for fear memory consolidation and synaptic plasticity in the lateral amygdala. *PLoS ONE* **6**, e19958 (2011).
30. Van Lint, C., Emiliani, S. & Verdin, E. The expression of a small fraction of cellular genes is changed in response to histone hyperacetylation. *Gene Expr.* **5**, 245–253 (1996).
31. Mariadason, J.M., Corner, G.A. & Augenlicht, L.H. Genetic reprogramming in pathways of colonic cell maturation induced by short chain fatty acids: comparison with Trichostatin A, sulindac, and curcumin and implications for chemoprevention of colon cancer. *Cancer Res.* **60**, 4561–4572 (2000).
32. Halsall, J., Gupta, V., O'Neill, L.P., Turner, B.M. & Nightingale, K.P. Genes are often sheltered from the global histone hyperacetylation induced by HDAC inhibitors. *PLoS ONE* **7**, e33453 (2012).
33. Ito, K. & Adcock, I. Histone acetylation and histone deacetylation. *Mol. Biotechnol.* **20**, 99–106 (2002).
34. Molfese, D.L. Advancing neuroscience through epigenetics: molecular mechanisms of learning and memory. *Dev. Neuropsychol.* **36**, 810–827 (2011).
35. Young, L.J., Lim, M.M., Gingrich, B. & Insel, T.R. Cellular mechanisms of social attachment. *Horm. Behav.* **40**, 133–138 (2001).
36. Hawk, J.D., Florian, C. & Abel, T. Post-training intrahippocampal inhibition of class I histone deacetylases enhances long-term object-location memory. *Learn. Mem.* **18**, 367–370 (2011).
37. Stefanko, D.P., Barrett, R.M., Ly, A.R., Reolon, G.K. & Wood, M.A. Modulation of long-term memory for object recognition via HDAC inhibition. *Proc. Natl. Acad. Sci. USA* **106**, 9447–9452 (2009).
38. Yang, X.-J. & Seto, E. Lysine acetylation: codified crosstalk with other posttranslational modifications. *Mol. Cell* **31**, 449–461 (2008).
39. Cho, M.M., DeVries, A.C., Williams, J.R. & Carter, C.S. The effects of oxytocin and vasopressin on partner preferences in male and female prairie voles (*Microtus ochrogaster*). *Behav. Neurosci.* **113**, 1071–1079 (1999).
40. Insel, T.R. & Hulihan, T.J. A gender-specific mechanism for pair bonding: oxytocin and partner preference formation in monogamous voles. *Behav. Neurosci.* **109**, 782–789 (1995).
41. Stimson, L. & La Thangue, N.B. Biomarkers for predicting clinical responses to HDAC inhibitors. *Cancer Lett.* **280**, 177–183 (2009).
42. Iannitti, T. & Palmieri, B. Clinical and experimental applications of sodium phenylbutyrate. *Drugs R D.* **11**, 227–249 (2011).

ONLINE METHODS

Subjects. Sexually naive female prairie voles (*Microtus ochrogaster*) from a laboratory breeding colony were weaned at 21 d 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 voles were randomly assigned into experimental groups when they reached 70–90 d of age. The number of voles used was based on previous studies in the field by our group and others, combined with a power analysis. Experimental procedures were approved by the Institutional Animal Care and Use Committee at Florida State University.

Drugs. NaB, dissolved in saline, and TSA, dissolved in artificial CSF (BioFluids), were both purchased from Sigma-Aldrich. NaB was injected intraperitoneally at a dose of 600 mg per kg of body weight, which is known to induce histone acetylation in several brain structures in mice[43,44]. Similarly, the dosage used for TSA was based on previous work determining its effectiveness in inducing local histone acetylation events and variations in gene expression in rodents[19,20]. The selective V1aR receptor antagonist V1aRA ($d(CH_2)_5$[Tyr(Me)]AVP) and the OTR antagonist OTA(B) ([$d(CH_2)_5$, Tyr(Me)^2, Thr^4, Tyr-NH_2^{9}]-OVT), were obtained from Bachem. A second, more selective OTR antagonist (OTA(T), *d*Gly-NH_2-$d(CH_2)_5$ [Tyr(Me)^2,Thr^4]OVT)[45] was kindly provided by M. Manning (University of Toledo). These antagonists and doses used were chosen on the basis of previous studies demonstrating their selectivity for either V1aR or OTR[35,39,46–49].

Stereotaxic cannulation and micro-injection. Females were anesthetized with sodium pentobarbital (1 mg per 10 g of body weight), and 26 gauge stainless steel guide cannulae (Plastics One) were stereotaxically implanted, aimed to the lateral ventricle (unilaterally; nose bar at −2.5 mm, 0.6 mm rostral, 1.0 mm lateral, and 2.6 mm ventral to bregma) or site specifically to the NAcc (bilaterally; nose bar at −2.5 mm, 1.7 mm rostral, ±1.0 mm bilateral, and 4.5 mm ventral to bregma). After 3 d of recovery, subjects received microinjections of either CSF or CSF containing different concentrations of TSA. When selective antagonists for OTR or V1aR were used, they were injected 30 min before TSA. Injections were made with a 33 gauge needle that extended 1 mm below the guide cannula into the target area, in an injection volume of 500 nl into the lateral ventricle (icv) or 200 nl per side into the NAcc. The needle was connected to a Hamilton Syringe (Hamilton) through polyethylene-20 tubing and plunger depression was performed slowly, requiring 1 min per injection. At the end of the experiment, all voles were killed by quick decapitation and the brains were extracted to verify cannulae placement by an observer blind to experimental conditions. Voles with misplaced cannulae were excluded from data analysis.

Cohabitation and partner preference test. Immediately following intraperitoneal, icv or intra-NAcc injections of drugs, the females were cohabited with a male for 6 h without mating. The absence of mating was verified by examining the videotaped behavior. To investigate the neuroadaptations triggered by cohabitation with mating, estrogen-primed females (2 µg per d, intraperitoneal, for 3 d) were cohabitated with a male during 6 or 24 h, and the presence of mating was verified a posteriori on videotape (ranging from 6–11 bouts during the first 6 h of cohabitation).

The partner preference test was performed immediately after the 6-h cohabitation, as previously described[11]. Briefly, the three-chamber testing apparatus consisted of a neutral cage connected to two parallel identical cages, each housing a stimulus animal: an unfamiliar male stranger or a familiar male partner used during the cohabitation period. Female subjects were free to move throughout the apparatus during the 3-h testing, and the stimulus males were tethered in their cages, allowing no direct contact with each other. The entire session was videotaped and the duration of the subject's side-by-side contact with either the partner or stranger was later quantified by a trained experimenter unaware of the biological groups. A partner preference was defined as subjects spending significantly more time in body contact with the partner versus stranger, as determined by a paired, two-tailed *t* test. In addition, the three-chamber apparatus was equipped with photobeam sensors, allowing the determination of locomotor activity indicated by the number of entries of the female into the stimulus chambers. This locomotor score allowed us to control for putative secondary effects of the drugs on the females' behavior, such as general activity, anxiety or altered exploration of a novel environment, as commonly used by our group and others[12].

RNA and proteins extraction. Females were killed by rapid decapitation, and brains were immediately extracted and frozen on dry ice. Coronal sections (200 µm) were cut on a cryostat and frost-mounted onto microscope slides. Bilateral tissue punches with a 1-mm diameter were taken from the entire NAcc and caudate putamen, the latter being a control area, and stored at −80 °C until processing. Total RNA and proteins were extracted using the TRI-Reagent protocol according to manufacturer's instructions (Molecular Research Center).

Protein expression analysis by Western blot. Following separation on a 10% polyacrylamide gel (15% for histones), proteins were transferred to a nitrocellulose membranes and incubated primary antibodies to OTR (sc-8102, 1:1,000), V1aR (sc-18096, 1:500), D1R (sc-33660, 1:1,000) and D2R (sc-9113, 1:1,000, Santa Cruz Biotechnology), actin (A2066, 1:1,000, Sigma Aldrich), and acetyl histone H3 (Lys14, #06-911, 1:1,000) and total H3 (#05-928, 1:1,000, Millipore). All antibodies are validated for their use in humans, rats and mice, with which prairie voles share high percentages of homology (ranging from 81 to 96%). After hybridization 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). Quantification was performed using AIS 6.0 Image software (Imaging Research), and all signals were normalized within the same membrane to actin, except for the acetyl-H3 signal which was normalized to the total histone H3 signal. Normalized data are then expressed as percentage of CSF-treated voles.

Semi-quantitative real-time PCR. 0.5 µg of total RNA was processed for complementary DNA synthesis and analyzed as previously described[50] with normalization to the nicotinamide adenine dinucleotide dehydrogenase (*NADH*) gene. All reactions were done in triplicates and their specificity was verified by melting curve analysis and separation on a 2% agarose gel. The primers sequences used were as follows: 5′-TCCAAGGCCAAAATCCGCACGG-3′ (Fwd) and 5′-GGCAGAAGCTTCCTTGGGCGC-3′ (Rev) for *oxtr*, 5′-GAGGTGAACAATGGCACTAAAACC-3′ (For) and 5′-CCAGATGTGGTAGCAGATGAAGC-3′ (Rev) for *avpr1a*, 5′- TTAACAACAATGGGGCTGTG-3′ (For) and 5′-GGCATGAGGGATCAGGTAAA-3′ (Rev) for *d1dr*, 5′-GTGAAGGCGCTGTAGAGGAC-3′ (For) and 5′-CGGTGTGTTCATCATCTGCT-3′ (Rev) for *d2dr*, and 5′-CTATTAATCCCCGCCTGACC-3′ (For) and 5′-GGAGCTCGATTTGTTTCTGC-3′ (Rev) for NADH. The normalized data are expressed as a percentage of CSF-treated voles.

Chromatin immunoprecipitation. Histone H3 acetylation (Lys14) in NAcc and caudate putamen tissue punches was analyzed by using the Magna ChIP protein G Tissue Kit (Millipore) following the manufacturer's instructions. Briefly, after crosslinking with 1% formaldehyde, chromatin was sheared using a Misonix XL-2000 to fragments of 200–600 bp. Immunoprecipitation of acetylated histone H3 (Lys14) was then realized with 10 µg of antibody to acetyl-H3(Lys14) (Millipore) overnight at 4 °C. After washes, elution from beads and reversal of cross-link, immunoprecipitated DNA was purified and analyzed in triplicates by real-time PCR on an iCycler platform (see above) with an internal standard curve made from pooled INPUT samples. The primers were designed to amplify a 236-bp-long region located 128-bp upstream of the first exon coding for the prairie vole OTR (*oxtr*, Genbank accession #AF079980), or 192-bp-long region located 141-bp upstream of the first exon coding for the prairie vole V1aR (*avpr1a*, Genbank accession #AF069304). The sequences were as follows: 5′-CTCCGGAGCCGGGGCTAAGT-3′ (Fwd) and 5′-ACCGCTTCCCCGAGAGTAGGG-3′ (Rev) for *oxtr*, and 5′-GGTGGACCAGCCAGACCCCA-3′ (Fwd) and 5′-TGCAGAGCCAGGCGCTTTCC-3′ (Rev) for *avpr1a*. Each sample was normalized by the respective INPUT value, and data are then expressed as a percentage of CSF-treated voles.

Statistical analyses and data processing. For analyses of partner preference, voles that displayed mating behaviors during the cohabitation period or with misplaced cannulae were excluded. For all other molecular analyses, a maximum of one data point per biological group was excluded when identified as outlier.

Most of the experiments were replicated, except when the results were very clear. The time spent in side-by-side contact with either stimulus animal during the partner preference test was analyzed with a two-tailed paired *t* test. The locomotion scores were analyzed using a two-tailed *t* test (for two groups) or a one-way ANOVA (for more than two groups), and when appropriate, Fischer's PLSD *post hoc* tests were conducted with a significance threshold of $P < 0.05$. After verification of normality, all other data were analyzed with a two-tailed *t* test assuming equal or unequal variances tested beforehand. All statistical analyses were performed using the StatView software (SAS Institute). When data were standardized to their respective controls (% of CSF, saline or mating groups), the statistical analyses were conducted on the raw data.

43. Kumar, A. *et al.* Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. *Neuron* **48**, 303–314 (2005).
44. Schroeder, F.A., Lin, C.L., Crusio, W.E. & Akbarian, S. Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. *Biol. Psychiatry* **62**, 55–64 (2007).
45. Manning, M. *et al.* Peptide and non-peptide agonists and antagonists for the vasopressin and oxytocin V1a, V1b, V2 and OT receptors: research tools and potential therapeutic agents. in *Progress in Brain Research* (eds. D.N. Inga & L. Rainer) 473–512 (Elsevier, 2008).
46. Witt, D.M. & Insel, T.R. A selective oxytocin antagonist attenuates progesterone facilitation of female sexual behavior. *Endocrinology* **128**, 3269–3276 (1991).
47. Winslow, J.T., Hastings, N., Carter, C.S., Harbaugh, C.R. & Insel, T.R. A role for central vasopressin in pair bonding in monogamous prairie voles. *Nature* **365**, 545–548 (1993).
48. Insel, T.R. Oxytocin–a neuropeptide for affiliation: evidence from behavioral, receptor autoradiographic, and comparative studies. *Psychoneuroendocrinology* **17**, 3–35 (1992).
49. Insel, T.R., Wang, Z. & Ferris, C. Patterns of brain vasopressin receptor distribution associated with social organization in microtine rodents. *J. Neurosci.* **14**, 5381–5392 (1994).
50. Hollis, F., Duclot, F., Gunjan, A. & Kabbaj, M. Individual differences in the effect of social defeat on anhedonia and histone acetylation in the rat hippocampus. *Horm. Behav.* **59**, 331–337 (2011).

doi:10.1038/nn.3420