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Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds

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The involvement of dopamine within the nucleus accumbens in the formation and maintenance of pair bonds was assessed in a series of experiments using the monogamous prairie vole. We show that dopamine transmission that promotes pair bond formation occurs within the rostral shell of the nucleus accumbens, but not in its core or caudal shell. Within this specific brain region, D1- and D2-like receptor activation produced opposite effects: D1-like activation prevented pair bond formation, whereas D2-like activation facilitated it. After extended cohabitation with a female, male voles showed behavior indicative of pair bond maintenance—namely, selective aggression towards unfamiliar females. These voles also showed a significant upregulation in nucleus accumbens D1-like receptors, and blockade of these receptors abolished selective aggression. Thus, neuroplastic reorganization of the nucleus accumbens dopamine system is responsible for the enduring nature of monogamous pair bonding. Finally, we show that this system may also contribute to species-specific social organization.

The monogamous prairie vole (*Microtus ochrogaster*)[1–3] has proven to be an excellent model for neurobiological investigations of pair bonding[4,5]. This species shows selective affiliation toward an established partner, and investigations of this partner preference have examined the neurochemical regulation of pair bond formation[6–12]. Although a partner preference is a prerequisite for pair bonding, this behavior alone does not entirely define a pair bond. A pair bond is only fully established when unfamiliar conspecifics, including potential mates, are aggressively rejected[3,6,13]. This selective aggression is the primary behavior responsible for the stable maintenance of a pair bond, and completion of the behavioral transition from partner-preference formation to selective aggression indicates that the bond is fully developed.

The neurobiology underlying the dramatic behavioral transformation—from affiliative approaches (such as approach behavior and copulation) associated with partner-preference formation to avoidance and selective aggression towards unfamiliar conspecifics—is poorly understood. Previous studies have shown that dopamine (DA) transmission within the nucleus accumbens (NAcc) mediates both approach and avoidance behaviors[14–22], making this system an ideal candidate to be involved in this behavioral transformation associated with pair bonding. We therefore systematically studied the involvement of NAcc DA in both pair bond formation and maintenance. We first identified the specific subregion of the NAcc wherein dopaminergic processing facilitates partner preferences; then we examined the specific functions of distinct DA receptor subtypes in the regulation of this behavior. Once we had established a more precise understanding of NAcc DA regulation of pair bond formation, we examined its role in pair bond maintenance. We show that the NAcc DA system of pair bonded males is significantly restructured and that this neural plasticity mediates selective aggression. Taken together, our data show that NAcc DA is critical for pair bond formation and maintenance and that reorganization of this system underlies the behavioral transition between these two stages of pair bond development. Having revealed the importance of NAcc DA in pair bonding in prairie voles, we also show that this system may contribute to species-specific social organization.

RESULTS

Site-specific dopamine regulation of pair bond formation

Previous studies have demonstrated that partner preferences emerge after 24 h of *ad libitum* mating, but not after 6 h of cohabitation without mating[6–10,23]. The blockade of NAcc D2-like, but not D1-like, DA receptors prevents mating-induced partner preferences, and activation of D2-like receptors induces this behavior in the absence of mating[8,9]. However, there are important details regarding the nature of the dopaminergic regulation of pair bond formation that remain unknown. For instance, the NAcc is an extremely heterogeneous structure consisting of functionally distinct subregions, most notably the core and shell[24]. Both subregions are important for motivated behavior and associative learning[17,25–28] and are therefore potentially important for pair bond formation; however, it is unknown which subregion is involved in the formation of a partner preference. Here, we used a drug known to induce partner preferences, the D2-like specific

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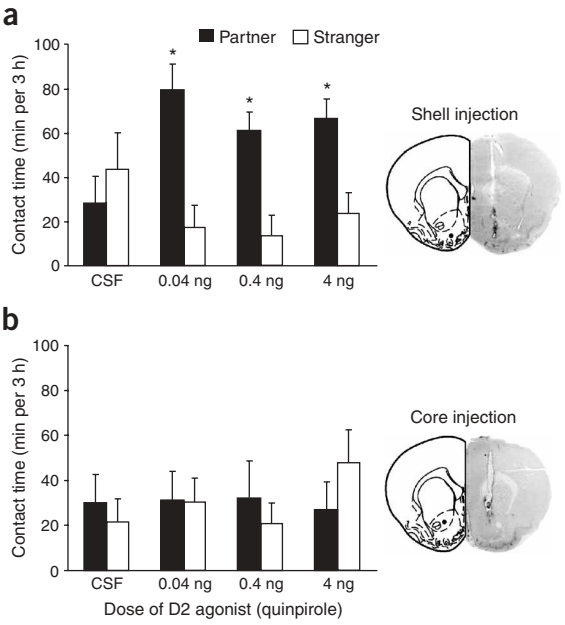

Figure 1 Activation of D2-like receptors within the rostral NAcc shell, but not core, is critical for partner preferences. (**a**) Intrashell administration of quinpirole, at all doses tested ($n = 9$–11), resulted in significantly more side-by-side contact with partners than with strangers. (**b**) Voles receiving core injections of quinpirole ($n = 8$–12) showed nonselective side-by-side contact between partners and strangers. Schematic illustrations (left) and representative photomicrographs of vole brain sections (right) demonstrating micro-injections into NAcc shell or core. $^*P < 0.05$. Error bars indicate s.e.m.

agonist quinpirole[7,8,10], to test which specific subregion of the NAcc is important for pair bond formation.

As shown previously[9,11], males injected with vehicle alone (artificial cerebrospinal fluid, CSF) and paired with a female for 6 h without mating did not show partner preferences: that is, they showed nonselective side-by-side contact with the stimulus females during the partner-preference test (**Fig. 1a**,**b**). However, administration of quinpirole into the NAcc shell induced partner preferences in the absence of mating (**Fig. 1a**). Quinpirole administration into the NAcc core was ineffective at all doses tested (**Fig. 1b**). Examination of injection tracks into the shell (**Fig. 1a**) and core (**Fig. 1b**) showed that these manipulations were rostral to the corpus callosum genu—a portion of the shell that has been shown to mediate appetitive associations[29] (**Fig. 2a**,**b**). To determine whether D2-like receptor facilitation of partner-preference formation is specific to the rostral shell, we injected the most effective dose of quinpirole (0.04 ng) caudal to the formation of the genu (**Fig. 2c**) into the caudal shell ($n = 8$) (**Fig. 2d**). Similar to the results of other studies showing that this portion of the shell does not mediate appetitive processing[29], caudal shell injections of quinpirole did not induce partner preferences (contact time in min per 3 h: partner = 36.5 ± 16.5; stranger = 38.9 ± 17.2; mean ± s.e.m.). Together, these data demonstrate that the rostral shell is the specific subregion of the NAcc important for DA regulation of partner-preference formation. Therefore, we used this site as the target for subsequent behavioral experiments and as the location for quantification of the anatomical data.

Opposing roles for D1- and D2-like receptors

The role of D2-like receptors in partner-preference formation is well established; in contrast, very little is known about the role of NAcc D1-like receptors in the regulation of this behavior. One study showed that the activation of NAcc D1-like receptors does not promote partner preferences[8]. In another study, low—but not high—doses of the nonselective DA agonist apomorphine promoted partner preferences, and it was suggested that high doses of this drug were ineffective because such manipulations activated D1-like receptors[9]. We therefore tested whether the activation of D1-like receptors in the rostral shell prevents partner-preference formation.

As shown previously[7,8,10], administration of quinpirole, but not the D1-like agonist SKF 38393, induced partner preferences in voles paired with a female for 6 h without mating (**Fig. 3a**). Therefore, quinpirole-induced partner preferences served as the control group for this experiment. Concurrent activation of D1-like receptors (by the administration of both SKF 38393 and quinpirole) blocked quinpirole-induced partner preferences (**Fig. 3a**). Coadministration of the D2-like antagonist eticlopride also prevented quinpirole-induced partner preferences (**Fig. 3a**); however, voles receiving both quinpirole and the D1-like antagonist SCH 23390 showed a robust partner preference (**Fig. 3a**). Thus, the activation of D1-like receptors not only did not induce a partner preference, but also prevented the partner-preference formation induced by D2-like activation.

Although the data described above show that D1-like activation prevents pharmacologically induced partner preferences, it is important to test whether D1-like activation interferes with naturally occurring (mating-induced) partner preferences. As expected from previous reports[6,9,11], vehicle-injected males that mated *ad libitum* for 24 h developed partner preferences (**Fig. 3b**). However, the administration

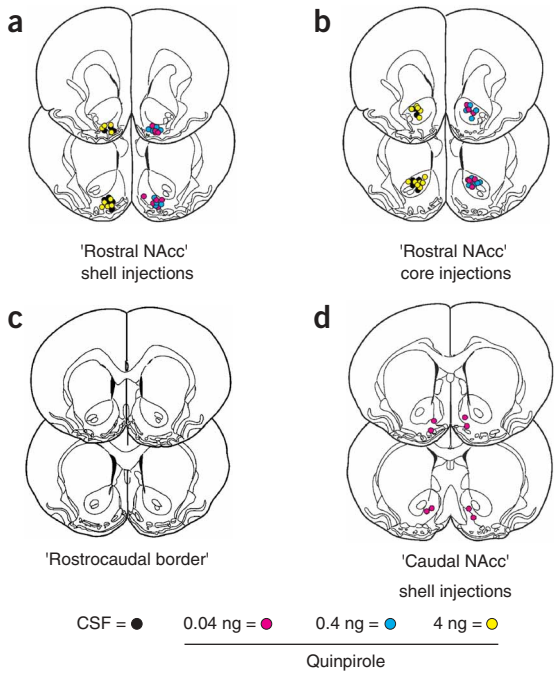

Figure 2 Histological verification of injection sites for experiment 1. (**a**) Effective quinpirole manipulations were within portions of the NAcc shell that were rostral to the corpus callosum genu, defined in previous studies as the 'rostral shell'[29]. (**b**) Ineffective core injections were located dorsal to this site, but along the same rostrocaudal gradient. (**c**) The portion of the NAcc near the formation of the corpus callosum genu was considered the 'rostrocaudal border'. (**d**) Ineffective 'caudal shell' injections were placed approximately 500 μm caudal to the effective rostral location.

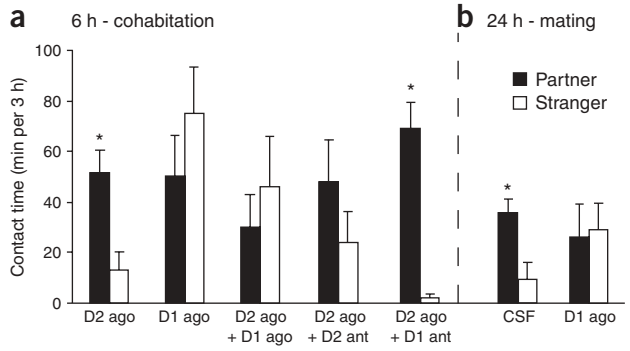

Figure 3 D1- and D2-like receptors within rostral NAcc shell have opposing effects on partner-preference formation. (**a**) Administration of the D2-like agonist quinpirole ('D2 ago'; 0.04 ng; $n = 9$), but not the D1-like agonist SKF 38393 ('D1 ago'; 0.4 ng; $n = 10$), induced partner preferences. Administration of quinpirole with the D1-like agonist (0.4 ng; $n = 8$) or with the D2-like antagonist eticlopride ('D2 ant'; 0.4 ng; $n = 9$) did not produce partner preferences. However, voles receiving quinpirole and the D1-like antagonist SCH 23390 ('D1 ant'; 0.4 ng; $n = 8$) showed partner preferences. (**b**) Mating induced partner preferences in control-injected voles ($n = 5$); however, voles treated with the D1-like receptor agonist (4 ng; $n = 8$) did not show partner preferences. $^*P < 0.05$. Error bars indicate s.e.m.

of SKF 38393 into the rostral shell of the NAcc before the 24-h mating period blocked partner-preference formation (**Fig. 3b**). Notably, the number of mating bouts did not differ between the two groups (control = 6.2 ± 1.0; D1-like agonist = 5.4 ± 0.9), suggesting that D1-like receptor activation disrupted the associative processing required for partner-preference formation without affecting normal mating behavior. Because D1-like receptor activation prevented both quinpirole-induced and mating-induced partner preferences, the activation of D1-like receptors within the rostral NAcc shell seems to antagonize pair bond formation. These findings indicate that the activation of D1- and D2-like receptors have opposing effects on partner-preference formation.

Nucleus accumbens reorganization in pair bonded males

In the next series of experiments, we examined the neurobiology underlying the stable maintenance of a pair bond. Field and laboratory studies have shown that extended exposure to a partner results in the rejection of potential mates, even if the partner is permanently removed[2,30]. Given the importance of NAcc DA in pair bond formation, we hypothesized that alteration of this system is involved in pair bond maintenance. Therefore, we tested whether a restructuring of this system is associated with pair bonding. We used receptor autoradiography to compare D1- and D2-like receptor binding within the rostral core and shell of the NAcc and a control area, the caudate putamen, between pair bonded and non–pair bonded males. Males were defined as pair bonded after 2 weeks of cohabitation with a female that resulted in pregnancy; non–pair bonded males were housed with same-sex siblings.

Pair bonded males had substantially more D1-like receptor binding in both the rostral core and shell of the NAcc, relative to non–pair bonded males (**Fig. 4a**,**b**). We found similar increases in the core (62.8 ± 12.4%) and shell (59.3 ± 12.8%): therefore, we combined these data. We inspected individual differences and found that two of seven voles (28%) did not show an increase beyond the amount of D1-like receptor binding observed in controls (**Fig. 4c**). Notably, there was no change in D1-like receptor binding in the caudate putamen, suggesting that the upregulation of D1-like receptors in the NAcc does not generalize to all dopaminergic areas (**Fig. 4a**,**b**). There were no group differences in D2-like receptor binding in either brain region (**Fig. 4a**,**b**).

The increase in D1-like receptor binding in pair bonded males might simply have been a result of social or sexual experience with a female. Thus, we conducted an additional experiment in which we compared the D1- and D2-like receptor binding in males that remained with their male sibling (control, $n = 6$), males that were paired with a female for 24 h without mating (cohab, $n = 6$) and males that mated *ad libitum* with a female for 24 h (mated, $n = 5$). Unlike males that were paired with a female for 2 weeks, those exposed to females for shorter periods (with or without mating) did not show altered binding for either receptor subtype within the NAcc (as expressed as a percentage of the values for the control group for D1-like receptors: control = 99.8 ± 22.0%, cohab = 97.0 ± 11.1%, mated = 121.1 ± 46.0%; for D2-like receptors: control = 99.6 ± 25.7%, cohab = 135.7. ± 37.4%, mated = 165.2 ± 39.5%) or the caudate putamen (data not shown).

These data showed that increased D1-like receptor binding in the NAcc was present after 2 weeks of exposure to a female, but not after only 24 h. Given that 24 h of mating resulted in a partner preference, this finding suggests that reorganization of the NAcc is not necessary for the initial formation of the partner preference. Rather, increased D1-like receptors in the NAcc seem to be indicative of a more fully established pair bond and may play a critical role in pair bond maintenance.

Nucleus accumbens reorganization and pair bond maintenance

Pair bonded voles showed increased D1-like receptor binding within the NAcc; therefore, we hypothesized that this upregulation of the receptors

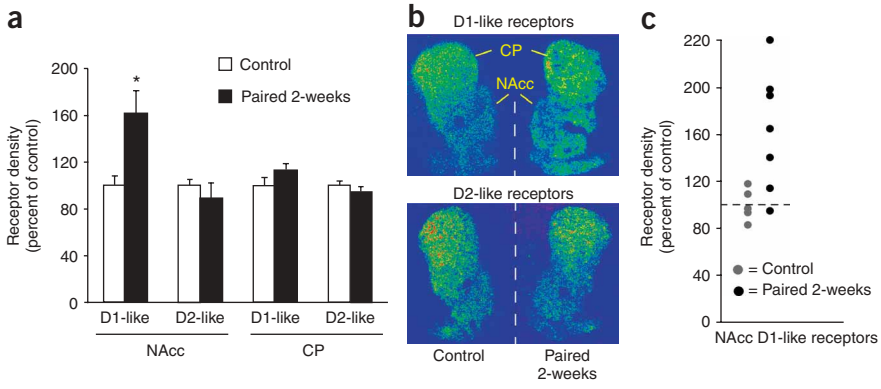

Figure 4 Pair bonded males have significantly more D1-like receptors within the NAcc than do non–pair bonded males. (**a**) The NAcc (both core and shell subregions) of pair bonded males ($n = 7$) showed D1-like receptor density that was 60% higher than that of sexually naive controls ($n = 6$). No group difference was found for D1-like receptor density within the caudate putamen, and D2-like receptors did not differ in either brain region. Comparisons were made in rostral portions of NAcc because pharmacological manipulations in this region effectively altered partner-preference behavior. (**b**) Representative photomicrographs of D1-like and D2-like receptor autoradiography for control (left) and pair bonded (right) males. (**c**) Raw data depicting individual variation in the increase of D1-like receptor binding in pair bonded voles. $^*P < 0.05$. Error bars indicate s.e.m.

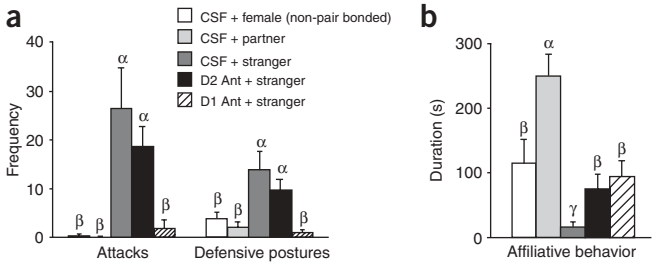

Figure 5 Blockade of D1-like receptors within the NAcc abolishes monogamous selective aggression. (**a**) Vehicle-treated pair bonded males showed significantly more attacks and defensive postures toward unfamiliar females (CSF + stranger; $n = 8$). These behaviors were abolished by blockade of D1-like receptors in NAcc (D1 ant + stranger; $n = 13$; 10 ng SCH 23390), but not by blockade of D2-like receptors (D2 ant + stranger; $n = 6$; 10 ng eticlopride). As expected, non–pair bonded males exposed to unfamiliar females (CSF + female; $n = 10$) and pair bonded males exposed to their familiar mates (CSF + partner; $n = 9$) showed very low levels of aggressive behavior. Attacks: the number of lunges, bites and chases in the 6-min test; defensive postures: offensive and defensive rears. (**b**) Pair bonded males engaged in side-by-side contact with their partners, and attacked or avoided unfamiliar females. Thus they showed significantly higher levels of affiliative behavior toward their familiar mates, and significantly lower levels of affiliative behavior toward unfamiliar females, than did sexually naive males. Pair bonded voles subjected to blockade of D1- or D2-like receptors showed levels of initial investigation of unfamiliar females that were similar to those shown by non–pair bonded males. Affiliative behavior: anogenital olfactory investigation or side-by-side contact. Symbols indicate group differences after the *post hoc* test. Bars with different symbols differ significantly ($P < 0.05$) from each other. Error bars indicate s.e.m.

that antagonize pair bond formation prevents the formation of a second pair bond and promotes the stable maintenance of the initial pair bond. The specific behavior that maintains a pair bond is selective aggression toward unfamiliar conspecifics[6,13]. We therefore tested whether upregulated NAcc D1-like receptors mediate this behavior.

Selective aggression was assessed by using a resident-intruder test[6,13]. The control group consisted of sexually naive (non–pair bonded) males that received intra-NAcc vehicle injections. As expected, these voles did not show aggression toward unfamiliar female intruders (**Fig. 5a**); instead, they approached the females and engaged in olfactory investigation (**Fig. 5b**). Pair bonded males, also vehicle-treated, likewise showed no aggression when the intruder was their familiar mate (partner) (**Fig. 5a**). They approached and huddled with their partners, resulting in a significantly greater duration of affiliative behavior than that shown by the non–pair bonded males (**Fig. 5b**; $P < 0.05$). In contrast, when the intruders were unfamiliar females (strangers), pair bonded males (also vehicle-treated) were extremely aggressive, showing significantly ($P < 0.05$) more attack behavior and defensive postures (**Fig. 5a**) and less affiliative behavior (**Fig. 5b**). Selective aggression was not altered by blockade of D2-like receptors within the NAcc (**Fig. 5a**). However, intra-NAcc blockade of D1-like receptors in pair bonded males abolished selective aggression (**Fig. 5a**). Decreased aggressive behavior was not due to a drug-induced decrement in locomotor activity: voles with a D1-like receptor blockade investigated intruders to the same extent as did those that received the D2-like antagonist (**Fig. 5b**). Additionally, voles across all groups showed no differences in the number of grid crosses in an open field test conducted before the selective aggression test (grid crosses per min: CSF + female = 21.2 ± 4.5; CSF + partner = 21.3 ± 4.2; CSF + stranger = 16.9 ± 3.8; D2 antagonist + stranger = 23.8 ± 3.5; D1 antagonist + stranger = 24.9 ± 3.7; mean ± s.e.m.). Thus, the blockade of the upregulated NAcc D1-like receptors prevented selective aggression and, therefore, the apparent increase in the number of NAcc D1-like receptors may be directly responsible for pair bond maintenance.

D1-like receptors and species-specific social organization

Our study demonstrates that D1-like receptors within the NAcc are critical to the social organization and behavior of monogamous prairie voles, in that upregulation of these receptors underlies the transformation from affiliative behavior toward unfamiliar females (**Fig. 6a**) to a behavioral state in which unfamiliar females are attacked or avoided. Unlike prairie voles, sexually naive males of a related but nonmonogamous species, the meadow vole (*Microtus pennsylvanicus*) (**Fig. 6b**), are more solitary by nature and show low levels of affiliative behavior[31]. Because increased numbers of D1-like receptors in the NAcc are associated with low affiliative behavior in prairie voles, we hypothesized that meadow voles would have higher basal levels of NAcc D1-like receptors, which would be consistent with their more asocial nature. We therefore used receptor autoradiography to compare D1-like receptor binding in sexually naive male prairie voles with that in meadow voles.

In support of our hypothesis, male meadow voles showed significantly ($P < 0.05$) greater D1-like receptor binding in the NAcc than did male prairie voles. We found similar species differences in D1-like binding in both the NAcc core and shell: therefore, we combined the data from these subregions (**Fig. 6c**). Meadow voles also seemed to have more D1-like receptors in the caudate putamen than did prairie voles, but this difference did not reach statistical significance ($P = 0.08$) (**Fig. 6c**).

We next tested the effects of NAcc D1-like receptor blockade on meadow vole social behavior. Male meadow voles received intra-NAcc injections of CSF ($n = 7$) or CSF containing the D1-like receptor antagonist SCH 23390 (25 ng per side; $n = 10$); they then cohabited with a female for 6 h, after which they were tested for partner

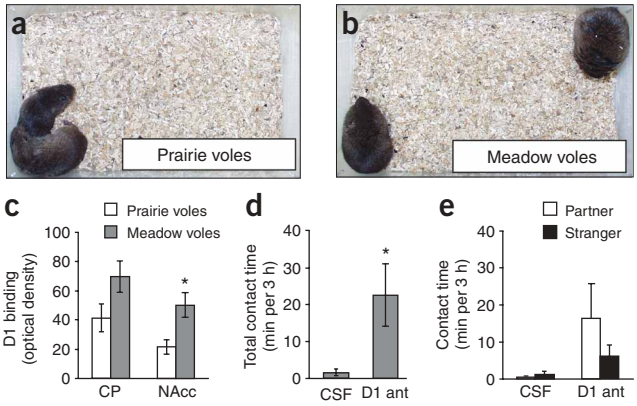

Figure 6 Species differences in D1-like receptor binding within the NAcc are consistent with species differences in social behavior. (**a**) A sexually naive male prairie vole showing typical affiliative behavior with a female. (**b**) Male and female meadow voles show no physical contact, maximizing the distance between them. (**c**) Male meadow voles have significantly more D1-like receptors within the NAcc than do male prairie voles. (**d**) Blockade of NAcc D1-like receptors increased the duration of nonselective side-by-side contact during the partner-preference test. (**e**) Blockade of NAcc D1-like receptors did not induce a partner preference in male meadow voles. *$P < 0.05$. Error bars indicate s.e.m.

preferences. Voles with a blockade of D1-like receptors showed significantly greater ($P < 0.05$) general affiliative behavior (side-by-side contact with either the partner or stranger) than did the CSF controls (**Fig. 6d**). Despite increased affiliative behavior, these voles did not form a partner preference: side-by-side contact was not significantly greater with the partner compared to the stranger ($P = 0.31$; **Fig. 6e**). Further, meadow vole affiliative behavior remained low compared to what is typically seen in prairie voles (compare with **Figs. 1** and **2**). Together, these data suggest that, although high basal levels of D1-like receptors in the NAcc are associated with the asocial nature of meadow voles and thus contribute to species-specific social organization, differences within this system cannot account for all species differences in social behavior.

DISCUSSION

This study provides the first description of a neural mechanism underlying the dramatic behavioral alterations seen in monogamous male prairie voles as they transition from sexually naive to fully pair bonded. Sexually naive males readily approached unfamiliar females and formed partner preferences if highly selective dopaminergic processing occurred within the rostral shell of the NAcc. Activation of D2-like receptors promoted partner-preference formation, whereas activation of D1-like receptors prevented this behavior. After prolonged cohabitation with their partner, males became very aggressive toward unfamiliar females; these male voles showed a significant upregulation of the 'antagonistic' D1-like receptors within the NAcc. Blockade of upregulated D1-like receptors within the NAcc prevented selective aggression toward unfamiliar females, indicating that this restructuring of the NAcc is directly responsible for the enduring nature of monogamous pair bonding. Together, these data demonstrate that NAcc DA differentially regulates the formation and maintenance of monogamous pair bonds depending on the stage of pair bond development; moreover, the behavioral alterations observed across bond development are mediated by neuroplastic reorganization of the NAcc. Finally, this system may not only regulate pair bonding but may also contribute to species differences in social organization—as evidenced by the fact that nonmonogamous male meadow voles have more NAcc D1-like receptors than do male prairie voles.

Nucleus accumbens dopamine and pair bond formation

Mating facilitates partner-preference formation[6–9,11,12,23], and dopaminergic transmission critical for this behavior is increased by mating[8,9]. Because mating is known to be rewarding to male rodents[32], it has been argued that reward learning is an important component of pair bond formation[4,5,9]. Indeed, a role of NAcc DA suggests that pair bond formation involves what has been referred to as reward learning[17,19,33], stamping-in motivational importance[21] or increased incentive salience[18]. Although the appropriate language for discussing such phenomena is still debated, the neurobiology of pair bond formation (an inherently associative process) is consistent with past studies that have examined associations of a positive nature.

The current study shows that DA regulation of partner-preference formation occurs within the NAcc shell, which is a key component of the neural circuitry that processes positive information[17–20,24,34]. Further, our data indicate that the rostral, but not the caudal, shell mediates partner preferences—consistent with recent neurochemical studies of positive association formation[29]. Additionally, several recent studies have demonstrated that another component of this circuitry, the ventral pallidum[18,19,24], is also important for pair bonding[11,12]. Together, these findings suggest that reward-related processing may be important for social attachment.

The opposing effects on partner-preference formation of D1- and D2-like receptors, within the rostral NAcc shell, also suggest the involvement of reward processing. Activation of D2-like receptors promotes partner-preference formation, whereas activation of D1-like receptors prevents this behavior. This is similar to the regulation of another behavior associated with reward: drug seeking. D2-like activation reinstates cocaine seeking, whereas D1-like activation prevents the reinstatement of drug seeking induced by cocaine[35]. Notably, DA receptor activation seems to mediate other reward-related behaviors differently[36–38]. However, DA regulation of partner-preference formation seems to be similar to drug seeking, and this regulation is consistent with the opposing regulation of intracellular signaling by D1- and D2-like receptors[37,39] and supports studies suggesting that DA acts in a very specific manner *in vivo*[22].

Together, our data suggest that NAcc DA regulation of pair bond formation is consistent with its well established role in the processing of positive information[20–22,33,40,41], and shares several similarities with dopaminergic regulation of drug reward[17,19,35]. These data, therefore, support the hypothesis that drugs of abuse target neural systems that evolved to mediate adaptive behaviors such as social bonding[34,42,43].

Nucleus accumbens dopamine and pair bond maintenance

Perhaps the most extraordinary aspect of the pair bonds formed by prairie voles is their enduring nature. In their natural environment, lost members of breeding pairs are rarely replaced[1–3], and a laboratory study using females showed that most voles will not form a second bond even if their partner is permanently removed and other males are available[30]. The current findings suggest that prairie voles are not capable of forming a second pair bond because antagonistic NAcc D1-like receptors are upregulated. We propose that interaction with unfamiliar conspecifics increases DA release in the NAcc[44], which activates upregulated D1-like receptors. This activation facilitates mate guarding, via selective aggression[3], which ensures stability of the initial pair bond.

In contrast to the initial formation of the bond, NAcc DA regulation of pair bond maintenance mediates behavior that is not regarded as rewarding (that is, aggressive mate guarding). This is consistent with studies suggesting that NAcc DA is important for highly motivated behavior that is not necessarily rewarding[15,16]. Further, this behavioral change is associated with upregulation of D1-like receptors within both the core and shell subregions of the NAcc. Therefore, although the core is not critical for DA-mediated pair bond formation, it seems to be important for pair bond maintenance. This is consistent with NAcc core involvement in the expression of other motivated behaviors[25–28]. It is also significant that D1-like receptors are important for pair bond maintenance, because these receptors have been shown to be critical for neural plasticity and memory[20,45]. Thus, the transition to mate guarding seems to involve neural processing that is generally related to learning and the expression of motivated behavior.

It has been argued that mate guarding is the selective pressure primarily responsible for the evolution of monogamy[46], and the present data are consistent with a key role for mate guarding as this behavior is maintained by a robust neural reorganization. The finding that such a stable behavior, essential for pair bonding, was abolished by the blockade of upregulated D1-like receptors strongly argues for the importance of this neural reorganization. Further, 28% of the voles in the pair bonded group did not show an increase in NAcc D1-like receptors over that seen in controls. Moreover, given that field studies indicate that approximately 20% of both males and females will form a second pair bond[2], it is an intriguing possibility that individual variability in D1-like receptor upregulation may be associated with

individual differences in the ability to form a second pair bond. This should be tested in future studies.

Compared to male prairie voles, nonmonogamous male meadow voles have significantly greater density of the antagonistic D1-like receptors within the NAcc, and blockade of these receptors increased the social behavior of this species. This is consistent with previous studies showing that neuroanatomical differences between these species are typically related to differences in social behaviors[47,48]. For example, prairie voles (but not meadow voles) have high levels of oxytocin receptors in the NAcc[47] and vasopressin V1a receptors in the ventral pallidum[48,49], and these receptors are important for pair bonding[10,11,50]. In fact, viral vector gene transfer of vasopressin V1a receptors into the ventral pallidum of meadow voles induces social behavior typical of prairie voles[12]. Notably, this effect is prevented by the blockade of D2-like DA receptors[12], demonstrating the complex multisystem nature of pair bonding[5]. As meadow voles differ in these neuropeptide systems essential for pair bonding[5], it is not surprising that D1-like receptor blockade alone did not facilitate partner preferences. However, given that oxytocin and vasopressin manipulations interact with NAcc DA to influence pair bond formation[10,12], dopaminergic processing within the NAcc is most likely important for species-specific social behavior.

Conclusion

This study clearly demonstrates that NAcc DA has at least two distinct roles in the regulation of monogamous pair bonding depending on the stage of pair bond development. Initially, in the sexually naive male, female-evoked dopaminergic processing facilitates a positive association: the partner preference. Such dopaminergic regulation is consistent with its role in attributing incentive salience to an unfamiliar stimulus[18] or stamping-in the motivational importance of such a stimulus[21], or perhaps with its role in reward processing in general[17,19,33,41]. However, in the pair bonded male, unfamiliar females induce DA release in a restructured NAcc, and increased DA transmission now signals the presence of an aversive stimulus and mediates aggressive behavior that promotes pair bond maintenance[3]. This finding supports arguments that DA regulates motivated behavior by its association with salient events, which are not necessarily positive in nature[15,16]. Our data, therefore, provide an example wherein NAcc DA mediates both superficially positive and negative types of adaptive behavior.

METHODS

Voles. Voles were capture-bred adult male prairie and meadow voles (90–120 d of age), weaned at 21 d and then housed in single-sex sibling pairs[9]. Experimental procedures were approved by the Institutional Animal Care and Use Committee at Florida State University.

Partner-preference test. The three-chamber apparatus consisted of a neutral cage joined with two identical cages, each housing a stimulus vole[7,9]. All voles (except for the stimulus voles) were free to move throughout the apparatus[9]; the stimulus voles were loosely tethered within separate cages. Stimulus females were ovariectomized. Partners and strangers used for 24-h manipulations were estrogen primed by subcutaneous implantation of estrodial benzoate pellets 4 d before the experiment[9]. The 3-h test was recorded, and an experimenter blind to treatment scored duration of side-by-side contact. Group data for contact time spent with the partner or stranger were compared using a *t*-test.

Administration of dopaminergic compounds. Voles were anesthetized with sodium pentobarbital (2.5 mg per 40 g body weight). Bilateral 26-gauge guide cannulae (Plastics One) were stereotaxically implanted, aimed at the NAcc (nose bar – 2.5 mm; 1.6 mm rostral, ± 1 mm bilateral, 4.5 mm ventral (for shell), 3.5 mm ventral (for core) from bregma). After 3–5 d of recovery, voles received 200-nl microinjections in each side[9]. Drugs used were quinpirole, eticlopride, SKF 38393 and SCH 23390 (Research Biochemicals). Specific doses for manipulation of partner preferences in prairie voles were based on previous experiments in males[9] and females[7,8,10]. Drug manipulations did not significantly alter locomotor activity during cohabitation periods or during the partner-preference tests (data not shown). For manipulation of selective aggression in prairie voles and partner preferences in meadow voles, we used doses that did not interfere with general locomotor activity.

DA receptor autoradiography. Brain sections (15-μm thick) at 90-μm intervals were rinsed twice, for 10 min each time, in 50 mM Tris-HCl (pH 7.4) and then incubated in a buffer containing 50 mM Tris-HCl (pH 7.4), 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and [^{125}I]SCH23982 (D1 ligand) or [^{125}I]2'-iodospiperone (D2 ligand) (PerkinElmer). We added 50 nM ketanserin (RBI) to preclude binding to 5-HT_2 receptors. After a 90-min (D2) or 45-min (D1) incubation at room temperature, sections were fixed in fresh ice-cold buffer containing 0.1% paraformaldehyde, rinsed in cold buffer several times, dipped in ice-cold double-distilled H_2O (ddH_2O), and dried under a stream of cool air. Nonspecific binding was defined by incubating adjacent sections in eticlopride and SCH23390, which displaced specific binding. Slides were exposed to BioMax MR film (Kodak) along with ^{125}I plastic standards (Amersham) for 4 h. Images were analyzed with the IMAGE program (IMAGE, NIH). Group differences in binding density were analyzed by a *t*-test.

Resident-intruder test. On day 14 of cohabitation with a female, pair bonded voles were fitted with bilateral guide cannula aimed at the NAcc shell. Voles then recovered for 3 d, with their mate present, in the chamber that served as the home cage during the resident-intruder test (45 cm × 22 cm × 20 cm). On the test day, voles received a microinjection and were returned to their home cage for 10 min. Because D1-like receptors were increased equally in the NAcc core and shell in pair bonded voles, micro-injection volume was increased to 500 nl per side; this allowed sufficient upward diffusion into NAcc core, resulting in blockade of D1-like receptors in both subregions. Microinjections consisted of CSF alone or CSF containing 10 ng SCH 23390 or 10 ng eticlopride. Voles were then placed in an open field apparatus (60 cm × 60 cm; 16 grids; 15 cm × 15 cm each) for 10 min to assess locomotor activity. Thereafter, voles were returned to their home cage (in isolation) for 10 min, and then the intruder was placed in the cage. Non–estrogen primed, sexually naive females served as intruders (except for the CSF + partner group). The 6-min resident-intruder test was videotaped and the following behaviors were scored: attacks (lunges, bites, chases); aggressive postures (offensive and defensive rears); affiliative approaches (nonaggressive attempts at anogenital investigation); and the duration of affiliative behaviors (affiliative olfactory investigation or side-by-side contact). Group differences were assessed by a one-way analysis of variance (ANOVA) followed by a *post hoc* analysis of the orthogonality of comparisons (contrasts).

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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