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Dopamine D_1 Receptor Stimulation of the Nucleus Accumbens or the Medial Preoptic Area Promotes the Onset of Maternal Behavior in Pregnancy-Terminated Rats

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There is good evidence that interference with the mesolimbic dopamine (DA) system results in impaired maternal responding in postpartum female rats. However, whether activation of the mesolimbic DA system is capable of promoting maternal behavior has not been investigated. This study examined whether increasing DA activity in various brain regions of pregnancy-terminated, naive female rats would stimulate the onset of maternal behavior. Experiments 1 and 2 examined the effects of microinjection of various doses (0, 0.2, or 0.5 μg/0.5 μl/side) of a D_1 DA receptor agonist, SKF 38393, or a D_2 DA receptor agonist, quinpirole, into the nucleus accumbens (NA) on latency to show full maternal behavior, and Experiment 3 determined the effects of SKF 38393 injection into a control site. Finally, because the medial preoptic area (MPOA) is also important for maternal behavior, receives DA input, and expresses DA receptors, the authors examined whether microinjection of SKF 38393 into MPOA was capable of stimulating the onset of maternal behavior. Results indicated that microinjection of SKF 38393 into either the NA or the MPOA facilitates maternal responding in pregnancy-terminated rats.

Keywords: dopamine, maternal behavior, nucleus accumbens, preoptic area

The hormonal events of pregnancy and parturition allow the postpartum female rat to show immediate maternal responsiveness in the presence of offspring (Numan & Insel, 2003). However, maternal behavior can also be induced in naive virgin female rats after 6–8 days of repeated exposure to pups during a process termed *sensitization* (Fleming & Rosenblatt, 1974; Numan & Insel, 2003; Rosenblatt, 1967). One interpretation of sensitization is that exposure to pregnancy hormones is not essential for maternal behavior to occur; however, as a consequence of hormonal stimulation, less exposure to pup stimuli is needed before appropriate maternal responses are initiated.

The medial preoptic area (MPOA) of the hypothalamus is one neural site where hormones act to promote the parturient female's responsiveness to pup-related stimuli: Both estradiol (Numan, Rosenblatt, & Komisaruk, 1977) and prolactin/placental lactogens (Bridges, Numan, Ronsheim, Mann, & Lupin, 1990; Bridges et al., 1997) have been shown to act on the MPOA to stimulate the onset of maternal behavior.

What neural processes are modified as a consequence of hormone action on the MPOA so that maternal responsiveness is facilitated? Fahrbach, Morrell, and Pfaff (1986) have shown that estradiol-binding neurons in the MPOA project to the ventral tegmental area (VTA), which is the origin of the mesolimbic dopamine (DA) pathway. One hypothesis is that hormone action on the MPOA allows pup stimuli to activate MPOA efferents to the VTA, in this way stimulating dopaminergic input to the nucleus accumbens (NA), with such input promoting the female's responsiveness to pups (Numan, 2006). It is possible that estrogenic stimulation of MPOA efferents to VTA is partially mediated by oxytocin. Pederson, Caldwell, Walker, Ayers, and Mason (1994) showed that oxytocin acts on the MPOA to stimulate maternal behavior, and other work indicates that estradiol can induce oxytocin receptor expression in MPOA (Champagne, Diorio, Sharma, & Meaney, 2001). Therefore, one aspect of the mechanism underlying estrogen stimulation of MPOA efferents may involve estrogen induction of oxytocin receptor expression, and this, coupled with prolactin stimulation of the MPOA, could result in increased activity of MPOA efferents to the VTA.

In support of the hypothesis that hormone action on the MPOA stimulates maternal behavior via efferent output to the VTA, there is ample evidence that both MPOA projections to the VTA and DA action at the level of the NA are part of the neural circuitry that underlies maternal responsiveness. With respect to the former, using an asymmetrical design, Numan and Smith (1984) showed that when a unilateral MPOA lesion (which alone does not disrupt maternal behavior) is paired with unilateral damage to the VTA on the contralateral side of the brain, but not the ipsilateral side, ongoing maternal behavior is disrupted in lactating female rats. In addition, the occurrence of maternal behavior is associated with Fos expression in the MPOA region, and many of these Fos-expressing neurons project to the VTA (Numan & Numan, 1996,

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1997). It is also worth noting that during maternal behavior, the expression of Fos in the MPOA occurs in neurons that also contain estradiol receptors (Lonstein, Greco, De Vries, Stern, & Blaustein, 2000). Finally, during maternal behavior, Fos is also expressed in the shell region of NA (Lonstein, Simmons, Swann, & Stern, 1998; Stack, Balakrishnan, Numan, & Numan, 2002). Of note, if the MPOA is lesioned with an excitotoxic amino acid on one side of the brain only, Fos expression is eliminated in the NA ipsilateral, but not contralateral, to the MPOA lesion (Stack et al., 2002). These results suggest that activity in the MPOA is functionally linked to NA Fos expression during maternal behavior.

Mesolimbic DA projections from the VTA to the NA have been hypothesized to modulate the impact of sensory inputs on behavior such that the ability of biologically significant stimuli to elicit appropriate and adaptive responses is facilitated (Berridge & Robinson, 1998; Horvitz, 2002; Kelley & Berridge, 2002). Not surprisingly, a substantial amount of evidence indicates that the mesolimbic DA system plays an important role in the regulation of maternal responsiveness: DA depletions by 6-hydroxydopamine lesions of either the VTA or the NA impair both the onset and the maintenance of maternal behavior (Hansen, Harthon, Wallin, Lofberg, & Svensson, 1991a, 1991b), and mother–pup interactions are associated with increased DA release into NA (Champagne et al., 2004; Hansen, Bergvall, & Nyiredi, 1993). Blockade of DA receptors via systemic administration of DA antagonists causes impairments in both the onset and the maintenance of maternal behavior (Byrnes, Rigero, & Bridges, 2002; Li, Davidson, Budin, Kapur, & Fleming, 2004; Pereira & Ferreira, 2006; Silva, Bernardi, & Felicio, 2001), which can be reversed with concurrent DA agonist treatment (Giordano, Johnson, & Rosenblatt, 1990). Furthermore, intra-accumbens infusions of DA antagonists disrupt maternal behavior in postpartum rats (Keer & Stern, 1999; Silva, Bernardi, Cruz-Casallas, & Felicio, 2003), and this effect appears to be selectively mediated by D_1 DA receptors (Numan, Numan, Pliakou, et al., 2005).

The above studies demonstrate that interference with mesolimbic DA activity at the level of the NA disrupts maternal behavior. However, it has not been investigated whether increased DA activity in NA can stimulate the onset of maternal behavior in females that would not normally show such behavior. The present series of experiments was designed to examine this possibility. We chose a 15-day hysterectomy and ovariectomy (15HO) pregnancy-termination model in order to partially prime naive females for maternal behavior. As established in our laboratory and others, artificial termination of pregnancy via hysterectomy (removal of the fetus and uterus) and ovariectomy on Day 15 produces a moderate facilitation of maternal behavior: 15HO females require approximately 2–3 days of pup exposure before they show complete maternal behavior (Rosenblatt, Olufowobi, & Siegel, 1998). Note that this latency, although not immediate, is substantially shorter than the 6–8-day sensitization latencies shown by virgin females (Rosenblatt, 1967). Most important, if 15HO females are systemically treated with estradiol, or if estradiol is applied locally to the MPOA, they show maternal behavior immediately upon pup presentation (0-day latency; Numan et al., 1977; Siegel & Rosenblatt, 1978).

The goal of our study was to determine whether microinjection of a DA agonist into the NA could substitute for estradiol administration and result in 15HO females displaying almost immediate maternal behavior. We hypothesized that agonists at the D_1 DA receptor would facilitate the onset of maternal behavior in 15HO females because our previous work in postpartum animals showed that D_1, but not D_2, DA receptor antagonists disrupted maternal behavior when injected into NA (Numan, Numan, Pliakou, et al., 2005). On the basis of the evidence supporting the view that estradiol action on the MPOA stimulates maternal behavior, in part, by activating the mesolimbic DA system, we rationalized that the addition of DA agonists to the NA might substitute for a lower level of estradiol priming of MPOA efferents in 15HO females (estradiol is secreted by the ovary prior to Day 15 of pregnancy, and so the MPOA and other regions would have been exposed to estradiol prior to hysterectomy and ovariectomy; Bridges, 1984).

The partially primed 15HO female model was used to test our hypothesis because it is not likely that DA action on D_1 receptors in NA, acting alone, would stimulate maternal behavior in naive virgin females who normally show long sensitization latencies. As reviewed by Numan (2006), multiple neural systems are affected by pregnancy hormones to stimulate the immediate onset of maternal behavior in parturient females, which includes an up-regulation of neural systems that promote maternal responsiveness and a down-regulation of neural systems that inhibit maternal behavior. The mesolimbic DA system is conceived of as a component of a facilitatory neural system, whereas medial amygdala projections to the caudal anterior hypothalamic region are viewed as components of the inhibitory system (see Numan, 2006). By partially priming females, it was hoped that the inhibitory system would be down-regulated sufficiently to allow an experimentally induced up-regulation of DA activity at D_1 receptors in NA to promote full maternal behavior.

Although our hypothesis is that stimulation of D_1 DA receptors in NA would promote the onset of maternal behavior in 15HO females, the current study also examined whether increased D_1 DA receptor stimulation in MPOA would promote maternal behavior. This possibility was investigated in light of the following evidence: (a) The MPOA is critical for the onset and the maintenance of maternal behavior in rats (Numan & Insel, 2003); (b) the MPOA receives DA input from diencephalic sources (Simerly, Gorski, & Swanson, 1986) and expresses both D_1 and D_2 receptors (Bakowska & Morrell, 1995); (c) DA activity in the MPOA fluctuates throughout pregnancy and lactation (Lonstein, Dominguez, Putnam, De Vries, & Hull, 2003; Olazabel, Abercrombie, Rosenblatt, & Morrell, 2004); and (d) D_1 antagonists injected directly into the MPOA have been found to disrupt maternal behavior in postpartum rats, and the nature of the disruption is similar to that observed after D_1 antagonist injection into NA (Miller & Lonstein, 2005).

General Method

Subjects and Housing

Experimental subjects were nulliparous female rats of the Charles River CD strain (Charles River, Wilmington, MA). Each female, 70–100 days of age, was mated with a male of the same strain. The day of mating was considered Day 0 of pregnancy. On the following morning, Day 1, females were transferred to clear polycarbonate cages (20 × 45 × 20 cm) that contained wood shavings as bedding material. On Day 14 of pregnancy, females were transferred to clear polycarbonate observation cages (50 ×

40 × 20 cm), which also contained wood shavings for bedding material. The floors of these observation cages were divided into four equal compartments by 5-cm-high Plexiglas dividers. These barriers served to prevent pups from crawling from one quadrant to another. All subjects were maintained under a 12-hr reversed light–dark cycle (lights off at 0600), and food and water were freely available. Behavioral observations occurred during the dark phase, and test rooms were illuminated with dim red light.

An additional group of female rats served as donor mothers that provided test pups for the experimental females. These Charles River CD females were mated and allowed to give birth naturally in polycarbonate cages (20 × 45 × 20 cm) containing wood shavings. These females and their litters were housed in a different room than the experimental females.

Surgery

Cannula implantation was performed on Day 1 of pregnancy. This stereotaxic surgery was carried out while the rats were under Nembutal anesthesia (50 mg/kg ip) and atropine sulfate pretreatment (0.2 mg/kg ip). Following surgery, females were injected with penicillin (50,000 units/kg sc) and were placed under a warming lamp to recover. Bilateral 22-gauge stainless steel guide cannulas (Plastics One, Roanoke, VA) were implanted into either NA, dorsal striatum (DS), or MPOA. The DeGroot (1959) stereotaxic atlas was used, and interaural zero served as the reference point. Coordinates for NA, MPOA, and DS guide cannula implants were, respectively: A 9.5, L ±1.0, V 7.0; A 7.5, L ±0.75, V 5.8; and A 8.6, L ±2.5, V 8.0. The implanted cannulas were cemented to the skull with dental acrylic and were occluded with stainless steel stylets that extended 2 mm beyond the end of the guide.

On Day 15 of pregnancy each female was hysterectomized and ovariectomized; the operations were performed by dorsolateral and ventral incisions with the animal under Nembutal anesthesia.

Intracranial Injection Procedures

To acclimate females to the intracranial drug administration procedure that would begin 48 hr after hysterectomy and ovariectomy, the following procedure was performed at 24 hr after hysterectomy and ovariectomy: Females were taken to a room outside of the maternal behavior testing room, and the experimenter, while holding the rat, removed and replaced the inner stylet that kept the guide cannula occluded.

To perform intracranial injections, the experimenter removed the inner stylet from the guide cannula of an awake, handheld rat and replaced it with a 28-gauge injector cannula that extended 2 mm beyond the end of the guide. The injector cannula was attached to a Hamilton microliter syringe with polyethylene tubing, and various doses of a DA agonist were injected into either the NA, the DS, or the MPOA over a 60-s interval with the aid of a Sage syringe pump (Model 341A, Orion Research, Inc., Cambridge, MA). The injector remained in place for an additional 45 s, at which time it was removed and the procedure was repeated on the other side of the brain. The injections were performed in a room outside of the maternal behavior testing room.

Maternal Behavior Measurements and Observations

Pup presentation and behavioral observations occurred between 1000 and 1100 on each test day and began 48 hr after hysterectomy and ovariectomy, deemed Day 0 of testing. On the days that females received intracranial injections (Days 0, 1, and 2 of testing), pup presentation occurred 20 min postinjection. Three stimulus pups aged 2–7 days, provided by donor mothers, were placed throughout the cage, one in each quadrant that the subject had not used as a sleeping quadrant. Care was taken so that younger pups were presented earlier in the testing period, whereas older pups were presented later. During the first hour of pup exposure, subjects were continuously observed for the first 15 min, spot-checked at 30 min, and observed continuously again for the last 15 min of the hour observation. Latency to sniff and approach pups was recorded. Females that did not sniff the pups by the end of the first 15-min observation were manually moved by the experimenter so that they would make snout contact with a pup (forced sniff), and such females were assigned a sniff latency of 15 min. During the 1-hr observation, the location of the female and the pups was noted, and the occurrence of retrieval responses, nursing behavior (crouching), pup grooming and licking, and infanticide were recorded.

On the first day of behavioral observations, the three stimulus pups were placed one at a time in the female's cage. At the start of the observation period, one pup was placed in the quadrant farthest away from the female's sleeping quadrant; at 15 min, a second pup was placed in a separate quadrant, also not the female's sleeping quadrant; and at the 30-min spot check, the third pup was placed. If the female attacked any of the pups on the first test day, all pups were removed and this process was repeated on Day 1 of testing. Subjects that attacked pups on 2 consecutive days were excluded from the experiment. (Across the various experiments, approximately 10–15% of females were removed from the study because of infanticide. The various groups did not differ in the frequency of this behavior.) Females were subsequently given all three freshly nourished pups at the start of behavioral observation on the remainder of the test days. Approximately 5 hr following pup presentation, subjects were spot-checked, and the locations of the female and the pups were recorded. To be considered fully maternal in a given test day, subjects had to retrieve all pups to a single location, lick and groom pups, and adopt a nursing posture over the grouped litter by the end of the 5-hr observation. At the 5-hr observation, if a female retrieved and grouped pups, she was observed continuously for 20 min to determine whether nursing behavior and pup grooming occurred. Behaviors observed during this 20 min were considered to have occurred on that test day.

The following morning, the location of the subject and the pups was noted, and the pups were removed and replaced with three freshly nourished pups (20 min postinjection). This procedure was repeated until subjects showed complete maternal behavior during the 1-hr a.m. observation for 2 consecutive mornings or until 5 days had elapsed. If complete maternal behavior occurred for 2 consecutive days during drug injections, an additional test was given without drug injection in order to determine whether or not the maintenance of the behavior was dependent on continued drug administration (as it turned out, all females that initiated maternal behavior while receiving intracranial injections maintained such behavior afterward).

The latency to show maternal behavior was taken as the number of days of pup contact preceding the first day on which full maternal behavior was displayed. Subjects that did not show maternal behavior during Test Days 0–5 were assigned latencies

of Day 6 for statistical purposes. If a subject showed maternal behavior for the first time on Day 5 of testing, the subject was tested again on Day 6 to ensure 2 consecutive days of complete maternal behavior. On the basis of our observations we were able to compute two main latency measures in each female: (a) latency to show full maternal behavior during the first hour of pup exposure (hour latency) and (b) latency to show full maternal behavior by the end of the test day (day latency).

Histology

Females were perfused with saline followed by formalin while under deep anesthesia. Brain sections 40 μm thick, cut on a freezing microtome, were stained with cresyl violet. Microscopic examination mapped the location of the cannula injection sites. Females with excessive brain damage or misplaced cannula were removed from the study.

Statistics

For all analyses, a minimum significance level of $p = .05$ was used. Ordinal scale data were analyzed with nonparametric statistics. The Mann–Whitney *U* test was used to analyze whether median sensitization latencies differed among the independent treatment groups. When more than two independent groups were analyzed, the Kruskal–Wallis test was used first, and if an overall significant difference was detected, then Mann–Whitney *U* tests were performed for multiple comparisons. For the maternal behavior latency measure, if an overall difference between groups was not significant by the Kruskal–Wallis test, we performed the following planned comparisons with the Mann–Whitney *U* test: Each drug treatment group was compared with the vehicle-injected control. The Fisher exact probability test was used for frequency data, and the same planned comparisons described above were analyzed. Interval scale data were analyzed with *t* tests or with analysis of variance (ANOVA) followed by the Newman–Keuls test. Interval scale data were analyzed with nonparametric statistics rather than ANOVA in cases in which there was a lack of homogeneity of variance across multiple groups.

Method for Experiment 1

The purpose of Experiment 1 was to determine the effects of bilateral injections of various doses of SKF 38393 (a D_1 DA receptor agonist obtained from Sigma Chemical, St. Louis, MO) into the NA on the sensitization latencies of 15HO female rats. This experiment was composed of three independent groups receiving either 0 μg (vehicle; $n = 12$), 0.2 μg (D_1-low; $n = 13$), or 0.5 μg (D_1-high; $n = 10$) of SKF 38393 into the NA. Each dose of SKF 38393 was dissolved in 0.5 μl of sterile H_2O and injected bilaterally on Days 0, 1, and 2 of behavioral testing. The doses of SKF 38393 were in the range used by other investigators (Ikemoto, Glazier, Murphy, & McBride, 1997).

For this experiment and all subsequent experiments, the number of subjects indicated for each group represents the number of subjects that were retained in the study. In Experiment 1, 2 subjects were removed because of inaccurate cannula placement or excessive brain damage, both of which were in the vehicle group.

Method for Experiment 2

Because Experiment 1 uncovered a facilitation effect of intra-accumbens SKF 38393 on maternal behavior, this experiment investigated the chemical specificity of those results. The effects of bilateral injections of quinpirole (a D_2 DA receptor agonist obtained from Sigma Chemical) on the sensitization latencies of 15HO female rats were examined. The methods for Experiment 2 were identical to those of Experiment 1 except that subjects received either 0 μg (vehicle; $n = 15$), 0.2 μg (D_2-low; $n = 10$), or 0.5 μg (D_2-high; $n = 12$) per 0.5 μl per side of quinpirole dissolved in sterile H_2O. Because quinpirole has a molecular weight close to that of SKF 38393 (256 and 292, respectively), the molar doses of these two drugs were approximately equivalent across the two experiments.

Method for Experiment 3

The purpose of Experiment 3 was to determine the anatomical specificity of the SKF 38393 effect found in the NA by examining the effects of SKF 38393 administration to the DS on maternal behavior. Over Days 0–2 of behavioral testing, 15HO female rats received bilateral microinjection of either SKF 38393 (0.5 μg/0.5 μl sterile H_2O per side) or sterile H_2O (0.5 μl/side) into DS (D_1-DS [$n = 8$] and vehicle-DS [$n = 8$] groups, respectively).

In Experiment 3, 1 subject was removed from the vehicle group because of excessive brain damage.

Method for Experiment 4

The purpose of Experiment 4 was to determine the effects of SKF 38393 microinjection into the MPOA of 15HO females on maternal behavior. In addition, we attempted to replicate the facilitatory effects of intra-accumbens SKF 38393 injection on the onset of maternal behavior, as was determined in Experiment 1. Four groups were formed according to the injections received on Days 0–2 of testing: D_1-MPOA ($n = 10$): 0.5 μg SKF 38393/0.5 μl sterile H_2O per side; vehicle-MPOA ($n = 10$): 0.5 μl sterile H_2O per side; D_1-NA ($n = 8$): 0.5 μg SKF 38393/0.5 μl sterile H_2O per side; vehicle-NA ($n = 8$): 0.5 μl sterile H_2O per side.

In this experiment we also determined whether SKF 38393 administration had an effect on general locomotor activity. On Days 0–2 of testing, over the 3 min prior to pup presentation (17–20 min postinjection), the absolute number of line crosses (movement of all four feet from one quadrant to another as defined by the Plexiglas dividers) performed by each female was recorded.

In Experiment 4, 3 subjects were removed because of inaccurate cannula placement or excessive brain damage, 1 of which was in the vehicle-NA group and 2 of which were in the D_1-NA group.

Results

Taken together, the results of these experiments indicate that increasing DA activity, specifically at D_1 receptors, in either NA or MPOA reduces the amount of exposure to pup stimuli needed to induce complete maternal behaviors.

Experiment 1

Experiment 1 examined the effects of microinjection of SKF 38393 (a D_1 DA receptor agonist) into the NA on sensitization

latencies to show complete maternal behavior in 15HO rats (see Table 1). Although statistical analyses did not reveal an overall significant difference among the three groups at either the end of the test day or the end of the first hour of observation, Kruskal–Wallis one-way ANOVA: $H(2) = 4.69$, $p = .09$; $H(2) = 4.92$, $p = .08$, respectively, planned comparisons were made between each dose of SKF 38393 and the vehicle-injected group. The median latency for females receiving the high dose (0.5 μg) of SKF 38393 ($n = 10$) to show complete maternal behavior by the end of the test day was 0.5 days, significantly shorter than the 2-day latency of the vehicle-treated group ($n = 12$; Mann–Whitney U test, $U = 26.5$, $p < .023$). When the median latency to show maternal behavior during the first hour of maternal behavior testing (rather than by the end of the test day) was analyzed, the 0.5-μg group still showed maternal behavior significantly sooner ($Mdn = 1$ day) than the 0-μg group ($Mdn = 2$ days; Mann–Whitney U test, $U = 25.5$, $p = .019$). The median latency for females receiving the low dose (0.2 μg) of SKF 38393 ($n = 13$) was 1 day and was not significantly shorter than the 2-day latency shown by the vehicle-treated group ($n = 12$) during the first hour of observation (Mann–Whitney U test, $U = 55.0$, $p = .19$) or by the end of the day (Mann–Whitney U test, $U = 53.5$, $p = .16$).

In addition to analyzing median latencies to onset of complete maternal behavior for either the day or the hour, we did the same analysis for each group on the latencies to retrieve all pups to the nest. In each group, these latencies were identical to those for complete maternal behavior. In other words, given our observational methods, fragmented maternal behavior did not occur. Once a female retrieved all of her pups to her nest, she also nursed and licked them.

The cumulative percentages of females in each group showing full maternal behavior during the day on each test day are shown in Figure 1. By Day 1 of testing, 90% of 15HO females microinjected with the high dose (0.5 μg) of SKF 38393 into NA showed full maternal behavior, compared with only 33% of vehicle-injected females. The Fisher exact probability test indicated that this difference was significant ($p \leq .05$). As shown in Table 1, the mean latency to approach and sniff pups (averaged over Days 0–2 of testing) did not differ between any of the groups: one-way ANOVA, $F(2, 32) = 0.15$, $p = .86$.

An interesting question is whether DA stimulation of NA caused a higher level of maternal behavior than would have been induced by sensitization processes alone. To get some information on this point, we examined the time it took a female to show complete maternal behavior on the first day during which maternal behavior occurred within the 1-hr morning observation period. Most females in the D_1-high group were receiving SKF 38393 microinjections into NA at this time, whereas the vehicle group did not receive such stimulation. Females in each of these groups did not differ in the time it took to show complete maternal behavior, most doing so in the first 15–30 min of the observation period.

Figure 2 shows reconstructions of the location of the NA injection sites, drawn onto the appropriate plates taken from the atlas of Paxinos and Watson (1997; on the microtome, the brain sections for both groups were cut in the plane of this atlas). Of note, microscopic analysis of brain sections indicated that for each group, all cannula placements were located in the NA, either in the shell region or the shell–core border.

Experiment 2

Experiment 2 examined the effects of microinjection of quinpirole (a D_2 DA receptor agonist) into the NA on sensitization latencies to show complete maternal behavior in 15HO rats (Table 1). The Kruskal–Wallis one-way ANOVA indicated there was not an overall significant difference between treatment groups by the end of the test day or the end of the first hour of observation, $H(2) = 2.65$, $p = .26$; $H(2) = 2.64$, $p = .27$, respectively. Planned comparisons examining each dose of quinpirole and the vehicle-treated group also indicated that group differences did not exist. The median latency for females receiving either the high dose (0.5 μg) of quinpirole (1.5 days; $n = 12$) or the low dose (0.2 μg) (3 days; $n = 10$) was not significantly different from that of females receiving vehicle injections ($Mdn = 2$ days; $n = 15$; Mann–Whitney U test, $U = 87.0$, $p = .84$; $U = 48.5$, $p = .13$, respectively). When the median latency to show maternal behavior

Table 1
Outcome Measures in 15HO Rats That Received 0-, 0.2-, or 0.5-μg Doses of SKF 38393 or Quinpirole Into the Nucleus Accumbens

Group	*N*	Mean ± *SE* latency to approach and sniff pups (s)	Median onset to full maternal behavior (days)	
			Day	Hour
SKF 38393				
Vehicle	12	232.95 ± 82.82	2 (1–4)	2 (1–4)
D_1-low	13	271.06 ± 113.30	1 (0–2.5)	1 (0.5–3.5)
D_1-high	10	256.40 ± 103.50	0.5* (0–1)	1* (0–1)
Quinpirole				
Vehicle	15	278.00 ± 66.41	2 (0–4)	2 (1–4)
D_2-low	10	182.00 ± 67.30	3 (2–5)	3 (2–6)
D_2-high	12	212.42 ± 64.93	1.5 (0–5)	1.5 (0–6)

Note. For each female, latency to sniff and approach pups was averaged over Days 0–2 of testing. Interquartile ranges are shown in parentheses. D_1 refers to SKF 38393, a DA-D_1 agonist; D_2 refers to quinpirole, a DA-D_2 agonist. 15HO = hysterectomized and ovariectomized on Day 15 of pregnancy.
* Significantly different from corresponding vehicle group, Mann-Whitney U test, $p < .05$.

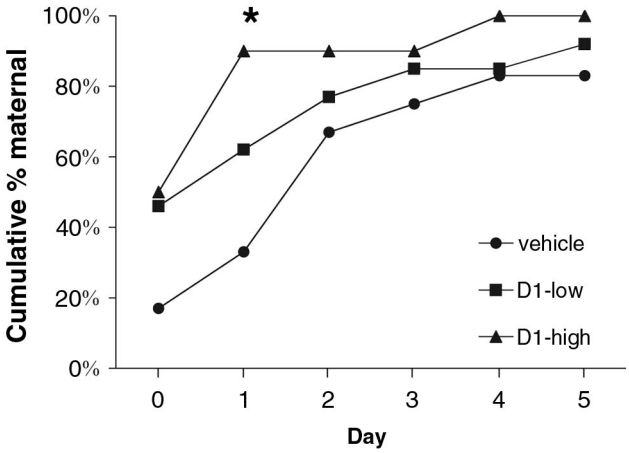

Figure 1. Cumulative percentage of female rats showing full maternal behavior on each test day. Females received bilateral microinjections of either 0 (vehicle), 0.2 (D_1-low), or 0.5 (D_1-high) μg SKF 38393 (*n*s = 12, 13, and 10, respectively) into the nucleus accumbens on Days 0, 1, and 2 of testing. *Significantly different from vehicle group, Fisher exact probability test, $p < .05$.

during the first hour of maternal behavior testing (rather than by the end of the test day) was analyzed, the differences were also not significant (Mann–Whitney *U* test, $U = 89.5$, $p = .94$; $U = 47.5$, $p = .12$, respectively). There were also no significant differences between the groups in mean latency to approach and sniff pups: one-way ANOVA, $F(2, 34) = 0.55$, $p = .58$.

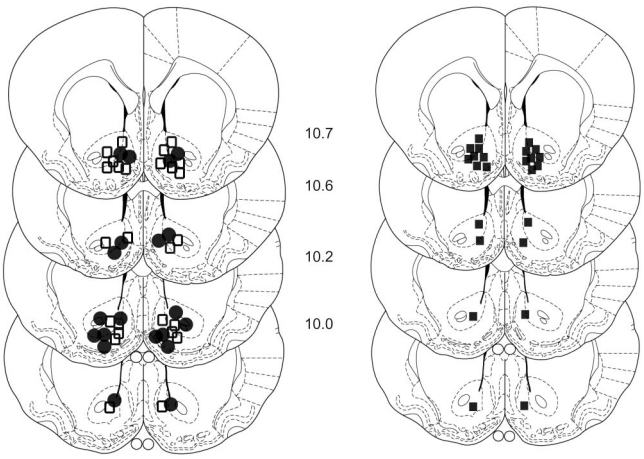

Figure 2. Reconstructions, based on the microscopic analysis of cresyl violet stained sections, of the location of SKF 38393 injection sites (solid squares = 0-μg dose; open squares = 0.2-μg dose; solid circles = 0.5-μg dose) into the nucleus accumbens (NA), which were drawn onto plates from the Paxinos and Watson (1997) atlas. The numbers next to the plates indicate the distance in millimeters anterior to the interaural plane. All implant sites were located in either the shell region of the NA or on the shell–core border. However, to facilitate viewing, the depicted locations were spread out slightly to eliminate the overlap that actually occurred. From *The Rat Brain in Stereotaxic Coordinates* (3rd ed.), by G. Paxinos and C. Watson, 1997, San Diego, CA: Academic Press. Copyright 1997 by Elsevier. Reprinted with permission.

The cumulative percentages of females in each group showing full maternal behavior during the day on each test day are shown in Figure 3. The Fisher exact probability test did not detect any significant differences. Figure 4 shows reconstructions of the location of the NA injection sites, which were all located near the shell–core border and within a range similar to NA injection sites for Experiment 1.

Experiment 3

Experiment 3 examined the effects of microinjection of SKF 38393 into the DS on sensitization latencies to show complete maternal behavior in 15HO rats (Table 2). The Mann–Whitney *U* test indicated there was not a significant difference between the vehicle-treated females (*Mdn* = 3 days; $n = 8$) and those treated with a 0.5-μg dose of SKF 38393 (*Mdn* = 6 days; $n = 8$) by the end of the test day (Mann–Whitney *U* test, $U = 16.5$, $p = .08$) or the end of the first hour of observation ($U = 16$, $p = .07$). Note, however, that these differences approached significance, implying that D_1 DA receptor stimulation of DS may actually depress maternal behavior. It is important to note that there was not a significant difference between the groups in mean latency to approach and sniff pups, $t(14) = 0.25$, $p = .80$ (Table 2).

The cumulative percentages of females in each group showing full maternal behavior during the day on each test day are shown in Figure 5. Significant differences did not occur. Figure 6 shows reconstructions of the location of the DS injection sites, drawn onto the appropriate plates taken from the atlas of Paxinos and Watson (1997).

Experiment 4

The results of Experiment 4 indicate two important points: First, the main effect of Experiment 1 was replicated in that the high dose of SKF 38393 (0.5 μg) was once again effective in reducing

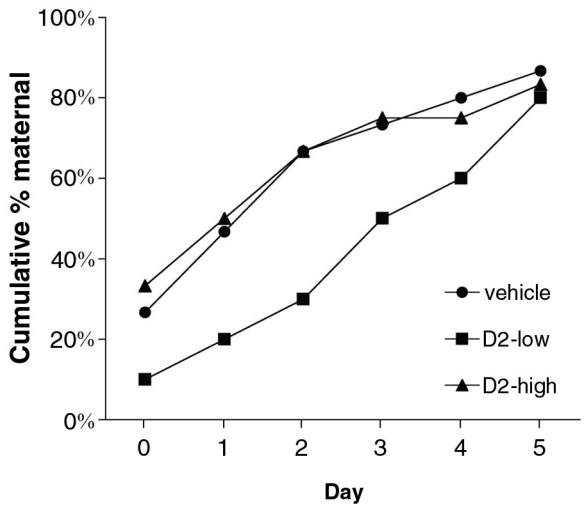

Figure 3. Cumulative percentage of female rats showing full maternal behavior on each test day. Females received bilateral microinjections of either 0 (vehicle), 0.2 (D_2-low), or 0.5 (D_2-high) μg quinpirole (*n*s = 15, 10, and 12, respectively) into the nucleus accumbens on Days 0, 1, and 2 of testing. Groups did not significantly differ.

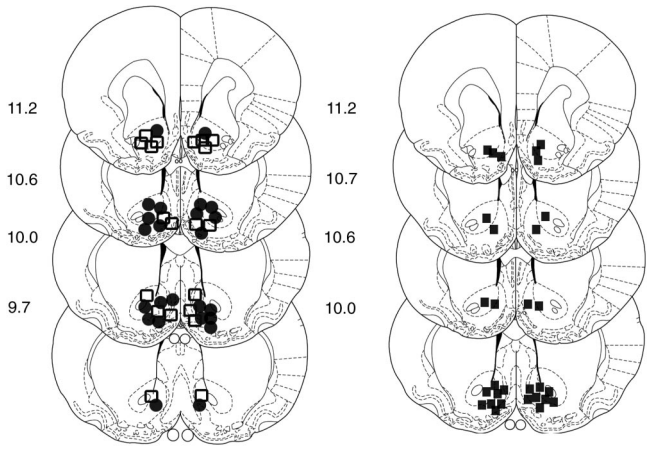

Figure 4. Reconstructions, based on the microscopic analysis of cresyl violet stained sections, of the location of quinpirole injection sites (solid squares = 0-μg dose; open squares = 0.2-μg dose; solid circles = 0.5-μg dose) into the nucleus accumbens (NA), which were drawn onto plates from the Paxinos and Watson (1997) atlas. The numbers to the left of the plates indicate the distance in millimeters anterior to the interaural plane. All implant sites were located in either the shell region of the NA or on the shell–core border. However, to facilitate viewing, the depicted locations were spread out slightly to eliminate the overlap that actually occurred. From *The Rat Brain in Stereotaxic Coordinates* (3rd ed.), by G. Paxinos and C. Watson, 1997, San Diego, CA: Academic Press. Copyright 1997 by Elsevier. Reprinted with permission.

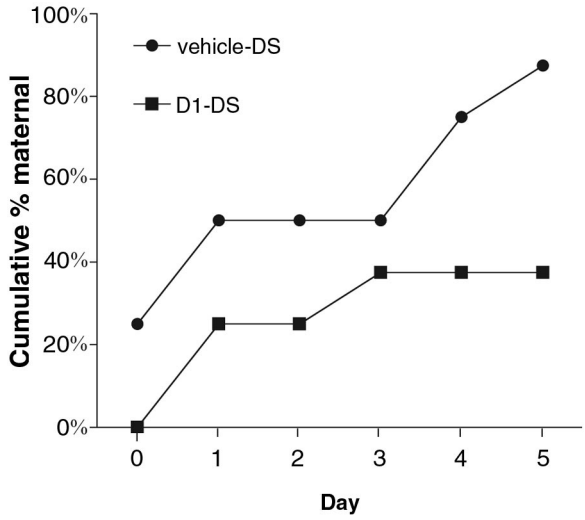

Figure 5. Cumulative percentage of female rats showing full maternal behavior on each test day. Females received bilateral microinjections of either 0 (vehicle-DS) or 0.5 (D_1-DS) μg SKF 38393 (*n*s = 8 and 8, respectively) into the dorsal striatum (DS) on Days 0, 1, and 2 of testing. Groups did not differ significantly.

sensitization latencies when injected bilaterally into NA, and second, this high dose of SKF 38393 was able to stimulate maternal behavior when injected into MPOA (Table 3). The Kruskal–Wallis one-way ANOVA indicated that there was an overall significant difference among the four groups during the observation hour, $H(3) = 9.73$, $p = .02$, and by the end of the observation day, $H(3) = 8.30$, $p < .05$. The median latency for females receiving 0.5 μg SKF 38393 into the NA ($n = 8$) was 0 days for both the hour and the day latency measure, and this was significantly shorter than the 2-day latency of the vehicle-treated group ($n = 8$) during the first hour of observation (Mann–Whitney *U* test, $U = 11.0$, $p < .05$) or the 1.5-day latency for the vehicle group when measured at the end of the test day (Mann–Whitney *U* test, $U = 12.5$, $p < .05$). The median latency for females receiving 0.5 μg SKF 38393 into MPOA ($n = 10$) was also significantly shorter than that of the vehicle-treated group during the first hour of observation (Mann–Whitney *U* test, $U = 21.5$, $p < .05$) or by the end of the observation day (Mann–Whitney *U* test, $U = 24.0$, $p <$

Table 2
Outcome Measures in 15HO Rats That Received 0- or 0.5-μg Doses of SKF 38393 Into the Dorsal Striatum (DS)

SKF 38393 group	*N*	Mean ± *SE* latency to approach and sniff pups (s)	Median onset to full maternal behavior (days)	
			Day	Hour
Vehicle-DS	8	249.21 ± 74.44	2.5 (0–5)	3 (0–5)
D_1-DS	8	222.17 ± 77.70	6 (1–6)	6 (3–6)

Note. For each female, latency to sniff and approach pups was averaged over Days 0–2 of testing. Groups did not differ significantly. Interquartile ranges are shown in parentheses. D_1 refers to SKF 38393, a DA-D_1 agonist. 15HO = hysterectomized and ovariectomized on Day 15 of pregnancy.

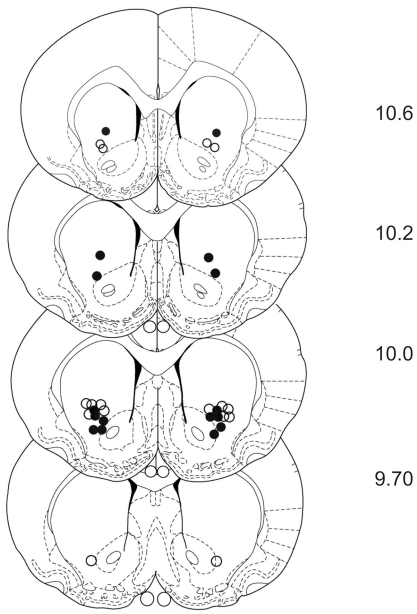

Figure 6. Reconstructions, based on the microscopic analysis of cresyl violet stained sections, of the location of SKF 38393 injection sites (open circles = 0-μg dose; solid circles = 0.5-μg dose) into the dorsal striatum (DS), which were drawn onto plates from the Paxinos and Watson (1997) atlas. The numbers next to the plates indicate the distance in millimeters anterior to the interaural plane. From *The Rat Brain in Stereotaxic Coordinates* (3rd ed.), by G. Paxinos and C. Watson, 1997, San Diego, CA: Academic Press. Copyright 1997 by Elsevier. Reprinted with permission.

Table 3
Outcome Measures in 15HO Rats That Received 0- or 0.5-μg Doses of SKF 38393 Into the Nucleus Accumbens (NA) or Medial Preoptic Area (MPOA)

Group	*N*	Mean ± *SE* latency to approach and sniff pups (s)	Mean ± *SE* number of line crosses	Median onset to full maternal behavior (days)	
				Day	Hour
Vehicle-NA	8	265.96 ± 82.61	0.71 ± 0.48	1.5 (1–6)	2 (1–6)
D_1-NA	8	153.21 ± 51.64	0.94 ± 0.95	0* (0–1)	0* (0–1)
Vehicle-MPOA	10	87.37 ± 38.38	0.20 ± 0.11	1 (1–4)	1.5 (1–4)
D_1-MPOA	10	179.29 ± 45.27	0.60 ± 0.31	0* (0–0)	0* (0–1)

Note. For each female, latency to sniff and approach pups and number of line crosses were averaged over Days 0–2 of testing. Interquartile ranges are shown in parentheses. D_1 refers to SKF 38393, a DA-D_1 agonist. 15HO = hysterectomized and ovariectomized on Day 15 of pregnancy.
* Significantly different from corresponding vehicle group, Mann-Whitney *U* test, $p < .05$.

.05). There were no significant differences between the median latencies for females receiving SKF 38393 injections into NA versus MPOA, nor were there significant differences between median latencies of vehicle-treated animals who received injections into NA or MPOA. A one-way ANOVA indicated there were not significant differences between treatment groups (see Table 3) in the latency to approach and sniff pups, $F(3, 32) = 1.80$, $p = .17$, or in home cage activity in the 3 min prior to pup presentation: Kruskal–Wallis one-way ANOVA, $H(3) = 3.37$, $p = .29$. Sniff latencies and home cage line crosses were averaged across Days 0–2 of testing.

In addition to analyzing median latencies to onset of complete maternal behavior for either the day or the hour, we did the same analysis for each group on the latencies to retrieve all pups to the nest. In each group, these latencies were identical to those for complete maternal behavior. Therefore, similar to the results obtained for Experiment 1, fragmented maternal behavior did not occur in Experiment 4. Once a female retrieved all of her pups to her nest, she also nursed and licked them.

The cumulative percentages of females showing complete maternal behavior on each test day are shown in Figure 7. By the end of Day 0 of testing, 80% of the females receiving SKF 38393 into MPOA showed complete maternal behavior, compared with only 20% of vehicle-treated animals. The Fisher exact probability test indicated that this difference was significant ($p < .05$). Although 62.5% of females injected with SKF 38393 into NA showed complete maternal behavior by the end of Day 0 of testing, compared with only 14% of vehicle-injected animals, this difference was not significant ($p = .07$, one-tailed). This difference approached significance, however, and if a larger number of subjects had been run, it is likely that a significant effect would have been observed. Figure 8 shows reconstructions of the location of MPOA injection sites, and Figure 9 shows reconstructions of the location of NA injection sites.

To examine whether DA stimulation of NA or MPOA caused a higher level of maternal behavior than would have been induced by sensitization processes alone, we examined the time it took a female to show complete maternal behavior on the first day during which maternal behavior occurred within the 1-hr morning observation period. Most females in the D_1-NA and D_1-MPOA groups were receiving SKF 38393 microinjections at this time, whereas the vehicle groups were not receiving such stimulation. Females in each of these four groups did not differ in the time it took them to show complete maternal behavior, most doing so in the first 15–30 min of the observation period. These results conform with those of Experiment 1.

Discussion

Previous research has shown that depression of DA action on the NA disrupts maternal behavior in postpartum female rats (Keer & Stern, 1999; Silva et al., 2003) and has emphasized the involvement of D_1 receptors (Numan, Numan, Pliakou, et al., 2005). As an important complement to these findings, the present study provides substantial evidence that increased DA activity at D_1 receptors in the NA promotes maternal behavior. In addition, our findings indicate that the MPOA, but not the DS, is another site where D_1 receptor activation promotes maternal behavior. These results suggest that increased DA activity at D_1 receptors in the NA or MPOA

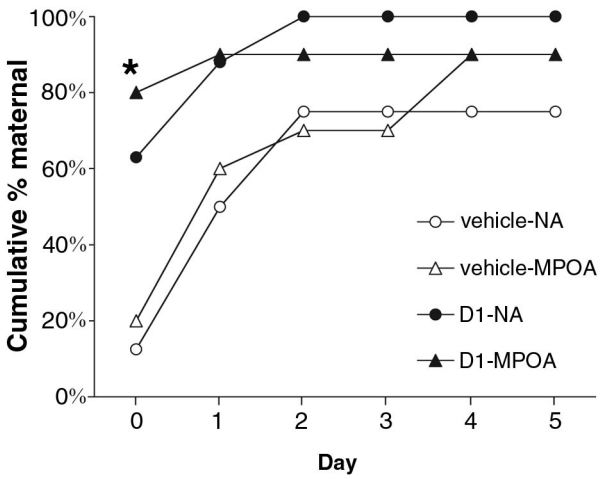

Figure 7. Cumulative percentage of female rats showing full maternal behavior on each test day. Females received bilateral microinjections of either 0 (vehicle-NA) or 0.5 (D_1-NA) μg SKF 38393 (*n*s = 8 and 8, respectively) into the nucleus accumbens (NA) on Days 0, 1, and 2 of testing, or 0 (vehicle-MPOA) or 0.5 (D_1-MPOA) μg SKF 38393 (*n*s = 10 and 10, respectively) into the medial preoptic area (MPOA). *Significantly different from vehicle-MPOA group, Fisher exact probability test, $p < .05$.

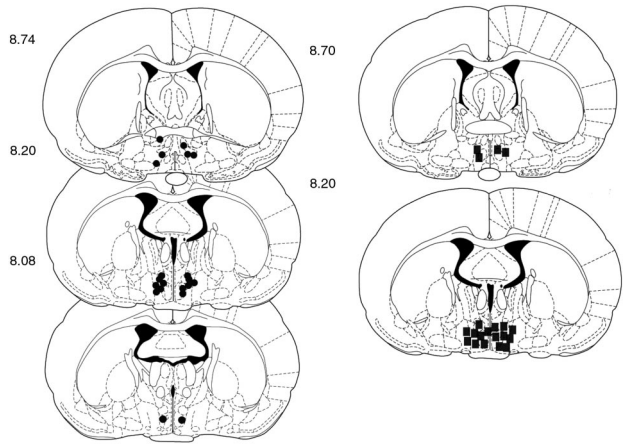

Figure 8. Reconstructions, based on the microscopic analysis of cresyl violet stained sections, of the location of SKF 38393 injection sites (solid squares = 0-μg dose; solid circles = 0.5-μg dose) into the medial preoptic area (MPOA), which were drawn onto plates from the Paxinos and Watson (1997) atlas. The numbers to the left of the plates indicate the distance in millimeters anterior to the interaural plane. All implants were located within the MPOA or the ventral bed nucleus of the stria terminalis. However, to facilitate viewing, the depicted locations were spread out slightly to eliminate the overlap that actually occurred. From *The Rat Brain in Stereotaxic Coordinates* (3rd ed.), by G. Paxinos and C. Watson, 1997, San Diego, CA: Academic Press. Copyright 1997 by Elsevier. Reprinted with permission.

can substitute for estradiol in the stimulation of maternal behavior in 15HO females.

Experiment 1 indicated that microinjection of a D_1 DA agonist, SKF 38393, into the NA promoted maternal responsiveness: The 0.5-μg dose resulted in a sensitization latency of about 0.5 days, significantly shorter than the 2-day latency of the control group. Of note, 90% of females receiving 0.5 μg SKF 38393 were maternal by Day 1 of testing compared with only 33% of females in the control group.

Experiment 2 indicated that within the NA, dopaminergic facilitation of maternal behavior is mediated primarily by D_1 receptors, as the D_2 receptor agonist quinpirole did not produce facilitatory effects at the doses tested, which were approximate molar equivalents of the SKF 38393 doses. Our results, of course, do not rule out the possibility that other doses of quinpirole might have been effective.

The facilitatory effect of D_1 activation in the NA on maternal behavior was also shown to be relatively site specific, as the dose of SKF 38393 that was effective in stimulating maternal behavior when injected into the NA failed to facilitate maternal behavior when injected into the DS. In fact, data obtained from Experiment 3 show that SKF 38393 injections into the DS tended to inhibit maternal responding compared with controls, although this trend was not significant.

Experiment 4 demonstrated that increased D_1 receptor activation in either the NA or the MPOA was capable of facilitating maternal behavior. Not only do these findings validate the results of Experiment 1, but they also suggest a role for dopaminergic mechanisms within the MPOA in the regulation of the onset of maternal behavior. Of note, there were no differences between the effects of SKF 38393 microinjection into the NA and MPOA, indicating that increased D_1 DA activation in each region produced equivalent effects on maternal responding.

In Experiment 1, 9 of the 10 females in the 0.5-μg SKF 38393 treatment group were showing maternal behavior by Day 1 of testing. Of these 9 females, 5 initiated maternal behavior on Day 0 and 4 showed maternal behavior on Day 1. Given that SKF 38393 was injected into the NA on Days 0, 1, and 2 of testing, what accounts for the observed variability in maternal behavior initiation? The best explanation is that there is an interaction between each female's baseline maternal responsiveness and the D_1 agonist: A certain level of maternal responsiveness may be necessary to allow D_1 DA activity in NA to push the female over the threshold for full maternal behavior. Some females may have had sufficient maternal responsiveness on Day 0, whereas others may have required a period of pup stimulation before the D_1 DA agonist could be effective. In this context, note that female rats typically give birth on Day 22 or 23 of pregnancy. Because all female rats were hysterectomized and ovariectomized on Day 15, it is possible that those females that would have given birth on Day

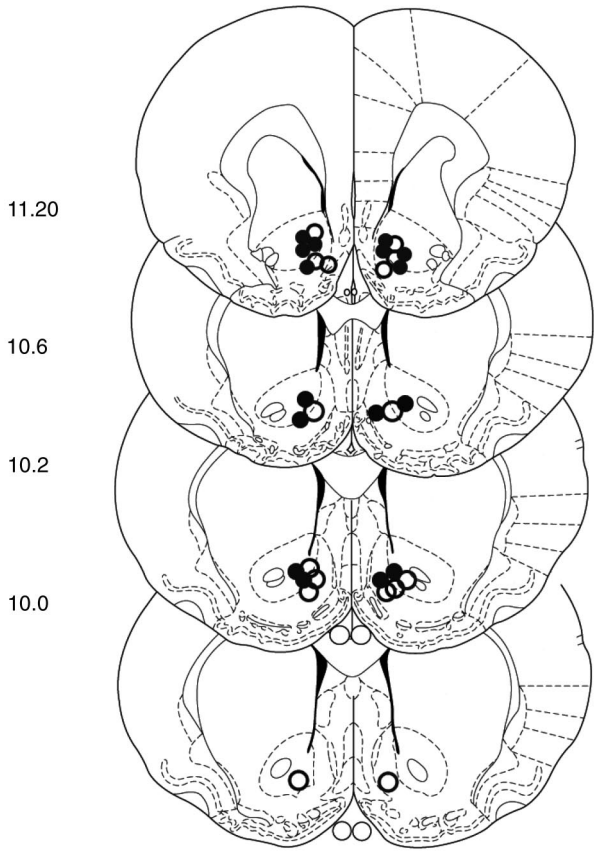

Figure 9. Reconstructions, based on the microscopic analysis of cresyl violet stained sections, of the location of SKF 38393 injection sites (open circles = 0-μg dose; solid circles = 0.5-μg dose) into the nucleus accumbens, which were drawn onto plates from the Paxinos and Watson (1997) atlas. The numbers next to the plates indicate the distance in millimeters anterior to the interaural plane. From *The Rat Brain in Stereotaxic Coordinates* (3rd ed.), by G. Paxinos and C. Watson, 1997, San Diego, CA: Academic Press. Copyright 1997 by Elsevier. Reprinted with permission.

22 received a higher level of partial hormonal priming than did the females who would have given birth on Day 23.

One possible interpretation of the finding that SKF 38393 significantly reduced sensitization latencies is that administration of the DA agonist altered motor activity and that therefore females treated with SKF 38393 had an increased opportunity to come into contact with pups. This interpretation is highly unlikely, however, because the latency to approach and sniff pups was not significantly different between treatment groups. Additionally, Experiment 4 showed that in the 3 min prior to pup presentation, groups did not differ in home cage activity. Finally, casual observation indicated that differences in general motor activity did not occur.

Concerning the location of the implant sites, histological analysis indicated that MPOA implant sites were similar between vehicle- and SKF 38393-injected animals. NA implant sites were all located within the shell region of the NA or the shell–core border in Experiments 1, 2, and 4. This finding fits with previous work that suggests the importance of the shell region for maternal behavior (Keer & Stern, 1999; Li & Fleming, 2003; Numan, Numan, Pliakou, et al., 2005; Stack et al., 2002). Analysis of the rostrocaudal location of the NA implant sites in the SKF 38393 treated females in Experiments 1 and 4 indicated that there was no relationship between anterior–posterior injection location and onset latency.

The 15HO suboptimal hormone preparation was chosen as our baseline condition for several reasons. As previously mentioned, it is not likely that increasing D_1 DA activity in NA alone would stimulate maternal behavior in virgins, who typically show 6–8-day sensitization latencies. Pilot data from our lab indicated that this assumption is correct. Virgin female rats who were implanted with bilateral cannula aimed at the NA and given microinjections of various doses of SKF 38393 did not show a reduction in sensitization latencies compared with control females receiving vehicle (H_2O) injections. These findings of course do not rule out the possibility that under certain conditions D_1 DA stimulation of NA alone could facilitate maternal behavior in virgin rats. However, under our treatment conditions, we were not able to produce those results. Whether microinjection of SKF 38393 into the MPOA of virgin female rats would produce a facilitatory effect is not known.

In support of the view that increases in DA activity within NA may be able to facilitate maternal behavior in virgins, Thompson and Kristal (1996) showed that a pharmacological manipulation that may have impacted the mesolimbic DA system was capable of stimulating maternal behavior in virgin female rats. Virgin females were implanted with bilateral guide cannula aimed at the VTA and injected with morphine sulfate on Days 0–2 of sensitization testing. Results indicated that 0.03 μg morphine effectively reduced sensitization latencies from a median of 10 days to a median of 5.5 days. It is important to note that the fact that onset of maternal behavior occurred after the drug had left the system suggests that the facilitation was not due to an acute effect of the drug. Rather, the drug may have indirectly stimulated maternal behavior by affecting some other neurotransmitter system. We have already made a case for the importance of VTA DA projections to the NA in promoting maternal behavior, and it is well established that opioid agonists in VTA can increase DA release within the NA (Kalivas, 1985). Therefore, perhaps the facilitatory effects of VTA morphine treatment were due to activation of the mesolimbic DA system (Thompson & Kristal, 1996).

In the present study, SKF 38393 was chosen as the D_1 receptor agonist because it is considered the complement of SCH 23390 (Missale, Nash, Robinson, Jaber, & Caron, 1998), the D_1 DA receptor antagonist that was found to disrupt retrieval behavior in postpartum animals when injected into the NA (Numan, Numan, Pliakou, et al., 2005). Although some work suggests that SKF 38393 is a partial agonist at D_1 DA receptors (Missale et al., 1998), other work has shown that its neurophysiological effects equal those of a full DA agonist (SKF 82598) and this neurophysiological effect can be blocked by the administration of SCH 23390 (Heidenreich, Mailman, Nichols, & Napier, 1995).

The results of present and previous experiments indicate that D_1 agonist action on NA or MPOA can stimulate the onset of maternal behavior whereas D_1 antagonist injections into either NA or MPOA can inhibit maternal behavior in postpartum females (Miller & Lonstein, 2005; Numan, Numan, Pliakou, et al., 2005). Therefore, D_1 DA activity at these sites seems necessary for both the onset of maternal behavior and the maintenance of established maternal behavior. In the present experiments, we injected SKF 38393 into the NA or MPOA on Days 0–2 of testing. We made the point, however, that once maternal behavior had been initiated or established, SKF 38393 injection into the brain was no longer essential for the continuance of maternal behavior. On the basis of the overall data, it appears that once maternal behavior is initiated, *endogenous* DA activity at D_1 receptors in either NA or MPOA, or both regions, maintains maternal behavior. These findings and this view are related to the well-known dichotomy between the onset and the maintenance of maternal behavior in rats and also related to the process called maternal memory (see Numan, 2006; Numan, Fleming, & Levy, 2006; and Numan & Insel, 2003, for reviews). Although ovarian hormones and lactogens are essential for the onset of maternal behavior at parturition, once maternal behavior is established it becomes relatively independent of hormonal control, even if a female is separated from her pups for varying intervals. The idea is that once maternal behavior is initiated and consolidated the brain mechanisms are reorganized in such a way that hormones are no longer necessary to prime the neural circuits that control maternal responsiveness. Although much research has been done on these issues and many hypotheses have been offered, at present it is not clear how the brain mechanisms underlying maternal behavior are changed by maternal experience. One potential mechanism that might contribute to maternal memory formation is that estradiol and lactogen priming of the MPOA allow pup stimuli to activate the MPOA efferents to the VTA, and the resultant stimulation of the mesolimbic DA system promotes the onset of maternal behavior. After the occurrence of maternal contact with pups, perhaps the MPOA is reorganized so that it becomes more sensitive to pup stimulation. This would allow the MPOA to continue to activate the mesolimbic DA system in the absence of continued hormonal priming (Numan, 2006; Numan & Insel, 2003).

In the present study we were primarily concerned with the mechanism regulating the onset of complete maternal behavior (retrieving, nursing, and licking pups), and we did not take detailed observations on the intensity of maternal behavior after it was initiated. An interesting question for future investigation might be whether the intensity of maternal behavior (amount of nursing and

licking pups) is superior in females that received SKF 38393 stimulation of either NA or MPOA when compared with the maternal behavior of control females. On the basis of the fact that once maternal behavior is initiated it is controlled by endogenous DA activity, our inclination, backed up by some observations during the current study, is that there would not be a difference in the nature of maternal behavior between SKF 38393- and vehicle-injected females. In particular, on the first day that females showed complete maternal behavior within the 1-hr morning observation, they typically showed the behavior within 15–30 min of pup presentation irrespective of whether they were receiving SKF 38393 microinjections into the brain. That is, SKF 38393- and vehicle-treated females did not differ in the speed to show the complete maternal behavior pattern once the behavior had been initiated. In further support, it has been found that in home cage tests, the maternal behaviors shown by sensitized virgins are virtually indistinguishable from those shown by postpartum lactating females (Fleming & Rosenblatt, 1974; Reisbick, Rosenblatt, & Mayer, 1975). Additionally, note that endogenous DA levels are higher in the MPOA of sensitized virgins compared with controls (Olazabel et al., 2004). However, we cannot rule out the possibility that SKF 38393 injection caused supernormal levels of D_1 stimulation, which might have caused higher levels of various maternal behaviors in terms of the durations of the behaviors (see Champagne et al., 2004).

Because the present study found that SKF 38393 microinjection into either the NA or the MPOA was capable of facilitating maternal behavior, probably the most central question that future research will have to resolve is whether SKF 38393 is actually capable of facilitating maternal behavior by acting at either of these sites. In other words, the possibility remains open that the D_1 agonist had its primary effect at only one of these two sites and that the facilitatory effect observed at the other site was due to spread of the drug to the primary site (the NA and the MPOA are separated by only about 2 mm). With respect to the possibility that the primary site of action of SKF 38393 is at the level of the NA, we should note the following. For postpartum females, Numan, Numan, Pliakou, et al. (2005) were able to depress maternal behavior with microinjection of SCH 23390 (a D_1 antagonist) into the NA at a dosage level (1–2 μg) that was ineffective when injected into the MPOA. The depressive effect of SCH 23390 into NA therefore cannot be explained by spread of the antagonist to the MPOA.

The effects of SCH 23390 microinjection into the MPOA on postpartum maternal behavior are less clear. Supporting the results of Numan, Numan, Pliakou, et al. (2005), Miller and Lonstein (2005) also found that bilateral injections of a 2-μg dose of SCH 23390 into the MPOA did not affect maternal behavior. However, these same authors found that bilateral MPOA injections of 5 μg of the D_1 antagonist did disrupt maternal behavior. These results suggest the possibility that the disruptive effects reported by Miller and Lonstein were due to spread of the 5-μg dose of SCH 23390 from the MPOA to the NA. We cannot rule out the possibility, however, that in postpartum females small doses of SCH 23390 in NA effectively depress maternal behavior, whereas direct action of this drug in the MPOA requires higher doses.

In spite of the difficulty interpreting the effects on maternal behavior of D_1 antagonism at the level of the MPOA, other research supports the possibility that in addition to an action on the NA, DA action on the MPOA is also important for maternal behavior. This research draws on parallels between the neural regulation of maternal behavior and male sexual behavior in rats. In particular, MPOA lesions disrupt both behaviors, and estradiol action on the MPOA not only facilitates maternal behavior but also promotes male sexual behavior (Numan, 1974, 1985). Significantly, DA activity in the MPOA is integral to the regulation of male sexual behavior (Hull & Dominguez, 2006). Blockade of DA receptors in the MPOA has been found to impair copulation, microinjection of DA agonists into the MPOA facilitates copulation, and DA is released into the MPOA both in the presence of a receptive female and during mating behavior (Dominguez & Hull, 2005). Furthermore, DA activity in the MPOA is regulated by male sex hormones, and this hormonal regulation of DA release has been linked to a mechanism involving the gaseous neuromodulator nitric oxide (NO) (Hull & Dominguez, 2006). Disruption of NO synthesis by microinjection of L-NAME (an NO synthase inhibitor) not only impairs copulation (Lagoda, Muschamp, Vigdorchick, & Hull, 2004) but also disrupts copulation-induced DA release (Dominguez, Muschamp, Schmich, & Hull, 2004).

Of note, there is also evidence to indicate that NO is important for the expression of maternal behavior in rats: Popeski and Woodside (2004) showed that intracerebroventricular injection of L-NAME disrupts maternal behavior. The recent finding that microinjection of L-NAME directly into the MPOA also disrupts maternal behavior, and that this effect can be reversed with simultaneous injections of SKF 38393 (Service & Woodside, 2006), suggests that NO may be similarly involved in the regulation of DA release in the MPOA during maternal behavior, and this may provide a mechanism through which the MPOA regulates the onset of maternal responsiveness.

In summary, a case can be made for the possibility that DA action on D_1 DA receptors in both the NA and the MPOA is important for both the onset and the maintenance of maternal behavior. Of interest, there is also evidence to suggest that estradiol action on NA can facilitate DA transmission in NA (Becker, 1999), and because the NA also contains nitric oxide synthase neurons and NO is involved in regulating NA function (West, Galloway, & Grace, 2002), an important question concerns the similarity in underlying mechanisms between DA action on these two structures.

Our working hypothesis, based on the literature reviewed, is that it is likely that D_1 DA receptor activation in both MPOA and NA is important for maternal behavior. In this context, recall that the MPOA and mesolimbic DA system are linked in the control of maternal behavior. Our lab has developed a model in which the MPOA plays a role in the regulation of maternal responsiveness through its activation of the mesolimbic DA system via projections to the VTA (see Numan, 2006; Numan, Numan, Schwarz, et al., 2005): As a result of hormonal stimulation, the MPOA is primed to respond to certain pup stimuli. The MPOA in turn activates the VTA-DA projections to NA, which ultimately, through a process of disinhibition, allow the ventral pallidum to process pup-stimuli-induced afferent input so that voluntary maternal responses can occur.

Given the linkage between the MPOA and the mesolimbic DA system in the control of maternal behavior, it is certainly possible that D_1 DA receptor activation in MPOA activates efferent projections to VTA, which in turn increases D_1 DA receptor activation

in NA. In the 15HO female that is not treated with estradiol, DA activity in both the MPOA and the NA may be relatively low. By up-regulating D_1 DA activity in either the MPOA or the NA, we circumvent the need for estradiol action on the MPOA and facilitate a rapid onset of maternal behavior.

Finally, it should be noted that MPOA lesions, D_1 antagonist action at the level of either the NA or the MPOA, or disconnection of the neural circuit between the MPOA and the VTA cause similar disruptions in behavior (Miller & Lonstein, 2005; Numan & Callahan, 1980; Numan, Numan, Pliakou, et al., 2005; Numan & Smith, 1984). These manipulations disrupt the proactive voluntary components of maternal behavior (retrieving behavior and pup licking) while leaving nursing behavior, a more reflexive aspect of maternal behavior, relatively intact and in some cases even enhanced. Proactive voluntary maternal behaviors undoubtedly involve the forebrain, and therefore it is not surprising that they are disrupted by the interventions described above. It is important to note that the MPOA has also been found to regulate pup-seeking behaviors. Postpartum females will learn to press a lever to gain access to pups, and MPOA lesions disrupt such operant responding (Lee, Clancy, & Fleming, 2000). Similarly, Fos expression is activated in the MPOA when a postpartum female is searching an environment that previously contained pups even when the pups are not currently present (Mattson & Morrell, 2005). One interpretation of these results is that MPOA efferents to the mesolimbic DA system increase maternal motivation in the sense of increasing voluntary responses to pup-related cues (Numan, 2006). In this context, it makes sense that D_1 activation of MPOA efferents or D_1 activation of NA would promote the onset of maternal behavior in 15HO female rats.

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