

Dopamine D2 Receptors in the Nucleus Accumbens Are Important for Social Attachment in Female Prairie Voles (*Microtus ochrogaster*)

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The prairie vole (*Microtus ochrogaster*), a monogamous rodent that forms long-lasting pair bonds, has proven useful for the neurobiological study of social attachment. In the laboratory, pair bonds can be assessed by testing for a partner preference, a choice test in which pair-bonded voles regularly prefer their partner to a conspecific stranger. Studies reported here investigate the role of dopamine D2-like receptors (i.e., D2, D3, and D4 receptors) in the nucleus accumbens (NAcc) for the formation of a partner preference in female voles. Mating facilitated partner preference formation and associated with an approximately 50% increase in extracellular dopamine in the NAcc. Microinjection of the D2 antagonist eticlopride into the NAcc (but not the prelimbic cortex) blocked the formation of a partner preference in mating voles, whereas the D2 agonist quinpirole facilitated formation of a partner preference in the absence of mating. Taken together, these results suggest that D2-like receptors in the NAcc are important for the mediation of social attachments in female voles.

The prairie vole (*Microtus ochrogaster*) is a monogamous rodent that forms long-lasting pair bonds (Getz, Carter, & Gavish, 1981; Getz & Hofman, 1986). In the field, pair bonds are characterized by preferential association with one partner and the failure to take a new partner after the loss of a mate (Pizzuto, 1998). In the laboratory, these bonds are assessed by testing for a partner preference, a choice test in which pair-bonded voles regularly prefer their partner to a conspecific stranger. Both male and female prairie voles that have mated for more than 14 hr will choose to huddle with their partner rather than with a novel stranger (Insel & Hulihan, 1995; Williams, Catania, & Carter, 1992; Winslow, Hastings, Carter, Harbaugh, & Insel, 1993). However, an equal period of cohabitation without mating fails to induce a partner preference (Insel & Hulihan, 1995).

Recently, we reported that systemic administration of dopaminergic drugs influenced the formation of a partner preference in female prairie voles (Wang et al., 1999). The D2-like (i.e., D2, D3, D4) receptor agonist quinpirole, but not the D1-like (i.e., D1, D5) agonist SKF 38393 or saline, given intraperitoneally, facilitated formation of a partner preference in the absence of mating. Conversely, the D2-like antagonist eticlopride, but not the D1-like antagonist SCH

23390 or saline, given intraperitoneally, was able to block a partner preference in voles allowed 24 hr of mating. Although the role of dopamine (DA) in locomotion and movement control is well established, we found that both the induction and blockade of a partner preference in female voles by systemically administered D2 drugs were independent of effects on locomotion. The demonstrated role of D2 receptors in the formation of partner preferences in female prairie voles raises the question of how the pertinent D2 receptors are involved and where these receptors are located.

Mesolimbic DA is known to be involved in the rewarding and/or reinforcing properties of natural stimuli and drugs of abuse (for a review, see Bardo, 1998). One such natural stimulus is mating, which can induce a place preference (Agmo & Gomez, 1993; Meisel & Joppa, 1994; Oldenburger, Everitt, & de Jonge, 1992; Paredes & Alonso, 1997) as well as act as a potent reinforcer (Everitt, Cador, & Robbins, 1989; Everitt, Fray, Kostarczyk, Taylor, & Stacey, 1987; Matthews et al., 1997). Within the nucleus accumbens (NAcc), DA is important for mediating these effects (Cador, Taylor, & Robbins, 1991; Everitt et al., 1989). Given the importance of mating in the formation of a partner preference, we hypothesized that DA receptors in the NAcc may be important for the formation of a partner preference. To test this hypothesis, we conducted three sets of experiments.

We began by using microdialysis to determine whether mating causes an increase in extracellular DA in the NAcc of female prairie voles. The second set of experiments utilized microinjected DA antagonists to determine whether activation of D2 receptors in the NAcc is necessary for a mating-induced partner preference. In a final set of experiments, we determined whether activation of D2 receptors in the NAcc is sufficient to induce a partner preference in the absence of mating. Together, our results suggest that activation of D2 receptors in the NAcc of female prairie voles is important for the formation of a partner preference.

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Method

Subjects

Sexually naive female prairie voles (*Microtus ochrogaster*) that were the F3 generation of a laboratory breeding colony were used in the study. Upon weaning (21 days of age), females were housed in same-sex sibling groups in plastic cages (20 × 25 × 40 cm) that contained pine chip bedding. Water and food were provided ad libitum. All voles were maintained on a 14:10-hr light–dark cycle with lights on at 0700. The temperature was maintained at approximately 20 °C. Subjects were housed with same-sex siblings until they were 80–100 days old, before being assigned to each experiment.

Cannulation and Microdialysis

Females were anesthetized (2.5 mg/40 g sodium pentobarbital), and a 21-gauge guide cannula (Plastics One, Roanoke, VA) was implanted, stereotaxically aimed at the NAcc (nosebar at –2 mm; 1.7 mm rostral, 0.85 mm lateral, and –4.5 mm ventral to bregma). After a minimum of 3 days for recovery, 1.0 µg estradiol benzoate (EB) or 0.1 cc sesame oil vehicle was injected subcutaneously on 3 consecutive days. On Day 4, a modified microdialysis probe, made according to the design of Pettit and Justice (1989), was inserted into the cannula and fastened. The probe consisted of two lengths of fused silica tubing (40 mm i.d.; 100 mm o.d.; Polymicro Technologies, Phoenix, AZ) inserted into a piece of cellulose dialysis fiber (220 mm o.d.; 13,000 MW cutoff; SMI, Houston, TX). The ends of the dialysis fiber were sealed with polyimide sealing resin (Alltech, Deerfield, IL) leaving a 1.5-mm active dialysis area. For each probe, recovery of DA was approximately 10%. Approximately 2 hr before probe implantation, the inlet line of the probe was connected to a 5-ml Hamilton syringe. The probe was perfused with artificial cerebrospinal fluid (CSF; 148 mM NaCl, 2.78 mM KCl, 1.2 mM CaCl₂·2H₂O, 1.2 mM MgCl₂·6H₂O, 1.4 mM Na₂HPO₄, 0.114 mM ascorbic acid) at 1 µl/min. A single-channel swivel and a counter-balanced lever arm (Instech, Plymouth Meeting, PA) allowed the vole to move freely about the cage while being perfused. In our pilot experiment, cannula implantation and connection of a microdialysis probe did not interfere with the female's behavior. After a minimum of 2 hr for equilibration, dialysate samples were collected in polyethylene tubes every 15 min for 90 min. A sexually experienced male was then introduced, and dialysate samples were continuously collected every 15 min for an additional 3 hr. Of the subjects that received EB, only those in estrus (defined as showing lordosis) were used ($n = 5$), and all of the oil-injected controls were used ($n = 5$, none showed lordosis). All samples were immediately frozen on dry ice and stored at –80 °C until assayed. Behavioral interactions between the female subjects and male partners were recorded and subsequently quantified.

High-Performance Liquid Chromatography (HPLC) with Electrochemical Detection

Dialysate samples were assayed for DA according to the procedure described previously (Pettit & Justice, 1989). Briefly, the dialysates were injected at 0.5 µl volume onto a small-bore HPLC system, which consisted of a 0.5 mm i.d. × 10 cm column (5 µm C-18 stationary phase). Electrochemical detection of DA was accomplished with an EG&G Princeton Applied Research amperometric detector using a working electrode (Model MF-1000, Bioanalytical Systems, West Lafayette, IN) with an applied potential of +700 mV relative to an Ag/AgCl reference electrode (Model

REI; BAS, West Lafayette, IN). To generate calibration curves, DA standard solutions were injected into the HPLC system.

Behavioral Studies

Several behavioral experiments were conducted to examine the role of NAcc D2 receptors in the regulation of partner preferences. These experiments were based on the observation that 24 hr of mating consistently induced a partner preference in female prairie voles, whereas 6 hr of cohabitation with a male without mating did not induce this behavior (Insel & Hulihan, 1995; Williams, Insel, Harbaugh, & Carter, 1994). The first experiment was designed to test the hypothesis that activation of D2 receptors in the NAcc is necessary for a mating-induced partner preference. Pilot data revealed that a unilateral injection of the D2 antagonist eticlopride (5–200 ng) into the NAcc did not block a mating-induced partner preference. Therefore, an additional experiment was conducted to determine whether bilateral antagonism of D2 receptors in the NAcc would block a mating-induced partner preference. Females were implanted bilaterally with cannulas aimed into the NAcc or medial prefrontal cortex (PLC), (NAcc: nosebar at –2 mm; 1.7 mm rostral, 0.85 mm lateral, and –4.5 mm ventral to bregma; PLC: nosebar at –2 mm; 1.7 mm rostral, 0.6 mm lateral, and –2.4 mm ventral to bregma) and allowed 3 days to recover. After injection with EB on 3 consecutive days, females were microinjected bilaterally into the NAcc or PLC with CSF (100 nl/side × 2; NAcc: $n = 9$; PLC: $n = 8$) or eticlopride (100 ng/side × 2 in 100 nl, NAcc: $n = 10$; PLC: $n = 8$) at the beginning (0 hr) and midway (12 hr) points of the 24 hr of mating. Microinjections were made with a 33-gauge needle that extended 1 mm below the guide cannula into the brain. The needle was connected to a Hamilton syringe through PE-20 tubing. Plunger depression was done slowly, requiring about 20 s per injection. Behavior was videotaped to verify mating, and at the end of 24 hr, the male partner was removed and the female subjects were placed in a three-chamber apparatus to test for a partner preference (see *Partner Preference Test* section).

A second experiment was carried out to determine whether activation of D2 receptors in the NAcc is sufficient to induce a partner preference in the absence of mating. Sexually naive females were stereotaxically implanted with a 26-gauge guide cannula aimed at the NAcc. After 3 days of recovery, females received a microinjection of 200 nl of either CSF (BioFluid, Rockville, MD; $n = 8$), or 50 pg ($n = 7$), 1 ng ($n = 9$), or 10 ng ($n = 8$) of the DA D2 receptor agonist quinpirole. Immediately after injection, females were housed with a sexually naive male for 6 hr while their behavior was videotaped to verify the absence of mating. Thereafter, the male partner was removed and the female subjects were tested for a partner preference.

After behavioral tests, females received an injection of 2% India ink (200 nl), and their brains were sectioned to verify proper cannula placement. About 20% of the females were excluded from data analysis due to cannula misplacement, and they were not included in the above-mentioned numbers of subjects for each experiment. All dopaminergic compounds were purchased from Research Biochemical (Natick, MA) and dissolved in CSF before each experiment.

Partner Preference Test

Partner preference was tested in a three-chamber apparatus as described previously (Williams, Catania, & Carter, 1992; Winslow et al., 1993). Briefly, the testing apparatus consisted of a central or neutral cage (20 × 25 × 45 cm) adjoined by hollow tubes (7.5 × 16.0 cm) to two parallel identical cages, each housing a

stimulus male. The female subjects were free to move throughout the apparatus, whereas stimulus males were loosely tethered within their separate cages and had no direct contact with each other. The familiar partner (male that was cohabited or mated with the female) and a stranger (male that had not previously encountered the female) were used as stimulus animals. The females were put into the neutral cage, and their behavior was recorded for 3 hr by a time-lapse video recording system.

Data Acquisition and Analysis

For the microdialysis study, the videotape was scored to verify the presence or absence of mating as well as the onset and frequency of copulation. To compare the sample taken immediately before the male was added with the sample taken immediately after the addition of the male, the level of DA in the first five samples collected was averaged and used as the baseline. For each treatment group, the pre-male, 15-min sample (relative to baseline) was compared with the post-male, 15-min sample by a paired *t* test. To further examine temporal and treatment effects, the actual DA level for the 1 hr before the male was added as well as each of the 3 hr after the addition of the male were compared by a two-factor (Treatment \times Time) repeated-measures analysis of variance (ANOVA). This was followed by a one-factor (time), repeated-measures ANOVA for each treatment group. A Scheffé's post hoc test was used when appropriate.

For the partner preference test, the duration and frequency of the female's side-by-side contact with either the partner or the stranger, the time that females spent in each cage, and the frequency of cage entry were recorded on a computerized data acquisition system. Treatment effects on the time that females spent in each cage within each experiment were analyzed by a Kruskal-Wallis nonparametric test (because of nonhomogeneity of variance). Differences in side-by-side contact with the partner or the stranger within each treatment group were analyzed by a Wilcoxon's signed rank sum nonparametric test. In addition, female voles were classified as having a partner preference if they spent more than twice as much time in contact with the partner as with the stranger and at least 10 min in contact with either male. In these experiments, no subject was excluded because of spending less than 10 min in contact with either male. A chi-square test was used to compare the number of females that displayed a partner preference between the treatment groups. Because DA's effects on partner preference could be secondary to its effects on locomotor activity during a preference test, frequencies of the female's entry into the partner's and stranger's cage were recorded and subsequently used as an index for locomotor activity. Treatment effects on cage entry were analyzed by a two-tailed, unpaired *t* test (antagonist experiment) or a one-way ANOVA (agonist experiment). Differences in partner preference could also be due to treatment effects on mating or other aspects of social interaction between the female and the male partner during the initial exposure. Therefore, frequency of mating was quantified during the first 6 hr. These data were analyzed by a repeated-measures ANOVA. An alpha level of .05 was used to reject the null hypothesis for all comparisons.

Results

Mating Accompanied an Increase in Extracellular DA in the NAcc

All of the estrous females injected with EB began mating within the first 15 min after the male was introduced. None

of the females that received oil injections mated. There was no difference in the basal level of DA between the two treatment groups. In estrous females, extracellular DA levels increased in the first 15-min sample by 51.0% (\pm 8.2%) above baseline. This increase was significantly greater than the pre-male sample (4.4% \pm 12.0), $t(4) = 2.989$, $p < .05$ (see Figure 1A). The females treated with oil were not in estrus and did not allow mating. In these females, exposure to the male did not significantly increase DA levels in the NAcc during the first 15-min, post-male sample (17.7% \pm 4.3%) compared with the pre-male sample (-0.8% \pm 13.9%); $t(4) = 1.715$, $p > .10$ (Figure 1A). To further investigate time and treatment effects, we used a two-factor (Treatment \times Time), repeated-measures ANOVA on the four 1-hr average time points (one pre-male, three post-male). There was no treatment effect, $F(1, 8) = 4.587$, $p > .1$, but both a significant time effect, $F(3, 24) = 13.066$, $p < .0001$, and a significant interaction were observed $F(3, 24) = 3.942$, $p < .05$. Because of the interaction, a one-factor, repeated-measures ANOVA was conducted on each treatment group individually. In the EB/mated group, a significant time effect was observed, $F(3, 24) = 20.168$, $p < .0001$, and a Scheffé's post hoc test revealed that the three post-male time points were different from the pre-male time point ($p < .05$). In contrast, no significant time effect was observed in the oil/nonmated group, $F(3, 24) = 1.969$, $p > .1$. These results are summarized in Figure 1B. For ease of display, the data are presented as the percentage of change from the pre-male baseline, which was the same for both groups. In the EB/mated group, there was no significant relationship between the amount of mating and the increase in extracellular DA levels.

Bilateral Treatment With a D2 Antagonist in the NAcc (but not the PLC) Blocked a Mating-Induced Partner Preference

As expected, females that mated for 24 hr and received CSF in the NAcc spent significantly more time in the partner cage (111.3 \pm 10.0 min) than either the stranger (32.0 \pm 8.8 min) or neutral cage (31.3 \pm 3.8 min; $H = 15.707$, $p < .001$; see Figure 2A). As a group, these females also spent significantly more time in contact with the partner than with the stranger ($Z = 2.547$, $p < .01$; see Table 1). In contrast to females that received identical injections of CSF, females that received two bilateral injections of eticlopride into the NAcc showed no difference in the time they spent in the partner (70.3 \pm 20.0 min), stranger (44.0 \pm 15.4 min), or neutral cage (61.8 \pm 17.6 min; $H = 0.637$, $p > .10$; Figure 2a). Additionally, these females showed no group difference in the time they spent in contact with the partner or the stranger ($Z = 0.840$, $p > .1$; Table 1). According to a categorical definition (twice as much time huddled with partner as stranger), 8 out of 9 females in the CSF group displayed a partner preference, whereas only 4 out of 10 of the eticlopride-injected females showed a preference, $\chi^2(18) = 4.866$, $p < .05$ (Figure 2C). No treatment effects were found for either the frequency of mating during the first

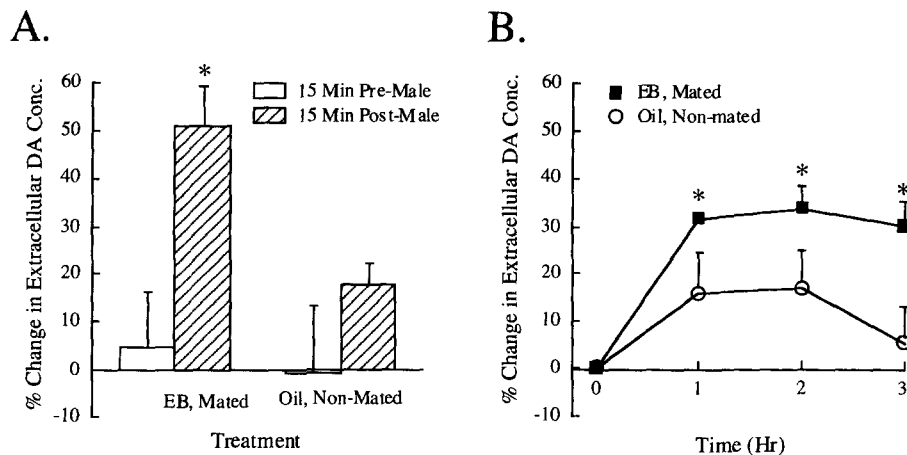


Figure 1. The effect of male exposure and mating on extracellular dopamine (DA) levels as measured by microdialysis and high performance liquid chromatography with electrochemical detection. **A:** Estrous females mated during the first 15 min after a male was placed in the cage, resulting in a 51.0% increase in extracellular DA above baseline. (* $p < .05$, paired t test.) Nonestrous females did not allow mating by the stimulus male, and no significant increase in DA level was observed in the first 15 min after the male was added. **B:** The level of DA remained significantly elevated in the mating group for the next 3 hr, compared with the pre-male sample. No significant increase was seen in the nonmating group during the 3-hr exposure to the male. A two-factor (Treatment \times Time), repeated-measures analysis of variance (ANOVA) revealed a significant interaction. After a repeated-measures ANOVA for each treatment, only the mated group showed a time effect. Conc. = concentration; EB = estradiol benzoate. * $p < .05$ based on a Scheffé's post hoc test. Error bars indicate SEM.

6 hr with the male, $F(1, 8) = 0.069$, $p > .1$ (Figure 2E), or for their locomotor activity during the preference test, cage entries: CSF = 72.3 ± 11.0 ; eticlopride = 73.2 ± 13.4 ; $t(18) = 0.049$, $p > .1$.

To test whether the antagonist's effect on partner preference formation were specific to the NAcc, two additional groups of females were injected bilaterally with CSF or eticlopride (100 ng/side \times 2) into the medial PLC. Females injected with CSF spent more time in the partner cage (115.6 ± 16.4 min.) than in the stranger (38.9 ± 13.4 min.), or neutral cage (25.2 ± 5.9 min; $H = 11.885$, $p < .001$; Figure 2B). A similar pattern was found in females injected with eticlopride into the PLC (partner: 96.7 ± 16.0 min; stranger: 47.4 ± 17.2 min; neutral: 35.5 ± 9.3 min; $H = 6.014$, $p < .05$; Figure 2B). The CSF group also spent more time in contact with the partner than with the stranger. ($Z = 1.960$, $p = .05$; Table 1) Although 6 of the 8 females in the eticlopride group spent at least twice as much time in contact with the partner as with the stranger, the difference in partner contact time compared with stranger contact time as a group was not significant ($Z = 1.40$, $p > .1$; Table 1) due to the exceptionally large amount of time 2 females spent with the stranger. However, when compared to the CSF group, there was no difference in the number of females showing a partner preference (7 of 8 vs. 6 of 8; $\chi^2 = 0.410$, $p > .1$; Figure 2D). As with the injections into the NAcc, no treatment effects were found on either the frequency of mating in the first 6 hr of male exposure, $F = 1.174$, $p > .1$ (Figure 2F), or locomotor activity during the preference test (cage entries: CSF = 81.1 ± 19.7 , eticlopride = 72.8 ± 11.0 ; $t = 0.371$, $p > .1$).

D2 Agonist in the NAcc Induced a Partner Preference Without Mating

As expected from previous studies, 6 hr of cohabitation with a male without mating did not induce a partner preference in female prairie voles (Insel & Hulihan, 1995; Wang et al., 1999; Williams et al., 1994). Females injected with CSF spent similar amounts of time in the partner, stranger, and neutral cages ($H = 0.195$, $p > .1$; see Figure 3A). Females injected with 50 pg of the D2 receptor agonist, quinpirole, spent more time in the partner's cage versus the neutral cage ($H = 10.442$, $p < .005$; Figure 3A) but not versus the stranger's cage. Females injected with 1 ng or 10 ng quinpirole spent significantly more time in the partner cage than either the stranger or neutral cage (1 ng: $H = 16.603$, $p < .001$; 10 ng: $H = 16.245$, $p < .001$; Figure 3A). An analysis of the time each treatment group spent in the contact with the partner or the stranger revealed a pattern similar to cage time (see Table 2). No significant difference was observed in the CSF or 50 pg group (CSF: $Z = 0.28$, $p > .1$; 50 pg: $Z = 1.014$, $p > .1$), but the 1 ng and 10 ng groups showed a significant difference (1 ng: $Z = 2.547$, $p < .05$; 10 ng: $Z = 2.380$, $p < .05$). These group differences are reflected in the number of females showing a categorical partner preference. There was no difference between the CSF and 50 pg groups (2 of 8 vs. 4 of 7; $\chi^2 = 9.932$, $p > .1$; Figure 3B). However, the 1 ng (8 of 9; $\chi^2 = 7.13$; $p < .01$; Figure 3B) and 10 ng (7 of 8; $\chi^2 = 6.349$, $p < .05$; Figure

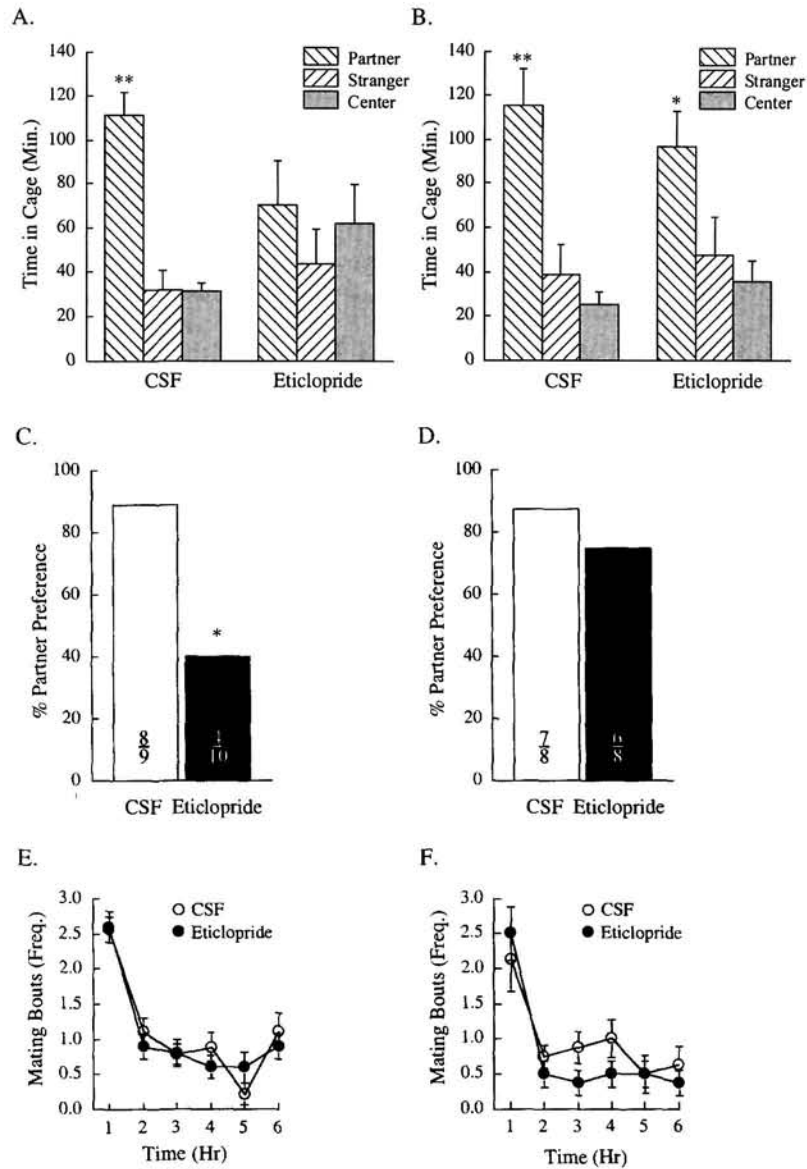


Figure 2. Effects of bilateral microinjections of artificial cerebrospinal fluid (CSF) or the D2 antagonist eticlopride into the nucleus accumbens (NAcc; Panels A, C, and E) and prelimbic cortex (PLC; Panels B, D, and F) on partner preference. **A:** Females injected with CSF into the NAcc spent more time in the partner's cage than in the stranger's cage after 24 hr of mating. However, injections of eticlopride blocked this preference. $**p < .01$, Kruskal-Wallis nonparametric test. **C:** Eticlopride reduced the number of females with a partner preference (4 of 10) compared with CSF-treated females (8 of 9). $*p < .05$, chi-square test. **E:** No effect on the average number of mating bouts during the first 6 hr were observed. **B:** Females injected with CSF or eticlopride into the PLC spent more time in the partner's cage than the stranger's cage after 24 hr of mating. $**p < .01$, $*p < .05$, Kruskal-Wallis nonparametric test. **D:** Eticlopride had no effect on the number of females showing a partner preference or **F:** the average number of mating bouts during the first 6 hr. Error bars indicate SEM.

3B) were different from the CSF control group. During the initial 6 hr of cohabitation, none of the females mated. No difference in locomotor activity during the preference test was observed (cage entries: CSF = 66.2 ± 16.9 , 50 pg = 90.1 ± 10.9 , 1 ng = 63.0 ± 8.5 , 10 ng = 78.5 ± 9.8 ; $F = 1.048$, $p > .1$).

Discussion

The monogamous prairie vole forms long-lasting pair bonds that are facilitated by mating (Carter & Getz, 1993; Insel & Hulihan, 1995; Shapiro & Dewsbury, 1990). An essential step in the establishment of a pair bond is the

Table 1
Effect of Eticlopride in the Nucleus Accumbens (NAcc) or Prelimbic Cortex (PLC) on Contact Time (in Minutes) During a Partner Preference Test After 24 Hr of Mating

Injection	NAcc		PLC	
	Partner	Stranger	Partner	Stranger
CSF	77.6 ± 9.8**	10.3 ± 7.2	81.2 ± 18.4*	9.7 ± 9.1
Eticlopride	38.0 ± 16.9	17.5 ± 12.5	57.7 ± 16.5	22.5 ± 13.7

Note. Values are means ± SEM. CSF = artificial cerebrospinal fluid.

* $p < .05$, ** $p < .01$, Wilcoxon's signed rank sum test.

formation of a partner preference. Females that mate for 24 hr reliably exhibit enduring partner preferences, whereas females that are exposed for 6 hr or less (without mating) fail to show a preference for the partner versus a stranger (Insel & Hulihan, 1995; Wang et al., 1999). We previously demonstrated that D2 (but not D1) receptor activation plays an important role in the formation of pair bonds in female voles (Wang et al., 1999). In that study, the D2 antagonist

eticlopride (but not the D1 antagonist SCH 23390), given intraperitoneally, blocked the formation of a partner preference associated with 24 hours of mating; and the D2 agonist quinpirole (but not the D1 agonist SKF 38393), given intraperitoneally, was sufficient to induce a partner preference in females with only 6 hr of nonmating social exposure to a male. The present study extended these previous results with site-specific studies of DA release as well as D2 blockade and activation. Mating (but not cohabitation with a male) increased extracellular DA in the NAcc in female prairie voles. Blockade of D2 receptors with eticlopride injected into the NAcc (but not the PLC) prevented the development of the partner preference usually observed after 24 hr of mating. Activation of D2 receptors by quinpirole injected directly into the NAcc facilitated the formation of a partner preference in the absence of mating.

DA Release With Mating

Extracellular DA increased significantly in the NAcc (approximately 50% in the first 15 min after copulation) and continued to stay elevated at approximately 30% above baseline for the next 3 hr. This increase in extracellular DA is unlikely to be entirely due to social (as opposed to sexual) experience because in the control group (nonestrous females exposed to a male) DA failed to increase significantly, either in the first 15 min or in the subsequent 3 hr. Nevertheless, in this control group, there was a trend toward an increase in DA (approximately 17% in the first 15 min, decreasing to approximately 8% by the 3rd hr). Although mating has been shown to be a potent stimulus for the release of DA in other rodents (Damsma, Pfaus, Wenkstern, Phillips, & Fibiger, 1992; Pfaus, Damsma, Wenkstern, & Fibiger, 1995; Wenk-

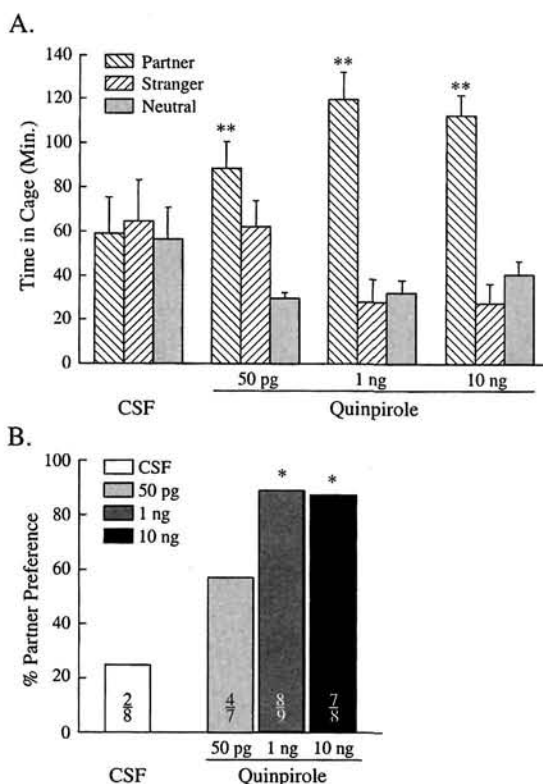


Figure 3. Effects of the dopamine D2 receptor agonist, quinpirole, on partner preference. A: Females injected with artificial cerebrospinal fluid (CSF) into the nucleus accumbens (NAcc) spent an equal amount of time in each cage after 6 hr of cohabitation with a male without mating. However, females injected with 1 ng or 10 ng quinpirole spent more time in the partner cage than in the stranger cage. The 50-pg group spent more time in the partner cage than in the neutral cage, but not more than in the stranger cage. * $p < .05$, ** $p < .001$, Kruskal-Wallis nonparametric test. B: Compared with the CSF group, significantly more females in the 1 ng and 10 ng groups had a partner preference. * $p < .05$, chi-square test. Error bars indicate SEMs.

Table 2
Effect of Quinpirole in the Nucleus Accumbens on Contact Time (in Minutes) During a Partner Preference Test After 6 Hr of Cohabitation Without Mating

Injection	Partner	Stranger
CSF	22.3 ± 9.2	26.4 ± 10.1
Quinpirole		
50 pg	40.8 ± 9.6	22.3 ± 10.5
1 ng	70.8 ± 9.1*	7.8 ± 5.8
10 ng	66.6 ± 8.5*	6.1 ± 4.9

Note. Values are means ± SEM. CSF = artificial cerebrospinal fluid.

* $p < .05$, Wilcoxon's signed rank sum test.

stern, Pfaus, & Fibiger, 1993), some reports have described a small but significant increase in DA in the NAcc of female rats and hamsters upon exposure to a male without copulation (Meisel, Camp, & Robinson, 1993; Mermelstein & Becker, 1995; Pfaus et al., 1995). Conceivably, with a much larger control group, the slight increase we observed in our nonmating females may have proven significant. Indeed, we failed to find a significant treatment effect in a direct comparison of the mated and nonmated females. Therefore, we cannot rule out the possibility that some portion of the increase in DA observed in the mating group may have been due to exposure to the male, rather than a consequence of mating. However, further analysis of the differences within our two treatment groups reveals that the lack of a treatment effect was due to the inclusion in the analysis of the pre-male baseline time point, which did not differ between the two treatments. If this baseline point is removed and the three post-baseline points are compared between the two groups, a two-factor, repeated-measures ANOVA shows a significant treatment effect, $F = 6.111, p < .05$. Thus, mating causes an additional release of DA, significantly above that released by exposure to the male without copulation in nonestrous females.

A potential confound in the comparison of the EB/mating group and the oil/nonmating group was the difference in hormone treatment and estrous status. Ideally, we should have included a second control group of estrogen-treated females exposed to males (without mating) to rule out the possibility that estrogen potentiated the effect of male exposure, irrespective of mating. Indeed, in previous reports, EB-treated female rats showed an increase in NAcc DA in response to male exposure without mating (Mermelstein & Becker, 1995; Pfaus et al., 1995). However, close examination of these data (Mermelstein & Becker, 1995) suggests that this increase was not significantly different than basal levels and was due to a slight decrease in DA levels in the oil-injected controls. Furthermore, an ingenious study in Syrian hamsters supports the conclusion that it is the onset of mating (specifically intromission, not mounting or hormone treatment) that causes the increase (Kohlert, Rowe, & Meisel, 1997). In that study, three groups of females were compared during exposure to sexually active males: estrous (EB-injected) with vaginas occluded by tape to preclude intromission, estrous (EB-injected) unoccluded, and nonestrous (oil-injected). Only the unoccluded estrous females showed any increase in NAcc DA, suggesting that EB treatment and male exposure were insufficient stimuli for DA release. A similar study still needs to be done in prairie voles to demonstrate conclusively the connection between copulation and DA release.

Finally, it should be noted that the increase we observed in extracellular DA concentrations was limited for technical reasons to the first 3 hr of male exposure, with or without mating. We know from behavioral studies that pair bond formation requires at least 14 hr of male exposure with as many as 10–20 bouts of copulation occurring during this interval (Insel & Hulihan, 1995). Therefore, it is not clear whether the observed increase is related to the onset of a

partner preference, nor is it known whether the increase in DA is limited to the NAcc.

D2 Blockade Prevents and D2 Activation Facilitates Partner Preference Formation

We hypothesized that the release of DA in the NAcc during mating was critical for the formation of a partner preference. Eticlopride injected bilaterally (but not unilaterally) into the NAcc successfully blocked the formation of a partner preference. This effect appears to be specific to the NAcc, as the same treatment in the PLC only slightly reduced the time females spent in the partner's cage and had no effect on the number of females showing a partner preference. In addition, the antagonist's effects are not secondary to effects on mating, as no change in mating behavior was observed in the first 6 hr. Nor can these effects be attributed to a change in locomotor behavior (no treatment effects on cage entries) or on the stress of injections (CSF group received identical handling). The total dose administered (400 ng) was 0.8% of the dose required by intraperitoneal injection (Wang et al., 1999). Although this does not establish the NAcc as the only active site for eticlopride's systemic effects, our findings are consistent with the hypothesis that the NAcc is an important target for the effects of eticlopride reported previously in female prairie voles (Wang et al., 1999).

It should be noted, however, that this blockade must be bilateral and continuous to be effective. The need for a second injection at 12 hr was surprising, as this was not required in our previous study that used intraperitoneal injections (Wang et al., 1999). The need for a second injection reinforces the possibility noted above: that a surge of DA release causing D2 activation after 12 hr of mating is important for pair bond formation. With a high dose of eticlopride given systemically, sufficient D2 blockade may have persisted throughout the 24-hr mating period to block this surge. In the current study, the antagonist was only effective when given bilaterally, suggesting that even a partial activation of D2 receptors could be sufficient for partner preference formation.

To further test our hypothesis that D2 receptors in the NAcc are important for partner preference formation, we attempted to induce a partner preference by microinjecting the D2 agonist quinpirole into the NAcc of female voles immediately before exposure to a male. Six hours with a male without mating did not result in a partner preference (CSF group in Figure 3, A and B). However, the D2 agonist dose-dependently induced a partner preference in the absence of mating, with an effective dose that was 0.002% of the dose previously used with systemic administration (Wang et al., 1999). As with the antagonist studies, these results cannot be attributed to drug effects on mating (none of the voles mated), locomotion (no differences in cage entries), or stress (no differences in handling between treatment and control groups).

Together, these experiments suggest that activation of D2 receptors in the NAcc is necessary and sufficient for the formation of a mating-induced partner preference. It should

be noted, however, that eticlopride and quinpirole bind to D3 and D4 as well as D2 receptors. Therefore, the precise cellular recognition site for either drug's action remains to be determined.

Mechanisms of Action

In considering how D2 (or D3/D4) receptors influence a complex behavior like partner preference formation, one needs to consider two levels of explanation: neurochemical and behavioral/cognitive mechanisms.

Neurochemical. The neurochemical consequences of D2 activation have not been reported in the vole, but an abundant literature in other rodents suggests the importance of regulation of intracellular signaling pathways and gene expression. Activation of D2 receptors is thought to inhibit adenylate cyclase, decrease Ca^{2+} influx, increase K^+ influx, and increase arachidonic acid levels (Caccavelli et al., 1992; Huff, 1996; Liu, Shen, Kapatos, & Chiodo, 1994; Picetti et al., 1997; Watts & Neve, 1996). Several mRNAs are regulated by D2 receptors as well, including those of preproenkephalin and prodynorphin, the μ -opioid receptor glutamic acid decarboxylase, and the immediate early genes *c-fos*, *jun B*, and *Zif/268* (Azaryan, Coughlin, Buzas, Clock, & Cox, 1996; Marshall, Cole, & LaHoste, 1993; Pollack & Wooten, 1992a, 1992b; Sirinathsinghji et al., 1994; Wang & McGinty, 1995, 1996). Which of these possible targets of D2 receptor activation are important for the formation of a partner preference in female voles is not known and remains the subject of investigation in our lab.

Previous studies in voles have reported that oxytocin (Insel & Hulihan, 1995; Williams et al., 1994), vasopressin (Winslow et al., 1993), and corticosterone (Carter, DeVries, & Getz, 1995) influence partner preference formation. Of these candidates, oxytocin appears to be important for mating-induced partner preferences in female prairie voles (Insel & Hulihan, 1995). It is not yet clear how D2 receptor activation in the NAcc might release oxytocin or activate oxytocin receptors, but there are three pieces of data that are relevant. First, the prairie vole (but not the nonmonogamous montane vole) has a dense concentration of oxytocin receptors in the NAcc (Insel & Shapiro, 1992). When injected directly into this region, an oxytocin antagonist blocks partner preference formation (Gingrich, Cascio, Liu, Insel, & Wang, 1998). Second, an oxytocin antagonist injected intracerebroventricularly blocks quinpirole's facilitation of a partner preference (Wang & Insel, 1999). Finally, in the rat, oxytocin injected intracerebroventricularly induces a profound and enduring increase in the firing rate of ventral tegmental area DA neurons (Yu, Stowe, & Insel, 1997). Taken together, these data suggest that DA may influence partner preference formation by interaction with oxytocin. This hypothesis is supported by numerous reports of DA-oxytocin interaction (Amico, Layden, Pomerantz, & Cameron, 1993; Argiolas et al., 1989; Argiolas, Melis, Vargiu, & Gessa, 1987; Melis, Argiolas, & Gessa, 1989; Melis, Argiolas, Stancampiano, & Gessa, 1990), including within the NAcc (Drago et al., 1986; Kovacs, Sarnyai, Barbarci,

Szabo, & Telegdy, 1990; Sarnyai, Szabo, Kovacs, & Telegdy, 1990).

Behavioral/Cognitive

A partner preference requires the olfactory detection of social cues (Williams, Slotnick, Kirkpatrick, & Carter, 1992) as well as processes of learning and memory (Wang et al., 1999). DA has been shown to have a role in all of these processes. For instance, DA is important for odor detection in general, with D2 agonists decreasing (Doty & Risser, 1989) and D1 agonists enhancing olfactory sensitivity (Doty et al., 1998). Although D2 antagonists might be expected to improve olfactory processing, it is possible that the antagonist in the NAcc blocked the formation of a partner preference by interfering with the olfactory processing of the male partner's odors. Indeed, stimulation of the accessory olfactory system has been shown to cause release of DA in the NAcc (Mitchell & Gratton, 1992), and DA receptors within the NAcc are important for social recognition in male rats (Ploeger, Willemen, & Cools, 1991). However, our previous experiments do not support this interpretation. We showed that, although the D2 antagonist given intraperitoneally does block formation of a partner preference, it does not interfere with its expression (i.e., it has no effect when given just before partner preference testing), a process that likely depends on olfactory discrimination of mate versus stranger (Wang et al., 1999).

An alternative explanation posits that DA in the NAcc is mediating some aspect of conditioning that occurs only after an extended period of mating. We suggest that mating is a potent rewarding stimulus that becomes associated with the male partner, causing some feature of the partner (likely his odor) to take on a rewarding significance, which leads to the formation of a partner preference. This hypothesized process is similar to the conditioned place preference (CPP) that occurs with reinforcing nonsocial stimuli such as psychostimulant drugs (Baker, Fuchs, Specio, Khroyan, & Neisewander, 1998; Carr & White, 1983; Hiroi & White, 1991) as well as quinpirole (White, Packard, & Hiroi, 1991). Indeed, others have demonstrated that sexual interaction can induce CPP in both male and female rats (Agmo & Gomez, 1993; Oldenburger et al., 1992; Paredes & Alonso, 1997), as well as female Syrian hamsters (Meisel & Joppa, 1994). In the case of female hamsters, D2 antagonists given intraperitoneally blocked mating-induced CPP (Meisel, Joppa, & Rowe, 1996). Given the evidence that mating releases DA in the NAcc of Syrian hamsters (Meisel et al., 1993) and that NAcc D2 receptors are important for drug-induced CPP (Hiroi & White, 1991), it seems plausible that CPP after mating is also dependent on NAcc D2 receptors. We suggest that in a similar manner, bilateral NAcc D2 receptor blockade in female voles prevents the formation of a partner preference induced by an extended period of mating.

Given that both monogamous and nonmonogamous rodents release DA in the NAcc with mating and that the NAcc appears important for conditioning, why do prairie voles form life-long pair bonds, whereas rats, hamsters, and almost all other rodents fail to develop enduring social

attachments? The answer to this question may provide a key to understanding the behavioral and neural mechanisms of monogamy and, more generally, social attachment. One possible behavioral explanation lies in the pattern of mating, which in the prairie vole includes repeated and regularly spaced bouts of copulation that can continue for 24–48 hr. The evidence that at least 14 hr of mating is required for the formation of an enduring pair bond (Insel & Hulihan, 1995) and the possibility of a concurrent sensitized release of DA would both be consistent with this explanation, but there are other vole species with similar patterns of mating that fail to develop pair bonds (Shapiro & Dewsbury, 1990), and we have yet to demonstrate that a sensitized release of DA is critical for pair bonding. Perhaps there is something about the stimulus processing that leads prairie voles to develop a social preference, whereas nonmonogamous species are more likely to exhibit a place preference. Indeed, in preliminary studies, we have been conspicuously unsuccessful in training prairie voles on the CPP. In tests of social memory (assessed by decrements in social investigation after repeated exposure to the same conspecific), prairie voles show a remarkable retention of 24 hr (Wang & Insel, 1999), whereas rats appear to “forget” a familiar conspecific within 1–2 hr (Ploeger et al., 1991). Such results, although preliminary, suggest that high levels of social rather than spatial discrimination have been selected in prairie voles. But this enhanced capacity for detecting social cues doesn’t explain the preference for the mate, unless one assumes that mating in the prairie vole is the reinforcing event that secondarily conditions the female to the specific social cues of her mate. Finally, some answers may come from more careful neuro-anatomical work to determine how the consequences of D2 activation differ in the prairie vole relative to other nonmonogamous rodents.

Taken together with a recent report on DA receptor blockade in the NAcc inhibiting maternal retrieval and licking of rat pups (Keer & Stern, in press), the experiments presented here suggest that D2 receptors in the NAcc may play an important role in the formation of social attachments. We have hypothesized that a simple conditioning model, similar to CPP, underlies DA’s involvement in partner preference formation. But exactly how this conditioning occurs, and how DA interacts with other neurotransmitters known to play a role in partner preference formation, is the subject of current investigations in our laboratory.

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