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NUCLEUS ACCUMBENS OXYTOCIN AND DOPAMINE INTERACT TO REGULATE PAIR BOND FORMATION IN FEMALE PRAIRIE VOLES

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Abstract—Although oxytocin (OT) and dopamine (DA) have been implicated in pair bond formation in monogamous prairie voles (*Microtus ochrogaster*), the nature of potential interactions between these two neurochemical systems and the brain circuits important for such interactions in the regulation of pair bonding have not been explored. Here, we demonstrated that access to both OT and DA D2-type receptors is necessary for pair bond formation, as blockade of either type of receptor prevented partner preferences induced by OT or a D2-type agonist. We also demonstrated that the nucleus accumbens (NAcc) is a brain area important for such OT–DA interactions. In NAcc, blockade of OT receptors prevented partner preferences induced by a D2-type agonist whereas blockade of D2-type, but not D1-type, DA receptors blocked OT-induced partner preferences. Together, our data suggest that concurrent activation of OT and DA D2-type receptors in NAcc is essential for pair bond formation in female prairie voles.

Key words: dopamine, oxytocin, nucleus accumbens, pair bonding, mating, monogamy.

Neurochemical interactions play important roles in regulation of behavior. For example, oxytocin (OT) and dopamine (DA) interact in the regulation of several behaviors including yawning, grooming, penile erections, mating, and drug-altered locomotor activity (Drago et al., 1986; Argiolas et al., 1988, 1989; Kovacs et al., 1990). However, no such experiments have been conducted in the study of social attachment. The limited efforts in this research area may be due to both the complexity of social attachment, which involves sensory processing, motivation, memory, and behavioral output, as well as the lack of an animal model demonstrating a reliable behavioral index of social attachment. Studies in recent years have demonstrated that the monogamous prairie vole (*Microtus ochrogaster*) provides an excellent model for the study of social attachment. This species forms long-term pair bonds in the field (Getz et al., 1981; Getz and Hofmann, 1986) and mates preferentially with one partner in the laboratory (Dewsbury, 1987; Carter and Getz, 1993; Getz and Carter, 1996). Following 24 h of mating, male and female prairie voles form pair bonds as indicated by subjects spending significantly more time with the familiar partner than with a conspecific stranger (partner preferences; Williams et al., 1992; Winslow et al., 1993).

Several neurochemicals and hormones have been implicated in partner preference formation (De Vries et al., 1995; Insel and Hulihan, 1995; Gingrich et al., 1998; Cho et al., 1999). In female prairie voles, i.c.v. administration of a selective OT receptor antagonist (OTA) blocks mating-induced partner preferences whereas administration of OT induces this behavior in the absence of mating, indicating the importance of OT in pair bond formation (Williams et al., 1994; Insel and Hulihan, 1995; Cho et al., 1999). Further, the nucleus accumbens (NAcc) in the prairie vole brain contains dense OT receptors (Insel and Shapiro, 1992; Young et al., 2001), and injections of OTA directly into NAcc block mating-induced partner preferences (Young et al., 2001). As pair bonding is induced by mating (Winslow et al., 1993; Insel and Hulihan, 1995), its formation likely involves reinforcing properties associated with mating, and thus can be viewed as a natural example of reward learning, a process with significant DA involvement (Robbins and Everitt, 1996; Wise, 1996; Berridge and Robinson, 1998; Di Chiara, 1999; Ikemoto and Panksepp, 1999). Administration of DA antagonists indeed blocks, whereas DA agonists induces, partner preference formation in female prairie voles (Wang et al., 1999; Gingrich et al., 2000). It is important to note that DA appears to act via a D2-type receptor-mediated mechanism to regulate pair bonding (Wang et al., 1999; Gingrich et al., 2000).

Although both OT and DA involvement in partner preference formation have been documented, it is by no means clear whether the two act in concert or independently to regulate behavior. Further, the nature of their interactions, if any, and the brain circuits in which such interactions may occur need to be determined. Here we demonstrate that access to both OT and DA D2-type receptors in NAcc is necessary for partner preference formation in female prairie voles.

EXPERIMENTAL PROCEDURES

Subjects

Subjects were sexually naive female prairie voles (*M. ochrogaster*) that were the F4 generation of a laboratory breeding colony started from field-captured animals. Subjects were housed in same-sex sibling pairs in plastic cages (20×50×40 cm) that contained cedar chip bedding. Water and food were provided *ad libitum*. The cages were maintained on a 14/10-h light/dark cycle with lights on at 07:00 h. The temperature was about 20±1 °C. Females at 70–90 days of age were used as subjects, while males of a similar age were used as stimulus animals.

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Abbreviations: CSF, artificial cerebrospinal fluid; DA, dopamine; NAcc, nucleus accumbens; OT, oxytocin; OTA, oxytocin receptor antagonist.

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Stereotaxic cannulation and microinjection

Subjects were anesthetized with sodium pentobarbital (1 mg/10 g body weight), and 26-gauge stainless steel guide cannulae (Plastics One Inc., Roanoke, VA, USA) were stereotaxically implanted aimed at the lateral ventricle (unilaterally; nose bar at −2.5 mm, 0.6 mm rostral, 1.0 mm lateral, and 2.6 mm ventral to the 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 the bregma). For the control animals, cannulae were aimed at the cingulate cortex which contains OT receptors (bilaterally; nose bar at −2.5 mm, 0.8 mm rostral, 0.6 mm bilateral, and 2.3 mm ventral to the bregma), or caudate putamen which contains DA receptors (bilaterally; nose bar at −2.5 mm, 1.7 mm rostral, 1.5 mm bilateral, and 3.0 mm ventral to the bregma). After 3 days of recovery, each subject received microinjections of either artificial cerebrospinal fluid carrier (CSF; BioFluids Inc, Rockville, MD, USA) or CSF containing different concentrations of OT (Peninsula Laboratory Inc., San Carlos, CA, USA), OTA ([*d*$(CH_2)_5$, Tyr(Me)^2, Thr^4, Tyr-NH_2^{9}]-OVT; Peninsula Laboratory Inc.), DA D2-type receptor agonist, quinpirole, or antagonist, eticlopride, D1-type receptor antagonist, SCH 23390, (Sigma, St. Louis, MO, USA), or combinations of these drugs (see procedures below). OTA is a high affinity, selective receptor antagonist that binds on OT receptors for at least 24 h in the prairie vole brains (Witt et al., 1991). Both quinpirole and eticlopride have high affinity for D2-type (including D2, D3, and D4) over D1-type (including D1 and D5) receptors whereas SCH 23390 has high affinity for D1-type over D2-type receptors (Missale et al., 1998). The injection volume was 200 nl into the lateral ventricle (i.c.v.) or per side region-specifically. The injection was performed by inserting a 33-gauge needle that extended 1 mm below the guide cannula. The needle was connected through PE20 tubing (Plastic One Inc) to a Hamilton syringe that was controlled by a manual injector (Fisher Scientific, Houston, TX, USA). The plunger was slowly depressed, requiring about 10 s per injection. Some animals also received i.p. injections (200 μl) of saline containing different concentrations of the DA agonist and/or antagonist. After the behavioral test, all subjects were injected with 200 nl of 2% India ink i.c.v. or site-specifically before killing. Their brains were then harvested and cut into 30 μm sections on a cryostat to verify injection sites histologically.

Minipump implantation and infusion

Subjects were anesthetized and then implanted stereotaxically with an L-shaped 25-gauge cannula aimed at the lateral ventricle. The cannula was connected to an osmotic minipump (Model 1007D; Alzet Corp., Cupertino, CA, USA) through PE20 tubing, both of which were filled with either CSF or CSF containing OT, OT with OTA or with the D2-type antagonist, eticlopride. The minipump was primed in sterile saline at 37 °C for 16 h prior to being used. The infusion flow rate was 0.5 μl/h.

Partner preference test

This test was initially developed in Dr. C. S. Carter's laboratory and has been adapted in several laboratories including our own. Briefly, the three-chamber testing apparatus consisted of a neutral cage (20 cm high×50 cm long×40 cm wide) joined by plastic tubes (7.5×16 cm) to two parallel identical cages, each housing a stimulus animal. Female subjects were free to move throughout the apparatus, and the stimulus males were tethered within their cages, allowing no direct contact with each other. The familiar partner (the male that had previously been housed with the subject) and a conspecific stranger (a male that had not previously encountered the subject) were used as stimulus animals. After the subjects were placed into the neutral cage, their behavior was recorded for 3 h with a time-lapse VCR.

Data quantification and analysis

To determine the partner preference, duration and frequency of the subject's side-by-side contact with either the partner or stranger were recorded. A partner preference was defined as subjects spending significantly more time in body contact with the partner versus stranger, as determined by a *t*-test ($P<0.05$). Videotapes of the 6 h of cohabitation were reviewed to confirm the absence of mating. In addition, as drug effects on partner preferences could be secondary to effects on locomotor activity during the preference test, group differences in the frequency of subject's cage entries were analyzed by a one-way analysis of variance. Although each treatment group originally started with eight to 15 subjects, some were excluded from data analysis because of misplaced cannulae determined by histological verification. Therefore, the number of subjects in each group mentioned in the experimental design (see below) represents the number used for data analysis.

RESULTS

Study 1: do DA and OT interact to regulate partner preference formation?

Previous studies have demonstrated that, in female prairie voles, administration of OTA or the D2-type DA receptor antagonist, eticlopride, blocks mating-induced partner preferences, whereas administration of OT or the D2-type DA receptor agonist, quinpirole, induces this behavior in the absence of mating (Williams et al., 1994; Insel and Hulihan, 1995; Cho et al., 1999; Wang et al., 1999; Gingrich et al., 2000). Although these data demonstrate OT and DA involvement in partner preference formation, it is unknown whether the two interact to regulate social attachment. The purpose of the present study was twofold: to further evaluate previous findings of DA and OT involvement in pair bonding and to examine possible interactions of DA and OT systems in regulation of pair bonding in female prairie voles.

Procedures. In experiment 1a, subjects were implanted with guide cannulae aimed at the lateral ventricle. After 3 days of recovery, subjects were randomly assigned to one of four treatment groups. In group 1 (control), subjects received i.c.v. administration of CSF (200 nl) and i.p. injection of saline (200 μl; $n=7$). In group 2, subjects received administration of CSF and saline containing 5 μg quinpirole ($n=7$). In group 3, subjects received administration of CSF and saline containing 5 μg quinpirole with 50 μg eticlopride ($n=8$). In group 4, subjects received CSF containing 50 ng OTA and saline containing 5 μg quinpirole ($n=8$). These doses (and doses in the following experiments) were chosen because they effectively induced or blocked partner preference formation in female prairie voles in previous studies (Insel and Hulihan, 1995; Wang et al., 1999; Gingrich et al., 2000; Young et al., 2001). Immediately following drug administration, subjects were housed with a male for 6 h in the absence of mating followed by the partner preference test. All subjects then were anesthetized and killed, and cannulation sites in their brains were verified histologically.

In experiment 1b, subjects were implanted with osmotic minipumps aimed at the lateral ventricle. They then

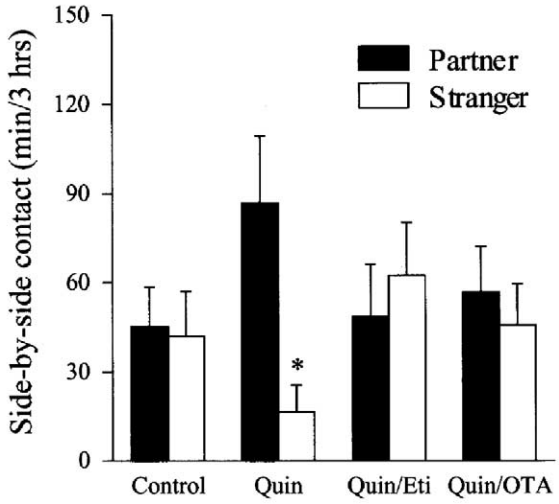

Fig. 1. Effects of the D2-type DA receptor agonist, Quin, on partner preference formation in female prairie voles. Control females did not show partner preferences following 6 h of cohabitation with a male without mating. Females that were injected (i.p.) with 5 μg of the D2-type receptor agonist, Quin, spent significantly more time with the partner versus a stranger. This induced partner preference formation was blocked by concurrent administration of 50 μg of the D2-type receptor antagonist, Eti or an OTA. * $P<0.05$. Error bars=S.E.M.

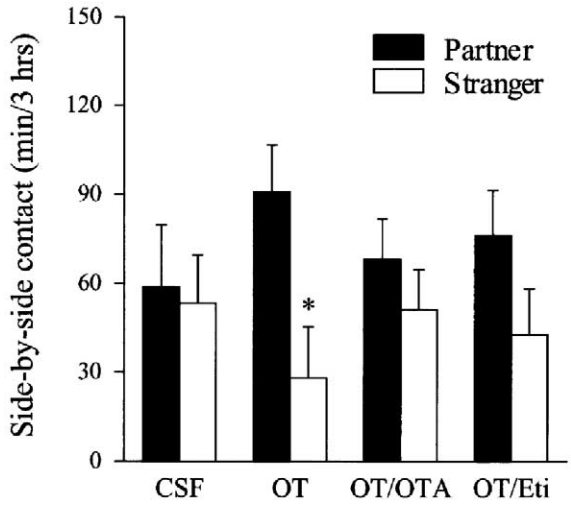

Fig. 2. Effects of central administration of OT on partner preference formation in female prairie voles. All females were implanted with an osmotic minipump aimed at the lateral ventricle. Control females infused with CSF (0.5 μl/h) did not show partner preferences following 6 h of cohabitation with a male without mating. Females infused with OT (1 ng/μl) spent significantly more time with the partner versus a stranger. This OT-induced partner preference was blocked by concurrent administration of an OTA (10 ng/μl) or the D2-type DA receptor antagonist, Eti (10 ng/μl). * $P<0.05$. Error bars=S.E.M.

were randomly assigned to one of four treatment groups that received i.c.v. infusions of either CSF alone (0.5 μl/h; $n=7$), or CSF containing OT (1 ng/μl; $n=9$), OT (1 ng/μl) with OTA (10 ng/μl; $n=11$), or OT (1 ng/μl) with eticlopride (10 ng/μl; $n=8$). After overnight recovery, each subject was housed with a male for 6 h in the absence of mating, followed by the partner preference test. Subjects then were killed and infusion sites were verified.

Results. In experiment 1a, control females exhibited approximately equal side-by-side contact with the partner or the stranger after 6 h of cohabitation (Fig. 1). Females injected with the D2-type receptor agonist, quinpirole, spent significantly more time preferentially with the partner than with the stranger ($t=2.88$, $P<0.05$). This quinpirole-induced behavior was blocked by co-administration of either the D2-type receptor antagonist, eticlopride, or OTA, suggesting that access to both OT and D2-type receptors was necessary for quinpirole induction of partner preference formation.

In experiment 1b, control females did not show partner preferences whereas females infused with OT spent significantly more time with the partner than with the stranger ($t=2.68$, $P<0.05$; Fig. 2). Co-administration of OT with either OTA or the D2-type antagonist, eticlopride, did not induce this behavior, suggesting that blockade of either type of receptor prevented OT-induced partner preference formation. In both experiments, no group differences were found in the frequency of subject's cage entries during the partner preference test (Table 1).

Study 2: is NAcc an important brain area for DA-OT regulation of partner preference formation?

Study 1 demonstrated that OT and DA interact in the regulation of partner preferences in female prairie voles. Previous studies have shown that NAcc contains both OT and DA receptors (Gingrich et al., 1998; Young et al., 2001) and activation of either OT or DA D2-type receptor in NAcc is involved in partner preference formation (Gingrich et al., 2000; Young et al., 2001; Aragona et al., 2003). In the present study, we tested the hypothesis that NAcc is a brain area in which OT and DA interact to regulate partner preferences in female prairie voles.

Procedures. In experiment 2a, female prairie voles were implanted with guide cannulae bilaterally aimed at NAcc. Subjects then were randomly assigned to one of seven treatment groups and received one of the following injections: CSF alone (200 nl per side; $n=8$), CSF containing 0.001 ng ($n=13$), 0.01 ng ($n=7$), 0.1 ng ($n=9$), or 1.0 ng ($n=10$) of quinpirole, or CSF containing 1.0 ng quinpirole with 10.0 ng eticlopride ($n=12$) or with 10.0 ng OTA ($n=12$). In the eighth group ($n=5$), subjects were implanted with guide cannulae bilaterally aimed at the caudate putamen and then injected with 1.0 ng quinpirole.

Abbreviations used in the figures

Cg cingulate cortex
CP caudate putamen
Eti eticlopride
Quin quinpirole

Table 1. Frequency of cage entries during the partner preference test

	Partner-cage	Stranger-cage	Total
Experiment 1a			
Control	45.5±8.1*	47.2±6.3	92.7±13.9
Quin	42.7±7.3	43.2±7.1	85.8±13.6
Quin/Eti	36.8±8.0	41.3±7.9	78.1±15.2
Quin/OTA	28.2±6.6	23.8±2.6	52.0±8.5
Experiment 1b			
Control	25.3±7.7	25.0±5.9	50.3±12.8
OT	30.0±6.5	30.2±5.6	60.3±11.9
OT/OTA	23.4±4.1	20.5±3.0	43.8±7.0
OT/Eti	23.9±5.5	22.3±5.2	46.1±10.4

* Mean±S.E.M.

Immediately after drug administration, all subjects were housed with males for 6 h without mating followed by a partner preference test. Thereafter, subjects were killed and their brains were sectioned to examine cannulation sites.

In experiment 2b, subjects were implanted with guide cannulae bilaterally aimed at NAcc, and were then assigned to one of seven treatment groups. Subjects received one of the following injections: CSF alone (200 nl per side; $n=7$), CSF containing 0.1 ng ($n=9$), 1.0 ng ($n=10$), or 10.0 ng OT ($n=7$), CSF containing 1.0 ng OT with 10.0 ng OTA ($n=7$), with 10.0 ng eticlopride ($n=9$), or with 10.0 ng D1-type receptor antagonist, SCH23390 ($n=11$). In the eighth group ($n=12$), subjects were implanted with guide cannulae aimed at the cingulate cortex and then injected with 1.0 ng OT. After 6 h of cohabitation with a male without mating, subjects were tested for partner preferences followed by histological verification of the cannulation sites. Although data from previous studies (Williams et al., 1994; Insel and Hulihan, 1995) and from our experiment 1b demonstrated that continues infusion of OT using an osmotic minipump reliably induced partner preference formation, this technique is limited to i.c.v. administration. Such manipulation in a particular brain region may cause excess diffusion of the drug outside of the desired target area, leading to a potentially ambiguous result with respect to site specificity. Therefore, acute administration was employed.

Results. Histological verification illustrated that most injection sites were located in the shell of the NAcc (Fig. 3). In experiment 2a, females that received injections of CSF (control) or lower doses of quinpirole (0.001 or 0.01 ng) did not show partner preferences (Fig. 4). However, females that received injections of higher doses of quinpirole showed significantly more side-by-side contact with the partner than with a stranger ($t=2.70$, $P<0.05$ for 0.1 ng quinpirole and $t=3.33$, $P<0.01$ for 1.0 ng quinpirole). Injections of the D2-type receptor antagonist, eticlopride, or OTA into NAcc blocked quinpirole-induced partner preferences. Further, quinpirole's effect on behavior seemed to be site-specific because administration of quinpirole into the caudate putamen did not induce partner preference formation. No treatment effects were found in animals' cage entries during the partner preference test.

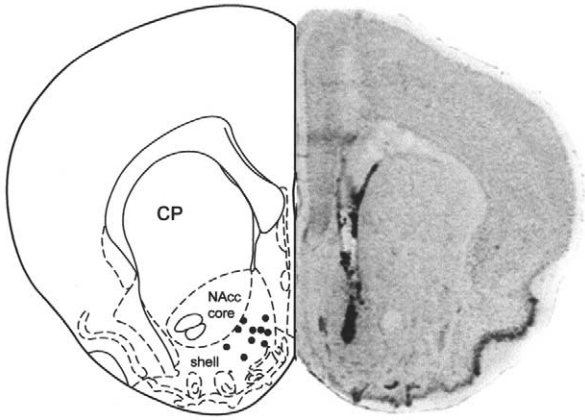

Fig. 3. A schematic illustration (left) showing locations of site-specific microinjections of 1.0 ng of the D2-type agonist, Quin, into the NAcc in experiment 2a, and a representative photomicrograph of vole brain section (right) displaying the site of microinjection into the NAcc.

In experiment 2b, control females and females that received injections of a low dose (0.1 ng) or a high dose (10 ng) of OT in NAcc did not show partner preferences (Fig. 5). In contrast, females that received injections of OT at a 1.0 ng dosage spent significantly more time with the partner than with a stranger ($t=5.39$, $P<0.01$). This behavior was blocked if animals received concurrent administration of OTA or the D2-type DA receptor antagonist, eticlopride. However, co-administration of the D1-type DA receptor antagonist, SCH 23390, did not block OT induced partner preference ($t=3.0$, $P<0.01$). OT administration in the cingulate cortex did not induce partner preferences. No group differences were found in animals' cage entries during the partner preference test.

DISCUSSION

Administration of OT or DA induces partner preference formation in female prairie voles in the absence of mating (Williams et al., 1994; Insel and Hulihan, 1995; Cho et al., 1999; Wang et al., 1999; Gingrich et al., 2000). Results from the present study not only confirmed but also extended these findings demonstrating that access to both OT and DA D2-type receptors is necessary for partner preference formation. We also found that NAcc is a brain area important for OT-DA interaction. In particular, blockade of OT receptors in NAcc blocked partner preferences induced by the D2-type agonist whereas blockade of D2-type, but not D1-type, DA receptors prevented partner preference formation induced by OT. Finally, in both studies drug administration had no significant effects on the animal's locomotor activity during the preference test, indicating that the treatment effect on pair bonding was not an artifact of hyper- or hypo-activity. Together, these results suggest that concurrent activation of OT and D2-type DA receptors in NAcc is essential for pair bond formation in female prairie voles.

A large body of literature has demonstrated behaviorally relevant interactions between neuromodulator (such as OT) and neurotransmitter (such as DA) systems.

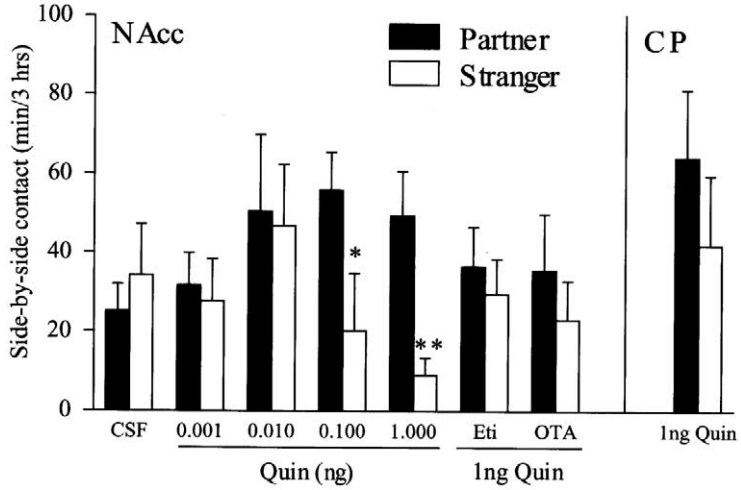

Fig. 4. Effects of the D2-type DA receptor agonist, Quin, in the NAcc on partner preference formation in female prairie voles. Control females injected with CSF and females injected with low doses (0.001 or 0.01ng) of the D2-type receptor agonist, Quin, in NAcc did not show partner preferences. Females injected with 0.1 ng or 1.0 ng of Quin in NAcc spent significantly more time with the partner versus a stranger. This induced partner preference was blocked by concurrent administration of the D2-type receptor antagonist, Eti (10.0 ng) or an OTA (10.0 ng). Site-specific administration of 1 ng Quin in the CP did not induce partner preferences. * $P<0.05$ and ** $P<0.01$. Error bars=S.E.M.

OT–DA interactions have been implicated in several behaviors including yawning, penile erections and mating (Drago et al., 1986; Argiolas et al., 1988, 1989; Kovacs et al., 1990), and such interactions in NAcc, in particular, are found to regulate grooming (Drago et al., 1986) and cocaine-induced locomotor hyperactivity (Kovacs et al., 1990). Here, for the first time, we show evidence that OT–DA interactions are also important for complex social interactions, specifically the formation of social bonds for monogamous mammals. We found that local administration of a D2-type agonist or OT in NAcc induced partner preference formation in a dose- and region-specific manner, supporting the notion that NAcc is a brain area important for DA and OT regulation of pair bonding (Gingrich et al., 2000; Young et al., 2001; Aragona et al., 2003). Further, administration of OTA in NAcc blocked partner preferences induced by the D2-type agonist whereas administration of the D2-type, but not D1-type, antagonist blocked the same behavior induced by OT. These data suggest that concurrent access to both OT and D2-type DA receptors in NAcc is necessary for partner preference formation. In support of this finding, neurochemical studies have shown that, in prairie voles, NAcc is enriched with DA terminals (Aragona et al., 2003) and D2-type receptors (Gingrich et al., 1998), and contains dense labeling of OT receptors (Young et al., 2001). Importantly, the effects of the drug treatment appeared to be specific to partner preferences and were not the secondary effect from altered

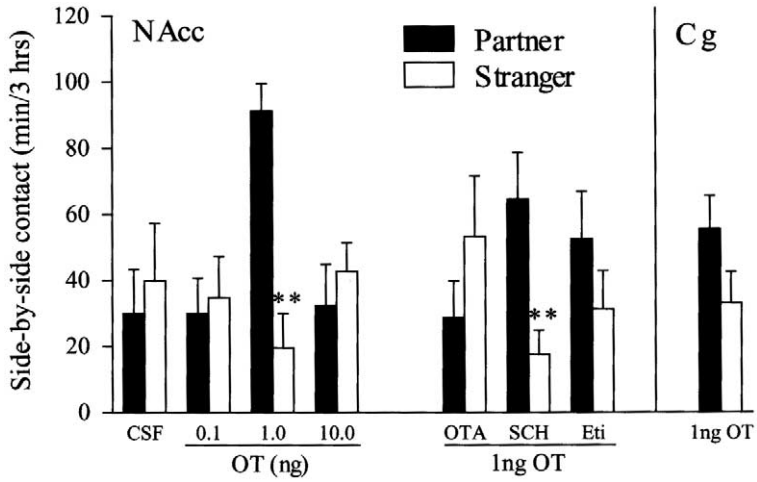

Fig. 5. Effects of OT administration in the NAcc on partner preference formation in female prairie voles. Control females injected with CSF and females injected with low (0.1 ng) or high (10.0 ng) dose of OT in NAcc did not show partner preferences. Females injected with 1.0 ng OT in NAcc spent significantly more time with the partner versus a stranger. This OT-induced partner preference was blocked by concurrent administration of 10.0 ng of the OTA or the D2-type DA receptor antagonist, Eti, but not the D1-type DA receptor antagonist, SCH23390 (SCH). Administration of 1 ng OT in the Cg did not induce partner preferences. ** $P<0.01$. Error bars=S.E.M.

locomotor activity as group differences were not found in animals' cage entries.

DA release in NAcc is generally believed to be reinforcing and involved in learning and memory (Ikemoto and Panksepp, 1999). In prairie voles, DA is released in NAcc upon mating (Gingrich et al., 2000) and released DA may act to facilitate pair bond formation (Aragona et al., 2003). However, mating induces DA release in NAcc also in other species of rodents that do not form pair bonds (Mermelstein and Becker, 1995; Pfaus et al., 1995). In addition, mating induces DA release (albeit in dorsal striatum) in a similar magnitude in both monogamous and non-monogamous voles (Curtis et al., 2003). Therefore, it is likely that species-specific mating strategy and social behavior are not the results of differences in mating-associated DA release. Instead, it may be due to species differences in OT systems and/or in OT-DA interactions. This is partially supported by the finding of a species-specific distribution pattern of OT receptors in voles: OT receptors are present in high densities in NAcc of monogamous prairie voles, but are almost absent in NAcc of promiscuous montane voles (*M. montanus*; Insel and Shapiro, 1992; Young et al., 2001) that do not form pair bonds (Insel et al., 1995).

How do OT and DA interact in regulation of pair bonding? One possible mechanism is that the two act sequentially. For example, OT is found to act downstream from DA receptors to regulate penile erection and yawning (Argiolas et al., 1988; Melis et al., 1989), whereas DA acts downstream from OT receptors to regulate grooming (Drago et al., 1986; Stivers et al., 1988). In the present study, blockade of either type of receptor prevented partner preference formation induced by either neurochemical, and thus it is unlikely that OT and DA act sequentially to regulate pair bonding. Another possibility is that the two systems interact and concurrent activation of both receptors is essential for pair bond formation. This notion is supported by the findings that DA administration induces OT release (Melis et al., 1989) whereas OT administration increases DA levels in rats (Pfister and Muir, 1989), and blockade of either OT or D2-type DA receptors blocks partner preferences in voles (Gingrich et al., 2000; Young et al., 2001; present study). OT–DA interactions are mediated by the specific types of DA receptors. For example, D2-type receptors are responsible for DA–OT regulation of yawning and penile erection (Argiolas et al., 1987; Melis et al., 1990; Sarnyai and Kovacs, 1993). In contrast, DA interacts with OT via a D1-type, but not D2-type, receptor-mediated mechanism to regulate grooming (Drago et al., 1999). Here we demonstrate that D2-type DA receptors are important for OT-DA interactions in the regulation of pair bonding. It is worthy to note that the D2-type agonist, quinpirole, and antagonist, eticlopride, used in the present study have been shown to act on all D2-type receptors, including D2, D3, and D4 receptors (Missale et al., 1998). Therefore, it will be interesting to further identify the specific subtype of D2 receptors that interacts with OT in the regulation of pair bonding.

It is also possible that DA and OT act on different aspects of the behavior; DA may act on the formation whereas OT may act on the expression of partner preferences. Indeed, the D2-type DA receptors are found to be important for the formation, but not expression, of partner preferences in female prairie voles (Wang et al., 1999) whereas OT is required for the expression of social recognition (Ferguson et al., 2000; Young et al., 2001). However, we cannot exclude the possibility that OT may also regulate pair bond formation by facilitating an association between rewarding NAcc activation and the male (Young et al., 2001).

As to the cellular mechanisms by which DA and OT could interact in the regulation of pair bonding, we can only speculate at present. Administration of OT or DA in NAcc induces pair bonding, mimicking the effects of mating. Therefore, a possible explanation is that repeated mating between male and female prairie voles induces increased DA release and turnover in NAcc (Gingrich et al., 2000; Aragona et al., 2003). Mating may also increase OT release in voles as it facilitates OT release in the medial preoptic area in female rats (Jirikowski, 1992). Released DA and OT then act in concert to regulate pair bonding. It has been suggested that the DA receptor activation increases OT release and axonal transport (Melis et al., 1989, 1990). On the other hand, OT may increase DA levels (Pfister and Muir, 1989), exert a region-specific action on the utilization of DA (Kovacs et al., 1990), modulate the pre-synaptic process of DA neurotransmission (Sarnyai, 1998), or alter DA receptors (Kovacs et al., 1986).

OT and DA induce yawning by activating nitric oxide synthesis and by interacting with several other neurochemical systems (Argiolas and Melis, 1998). Thus, it would not be surprising to see complex social behaviors, such as pair bonding, under the control of several neurotransmitters. Studies have shown that DA, OT, vasopressin, and glucocorticoids all are involved in the regulation of pair bonding (De Vries et al., 1995; Insel and Hulihan, 1995; Gingrich et al., 1998; Cho et al., 1999). Instead of operating on separate systems, it is possible that these neurochemicals interact and share some common mechanisms in regulation of behavior (Cho et al., 1999; Liu et al., 2001). Studies have also shown that NAcc is an important area in brain circuits implicated in DA mediation of reward learning (Wise, 1996; Berridge and Robinson, 1998; Waelti et al., 2001), and OT–DA interactions in NAcc may be among the mechanisms modulating neuroadaptation to other rewards, such as drugs of abuse (Sarnyai, 1998). Our data from the present study demonstrate that OT and DA interact in NAcc to regulate pair bond formation in the monogamous prairie vole. Therefore, these data support the contention that neural pathways and biochemical mechanisms implicated in drug addiction likely also mediate reinforcement produced by natural rewards (Wise, 1996; Nesse and Berridge, 1997; Young et al., 2001; Kelley and Berridge, 2002; Aragona et al., 2003).

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REFERENCES

Aragona BJ, Liu Y, Curtis TJ, Stephan FK, Wang ZX (2003) A critical role for nucleus accumbens dopamine in partner preference formation of male prairie voles. J Neurosci 23:3483–3490.

Argiolas A, Melis MR (1998) The neuropharmacology of yawning. Eur J Pharmacol 343:1–16.

Argiolas A, Melis MR, Gessa GL (1988) Yawning and penile erection: central dopamine-oxytocin-adrenorticotropin connection. Ann NY Sci 525:330–337.

Argiolas A, Melis MR, Mauri A, Gessa GL (1987) Paraventricular nucleus lesion prevents yawning and penile erection induced by apomorphine and oxytocin but not by ACTH in rats. Brain Res 421:349–352.

Argiolas A, Collu M, D'Aquila P, Gessa GL, Melis MR, Serra G (1989) Apomorphine stimulation of male copulatory behavior is prevented by the oxytocin antagonist $d(CH_2)_5Tyr(Me)$-Orn^8-Vasotocin in rats. Pharm Biochem Behav 33:81–83.

Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Rev 28:309–369.

Carter CS, Getz LL (1993) Monogamy and the prairie vole. Sci Am 268:100–106.

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

Curtis TJ, Stowe JR, Wang ZX (2003) Differential effects of intraspecific interactions on the striatal dopamine system in social and non-social voles. Neuroscience 118:1165–1173.

De Vries AC, De Vries MB, Taymans SE, Carter CS (1995) Modulation of pair bonding in female prairie voles (*Microtus ochrogaster*) by corticosterone. Proc Natl Acad Sci USA 92:7744–7748.

Dewsbury DA (1987) The comparative psychology of monogamy. Nebraska Symp Motivation 35:1–50.

Di Chiara G (1999) Drug addiction as dopamine-dependent associative learning disorder. Eur J Pharmacol 375:13–30.

Drago F, Contarino A, Busa L (1999) The expression of neuropeptide-induced excessive grooming behavior in dopamine D1 and D2 receptor-deficient mice. Eur J Pharmacol 365:125–131.

Drago F, Caldwell JD, Pedersen CA, Continella G, Scapagnini U, Prange AJ (1986) Dopamine neurotransmission in the nucleus accumbens may be involved in oxytocin-enhanced grooming behavior of the rat. Pharmacol Biochem Behav 24:1185–1188.

Ferguson JN, Young LJ, Hearn EF, Insel TR, Winslow JT (2000) Social amnesia in mice lacking the oxytocin gene. Nat Genet 25: 284–288.

Getz LL, Hofmann JE (1986) Social organizations in free-living prairie voles, *Microtus ochrogaster*. Behav Ecol Sociobiol 18:275–282.

Getz LL, Carter CS (1996) Prairie-vole partnerships. Am Sci 84:56–62.

Getz LL, Carter CS, Gavish L (1981) The mating system of the prairie vole, *Microtus ochrogaster*: field and laboratory evidence for pair-bonding. Behav Ecol Sociobiol 8:189–194.

Gingrich B, Liu Y, Cascio C, Wang ZX, Insel TR (2000) Dopamine D2 receptors in the nucleus accumbens are important for social attachment in female prairie voles. Behav Neurosci 114:173–183.

Gingrich B, Cascio C, Young LJ, Liu Y, Wang ZX, Insel TR (1998) Oxytocin, dopamine, and enkephalin in the nucleus accumbens: a neurochemical cascade for pair bonding. Soc Neurosci Abs 24: 1072.

Ikemoto S, Panksepp J (1999) The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. Brain Res Rev 31:6–41.

Insel TR, Shapiro LE (1992) Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. Proc Natl Acad Sci USA 89:5981–5985.

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

Insel TR, Preston S, Winslow JT (1995) Mating in the monogamous male: behavioral consequences. Physiol Behav 57:615–627.

Jirikowski GF (1992) Oxytocinergic neuronal systems during mating, pregnancy, parturition, and lactation. Ann NY Acad Sci 652:253–270.

Kelley AE, Berridge KC (2002) The neuroscience of natural rewards: relevance to addictive drugs. J Neurosci 22:3306–3311.

Kovacs GL, Faludi M, Falkay G, Telegdy G (1986) Peripheral oxytocin treatment modulates central dopamine transmission in the mouse limbic structures. Neurochem Int 9:481–485.

Kovacs GL, Sarnyai Z, Babarczi E, Szabo G, Telegdy G (1990) The role of oxytocin-dopamine interactions in cocaine-induced locomotor hyperactivity. Neuropharmacology 29:365–368.

Liu Y, Curtis JT, Wang ZX (2001) Vasopressin in the lateral septum regulates pair bond formation in male prairie voles (*Microtus ochrogaster*). Behav Neurosci 115:910–919.

Melis MR, Argiolas A, Gessa GL (1989) Evidence that apomorphine induces penile erection and yawning by releasing oxytocin in the central nervous system. Eur J Pharmacol 164:565–570.

Melis MR, Argiolas A, Stancampiano R, Gessa GL (1990) Effects of apomorphine on oxytocin concentrations in different brain areas and plasma of male rats. Eur J Pharmacol 182:101–107.

Mermelstein PG, Becker JB (1995) Increased extracellular dopamine in the nucleus accumbens and striatum of the female rat during paced copulatory behavior. Behav Neurosci 109:354–365.

Missale C, Nash SR, Robinson SW, Jaber M, Caron MG (1998) Dopamine receptors: from structure to function. Physiol Rev 78: 189–225.

Nesse RM, Berridge KC (1997) Psychoactive drug use in evolutionary perspective. Science 278:63–66.

Pfaus JG, Damsma G, Wenkstern D, Fibiger HC (1995) Sexual activity increases dopamine transmission in the nucleus accumbens and striatum of female rats. Brain Res 693:21–30.

Pfister HP, Muir JL (1989) Influence of exogenously administered oxytocin on central noradrenaline, dopamine and serotonin levels following psychological stress in nulliparous female rats (*Rattus norvegicus*). Intern J Neurosci 45:221–229.

Robbins TW, Everitt BJ (1996) Neurobehavioural mechanisms of reward and motivation. Curr Opin Neurobiol 6:228–236.

Sarnyai Z (1998) Oxytocin and neuroadaptation to cocaine. Prog Brain Res 119:449–466.

Sarnyai Z, Kovacs GL (1993) Role of oxytocin in the neuroadaptation to drugs of abuse. Psychoneuroendocrinology 19:85–117.

Stivers JA, Kaltwasser MT, Hill PS, Hruby VJ, Crawley JN (1988) Ventral tegmental oxytocin induces grooming. Peptides 9 (Suppl 1):223–231.

Waelti P, Dickinson A, Schultz W (2001) Dopamine responses comply with basic assumptions of formal learning theory. Nature 412:43–48.

Wang ZX, Yu GZ, Cascio C, Liu Y, Gingrich B, Insel TR (1999) Dopamine D2 receptor-mediated regulation of partner preferences in female prairie voles: a mechanism for pair bonding. Behav Neurosci 113:602–611.

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

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

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

Wise RA (1996) Neurobiology of addiction. Curr Opin Neurobiol 6:243–251.

Witt DM, Carter CS, Insel TR (1991) Oxytocin receptor binding in female prairie voles: endogenous and exogenous oestradiol stimulation. J Neuroendocrinol 3:155–161.

Young LJ, Lim MM, Gingrich B, Insel TR (2001) Cellular mechanisms of social attachment. Horm Behav 40:133–138.

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