2008

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Mayte Parada
University of Toronto, Mississauga

Samantha King
University of Toronto, Mississauga

Ming Li
University of Nebraska-Lincoln, mli2@unl.edu

Alison S. Fleming
University of Toronto, Mississauga, afleming@utm.utoronto.ca

The Roles of Accumbal Dopamine D_1 and D_2 Receptors in Maternal Memory in Rats

Mayte Parada,[1] Samantha King,[1] Ming Li,[2] and Alison S. Fleming[1]

1. Department of Psychology, University of Toronto, Mississauga, Ontario, Canada
2. Department of Psychology, University of Nebraska–Lincoln

Mayte Parada and Samantha King contributed equally to this project and should be considered co-first authors.

Corresponding author — Alison S. Fleming, Department of Psychology, University of Toronto at Mississauga, 3359 Mississauga Road North, Mississauga, Ontario, Canada L5L 1C6; email afleming@utm.utoronto.ca

Abstract

Female rats show enhanced maternal responsiveness toward their young if they have had maternal experiences before. This kind of maternal experience-based memory is critically dependent on the mesolimbic dopamine (DA) system, especially the nucleus accumbens (NA) shell. However, the relative contributions of the two main DA receptor systems (D_1 and D_2) within the shell have not been delineated. This study investigates the roles of dopamine D_1 and D_2 receptors in maternal memory by infusing a selective D_1 antagonist, SCH-23390; a selective D_2 antagonist, sulpiride; or a combination D_1/D_2 antagonist, cis-Z-flupenthixol, into the NA shell of postpartum female rats. Sulpiride-infused rats showed a significantly longer latency to exhibit full maternal behavior following a 10-day pup isolation period in comparison to the controls that received a vehicle. Cis-Z-flupenthixol disrupted maternal memory to a greater extent, as rats receiving this showed the longest latencies to express maternal behavior. SCH-23390 infusions had only marginal effects. These findings suggest that both the D_1 and the D_2 receptor subtypes play a role in the consolidation of maternal memory and they might do so by mediating the motivational salience of pup stimulation.

Keywords: maternal memory, NA shell, dopamine D_1 receptor, dopamine D_2 receptor

Female rats are maternally responsive to their pups immediately after parturition. The dam exhibits high levels of pup-directed behaviors including retrieving pups to the nest site, licking pups, and nursing them (Fleming & Rosenblatt, 1974a, 1974b, 1974c; Numan, Fleming, & Levy, 2006; Wiesner & Sheard, 1933). She continues to respond maternally toward her offspring for about 3–4 weeks, until they are weaned (Numan, 1994; Rosenblatt & Lehrman, 1963; Wiesner & Sheard, 1933).

It is known that maternal experiences obtained by the mother rat when interacting with the young play a critical role in maintaining the high level of maternal responsiveness well beyond the immediate postpartum period. As little as 30 min of proximal interaction with pups immediately following parturition enables the primiparous female rat to retain her maternal behavior for a period of days to several weeks (Bridges, 1975, 1978; Cohen & Bridges, 1981; Orpen & Fleming, 1987). If pups are removed from the dam after parturition, the retention of maternal behavior is attenuated and, after a 10- to 25-day period of isolation, the dam behaves toward foster pups in a manner similar to that of a virgin female (Bridges, 1975, 1978; Cohen & Bridges, 1981). The retention of maternal responsiveness as a result of prior maternal experience is termed *maternal memory* or the *maternal experience effect* (Bridges, 1975, 1977; Bridges & Scanlan, 2005; Cohen & Bridges, 1981; Li & Fleming, 2003a, 2003b).

Research on the neural and neurochemical basis of maternal memory has identified that the nucleus accumbens (NA), more specifically the shell region, is critical for the consolidation process of maternal memory. Thus, maternal memory is significantly disrupted when the NA, or the shell region, is lesioned before or immediately after a brief pup experience (Lee, Li, Watchus, & Fleming, 1999; Li & Fleming, 2003a, 2003b). Females given experience on Postnatal Day 1 (PND1) and tested after a 10-day isolation period show longer latencies to become maternal following NA lesions. Lesions to other areas including the dorsal hippocampus, cortical amygdala, and dorsomedial thalamus have no effect on maternal responsiveness (Lee et al., 1999). The latency to retrieve pups is significantly increased after electrolytic lesions of the NA shell, when performed both before parturition or immediately after a brief maternal experience (Li & Fleming, 2003a, 2003b). On the other hand, lesions to the NA core have no significant effects on maternal memory (Li & Fleming, 2003a, 2003b). Other maternal behaviors, including licking and nursing, are unaffected by such lesions (Li & Fleming, 2003a, 2003b). Maternal memory is also impaired following central infusions of cycloheximide (a protein synthesis inhibitor) into the NA shell or the medial preoptic area immediately after a brief maternal experience. Infusions to the NA core do not show this same impairment. This suggests that maternal memory, like many other memories, requires new proteins for its consolidation (Li & Fleming,

2003a, 2003b; Runyan & Dash, 2005; Touzani, Puthanveettil, & Kandel, 2007). This finding is consistent with the view that the NA is important for the expression of a number of motivated behaviors, including sexual behavior (Moses, Loucks, Watson, Matuszewich, & Hull, 1995), and is involved in learning and memory consolidation (Cheng, de Bruin, & Feenstra, 2003; Setlow & McGaugh, 1998, 2000).

The NA is densely innervated by dopaminergic neurons of various dopamine (DA) receptor subtypes, many of which originate in the ventral tegmental area (VTA; Setlow, 1997). In addition, the DA pathway projecting from the VTA to the NA shell is believed to play a large role in the mediation of the rewarding effects of various stimuli (Ikemoto, Glazier, Murphy, & McBride, 1997). It is possible that the NA's involvement in the mediation of maternal memory consolidation is through a dopaminergic receptor system. Recent work from Bridges's group suggests that the D_2 receptor systems, but not the D_1, are necessary for the retention of maternal memory (Byrnes, Rigero, & Bridges, 2002). Peripheral administration of D_2 blocker clebopride (0.5 or 1.0 mg/kg/day) via osmotic minipump during parturition significantly delays the onset of maternal behavior when tested 7 days later. Animals treated with D_1 blocker SCH-23390 do not show the maternal memory deficit.

On the basis of these findings, and the fact that high levels of both D_1 and D_2 receptor subtypes within the NA shell are detected (Bardo & Hammer, 1991), we hypothesized that D_1 and D_2 receptors within the NA shell are involved in mediating the expression of maternal behavior and that D_2 receptors are critically involved in the consolidation of maternal memory. We designed the present study to test this hypothesis. We microinfused a D_1, a D_2, or a D_1/D_2 antagonist into the NA shell and examined how these treatments affected maternal memory. We hypothesized that animals treated with a D_2 antagonist or a D_1/D_2 antagonist immediately following a 1-hr experience with pups would exhibit a delayed latency to perform maternally after 10 days of pup separation; however, animals treated with a D_1 receptor antagonist would not show a maternal memory deficit.

Method

Subjects and Housing

Fifty-eight nulliparous female Sprague–Dawley rats (about 70–90 days of age), housed and mated at the University of Toronto at Mississauga animal vivarium, were used in this study. Animals were derived from a stock originally obtained from Charles River Laboratories (St. Constant, Quebec, Canada). The rats were individually housed in transparent Plexiglas cages (47 cm × 26 cm × 20 cm) in a temperature- and humidity-controlled environment (22 °C and 45%–55%, respectively) on a standard 12-hr light–dark cycle (lights on at 0800). Purina Rat Chow and water were available ad libitum, and bedding consisted of wood shavings. Dams used to provide donor pups for retention testing were also maintained. All methods used complied with the Canadian Council on Animal Care guidelines for care of laboratory animals and were approved by the Local Animal Care Committee.

Procedure

Females were placed in a cage with a stud male for 7 days to ensure pregnancy. Between Gestation Days 10 to 14, bilateral guide cannulas aimed at the NA shell were implanted. Beginning on Gestation Day 21, subjects were checked for parturition at 30-min intervals, between 0800 and 2000. When signs of parturition were evident, pups were "caught" (systematically removed from the mother at 15-min intervals) until the entire litter had been delivered. Once parturition was assessed as complete (no more pups caught following three more intervals), the dam was removed from the birthing cage and placed into a large transparent maternal observation cage. Two shredded paper towels were placed in the cage to provide the dam with nesting material. Dams were then housed in a room without any pups to ensure complete pup deprivation.

On PND1, 1 day following mother–pup separation, all rats were exposed to 6 donor pups (3 male, 3 female) for a 1-hr period. A 10-min baseline maternal behavior test was performed at the initiation of the 1-hr period to ensure maternal behavior was present before any manipulations. Rats were then randomly assigned to receive infusions of either a drug or a vehicle immediately following this exposure period. Following infusions, the dams were placed in pup isolation for a period of 10 days. On PND11, maternal retention tests were conducted on a daily basis for a maximum of 11 days. Animals that became maternal (showing the full range of maternal behaviors) immediately on reintroduction of pups were given a maternal latency score of 0. An animal that did not become maternal by the 10th day of retention testing was given a maximum score of 10. On completion of behavioral observation, rats were overdosed using sodium pentobarbital (Somnotol, 0.9 ml/kg; MTC Pharmaceuticals, Cambridge, Ontario, Canada) and perfused intracardially, and cannula placements were verified using histological analysis.

Stereotaxic Surgeries

Females were anesthetized with a Ketamine (MTC Pharmaceuticals) and Xylazine (Rompun, Bayer Inc., Etobicoke, Ontario, Canada) cocktail (1.5 ml/kg, ip). Rats were also injected (ip) with Ketoprofen (Anafen, MERIAL Canada Inc., Morgan Baie d'Urfe, Quebec, Canada; 0.5 ml/kg, sc) to provide analgesia. Following injections, the rat's head was shaved and mounted into a small-animal stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) with the incisor bar adjusted to −3.0 mm relative to the interaural line.

Following placement into the apparatus, a midline incision was made, and the skin and periosteum were retracted to make bregma clearly visible. Two holes were drilled for implanting surgical screws, to serve as anchors for the guide cannulas. Bilateral guide cannulas were implanted, using coordinates obtained from a standard atlas of the rat brain (Paxinos & Watson, 1998).

Twenty-six-gauge stainless steel bilateral guide cannulas (Plastics One, Roanoke, VA) were then implanted into the NA shell region (coordinates: AP +1.56 mm; ML ±0.75 mm; DV −6.5 mm from the skull surface). A control group of females were implanted with cannulas in the dorsal hippocampal region (coordinates: AP −3.8 mm; ML ±1.5 mm; DV −3.8 mm). This site was chosen as a control for testing whether the D_1/D_2 neurotransmitter system in the NA shell is specific for maternal memory. The cannula and the screws were affixed to the skull using dental cement. Solid steel 6.2-mm dummy cannulas (Plastics One) were inserted into the guide cannulas to maintain patency and were subsequently removed only during the infusion procedure; a dust cap was screwed onto the

guide cannula to keep the dummy in place. Lidocaine (analgesic; AstraZeneca, Mississauga, Ontario, Canada) and Hibitane (antibacterial; Ayerst, Guelph, Ontario, Canada) were applied to the surgical site to prevent pain and bacterial infection, respectively.

Following surgery, rats were placed in clean cages and maintained in a recovery area on heating pads until they awoke. Rats perceived to be in good health were placed back into the animal housing area. Postoperative checks were done for 1 week after surgery to ensure the optimal healing of wounds and minimal weight loss.

Maternal Experience (Baseline–Pup Exposure) Test

All subjects were permitted to interact with 6 (3 male and 3 female) freshly fed 1- to 3-day-old pups for 1 hr between 0900 and 1100. During the initial 10 min of pup exposure the dams' maternal responses were recorded in their home cages. All 6 pups were placed diagonally to the nest site. Using BEST Collection software (University of Toronto at Mississauga, Mississauga, Ontario, Canada), the experimenter recorded the frequency and duration of several behaviors over a 10-min period: (a) retrieval, in which the dam lifted a pup with her mouth and carried it across at least one quadrant (typically to the nest site); the total number of pups retrieved, as well as the latency to retrieve the first through sixth pup, was recorded; (b) pup sniffing, in which the rat places the tip of her snout close to or in contact with the pups; (c) pup licking, opening the mouth and placing the tongue directly on the pup; this can consist of general body licking or licking of the anogenital region; (d) nursing posture, when the dam positions herself over the pups and arches her back in such a way that permits suckling; high and low crouch postures are differentiated here; (e) hovering, when the rat is on top of the pups performing other maternal behaviors (e.g., licking); (f) nest building, when the dam picks up nesting material in her mouth and carries it back to the nest site or pushes nesting material closer to the nest site with her forepaws; and (g) general non-pup-directed behaviors; eating, self-grooming, and sniffing air are all included in this category.

Drugs and Infusions

Fifty-eight rats were handled daily to acclimate them to the handling procedure, allowing infusions to be performed without use of anesthesia. Immediately after the 1-hr experience period, pups were removed from the cage, and rats were given bilateral microinfusions (0.5 μl/side) of one of the following: (a) 3.0 μg/μl of (-) Sulpiride (SUL); (b) 5.0 μg/μl of SCH-23390 (SCH); (c) 10.0 μg/μl of cis-Z-flupenthixol (FLU); (d) 10.0 μg/μl of cis-Z-flupenthixol to the control site (CON-FLU); or (e) 0.9% physiological saline (VEH). Drugs were dissolved in physiological saline before being infused. Drug doses were based on previous studies (Keer & Stern, 1999; Miller & Lonstein, 2005; Setlow & McGaugh, 1998) and on pilot studies from our laboratory that caused a deficit in maternal behavior that lasted less than 24 hr. Infusions were delivered over a 60-s period (at a rate of 0.5 μl/min) via 500 μl Bas gas-tight syringes (MD-0050, Bio Analytical Systems) connected to PE50 tubing with 26-gauge internal cannula needles extending 1 mm further than the length of the guide cannula. Infusions were automated by a Harvard infusion pump (Harvard Apparatus Inc. 22, Natick, MA), which delivered drug solutions over the 1-min period. Injection cannulas remained in place for an additional 60 s to minimize backflow into the cannula tracks.

Maternal Retention Tests

Subjects were tested for maternal behavior 10 days following the initial exposure to and subsequent isolation from pups (Retention Test 1, or RT1) between 1000 and 1200. Again, 6 freshly fed donor pups (1 to 2 days old) were placed into the cage in the quadrant diagonal to the nest site. The basic procedure was identical to the one used during the Maternal Experience Test. Following this maternal test, spot checks were conducted every hour after the first retention test, in which the position of the pups relative to the nest site and the mother were recorded on a two-dimensional representation of the maternal cage. Donor pups were returned to their mother the following morning, and subjects were provided with 6 new freshly fed pups.

Spot checks continued until the female was designated as maternal or for a maximum of 11 days of retention. If a rat appeared to have retrieved pups into the nest site, a maternal retention test was conducted and testing continued until the female reached maternal criterion. A rat was designated as maternal if, for 2 consecutive days, she retrieved all pups into the nest during the 10-min test and was observed in the nest with pups on spot checks. The latency to become maternal was considered to be the 1st of 2 consecutive days that the female fulfilled the above criteria. Pups were between 1 and 4 days old during maternal retention testing.

Statistical Analysis

Because of the variability in latency data and the absence of homogeneity of variance in the data, latencies to express maternal behaviors were compared across each of the five groups (SUL, SCH, FLU, VEH, and CON-FLU) using a nonparametric between-group Kruskal-Wallis test. Mann–Whitney U tests were conducted as post hoc analyses when main effects were found. Latency to express maternal behavior after the isolation period was analyzed in days. The cumulative percentages of rats that responded maternally across testing days overall and within each of the four groups were also calculated.

The duration and frequency of pup-directed maternal behaviors, as well as non-pup-directed behaviors, were analyzed using analyses of variance (ANOVAs). Repeated measures ANOVAs were conducted for baseline maternal behaviors and for the 1st day of retention testing and the first 2 days of maternal behavior to analyze group differences in behavior across the testing days.

Histological Analysis

On completion of behavioral observations, animals were overdosed with sodium pentobarbital (Somnotol, 0.9 ml/kg; MTC Pharmaceuticals) and perfused intracardially with 0.9% saline followed by a 10% paraformaldehyde solution. Extracted brains were put into vials containing a 30% sucrose–formalin solution for at least 24 hr, or until the brain had sunk. Brains were frozen and sliced at 30-μm sections on a cryostat (Leica CM 1850, Nussloch, Germany). Slices were mounted on gel-coated slides and stained with cresyl violet. Microscopic examination was used to determine location of microinfusion sites.
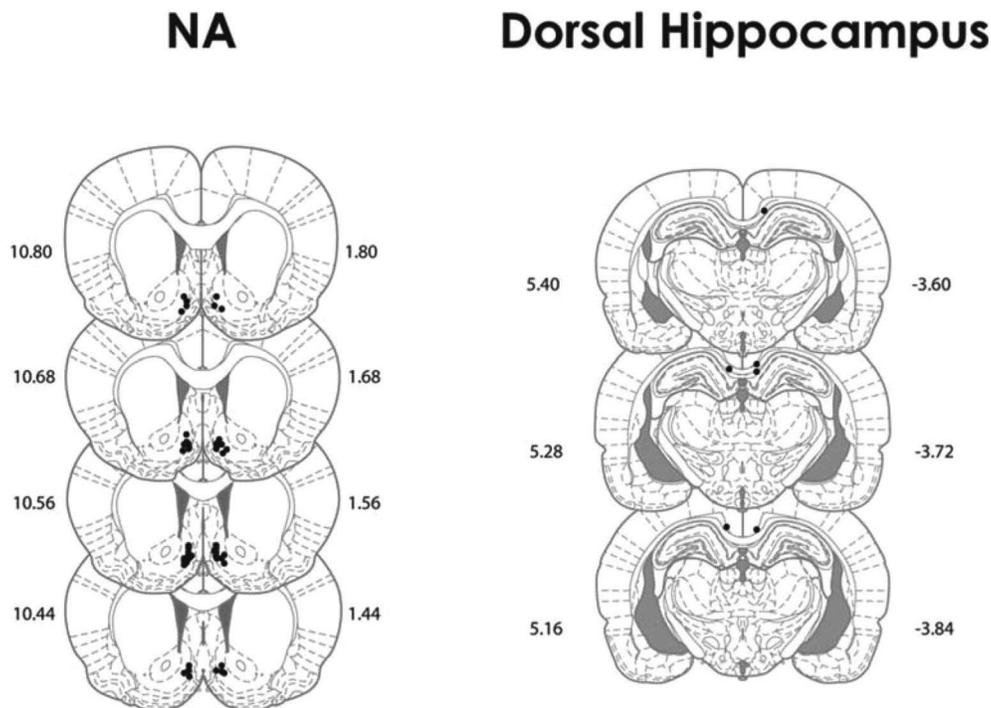
NA

Dorsal Hippocampus

Figure 1. Verification of infusions to nucleus accumbens (NA) shell and control region. Of the 58 animals cannulated, 49 were accurately located in the NA shell. The control flupenthixol group consisted of 6 animals that were cannulated accurately. From *The Rat Brain in Stereotaxic Coordinates* (4th ed.; Fig. 35), by G. Paxinos and C. Watson, 1998, San Diego, CA: Academic Press. Adapted with permission.

Results

Histological Verification

Histological analysis revealed that of the 58 subjects, 49 bilateral cannulas were accurately located in the NA shell region (see Figure 1). Any of the animals that had cannulas external to the NA shell were excluded from statistical analysis.

Maternal Behaviors Exhibited During Pup Exposure

All dams except 1 gave birth to healthy litters and exhibited normal maternal behaviors during the initial experience session. One dam did not behave maternally during this baseline test period and was subsequently removed from the study. There were no between-subjects differences in displays of retrieval, crouching, hovering, licking, or nest-building behaviors during the baseline test

Effect of Drug Treatment on Maternal Memory

Latency to reach maternal criterion. In this set of analyses, the five groups of rats receiving infusions of SUL, SCH, FLU, VEH, or CON-FLU were assessed for their latency to become maternal following a 10-day pup isolation period. A five independent-samples test comparing latency to reach maternal criterion for all drug treatment groups revealed a significant effect of treatment group on latency in days, $\chi^2(4) = 19.808$, $n = 49$, $p = .001$.

Post hoc Mann–Whitney U tests revealed a significant difference in maternal latency between the SUL and VEH groups ($U = 40.0$, $p = .040$), where SUL-treated rats had longer laten-

cies. Animals that received SCH infusions had latencies that were no different from those of the VEH group. However, SCH infusions resulted in a greater variability in maternal memory in which some subjects ($n = 3$, 50%) showed longer latencies and others ($n = 3$, 50%) showed shorter latencies. When both D1 and D2 receptors were antagonized, the FLU group ($n = 10$) showed a significantly longer mean latency than the three other groups (SUL $U = 24.0$, $n = 10$, $p = .017$; SCH $U = 20.5$, $n = 6$, $p = .081$; and VEH $U = 4.0$, $n = 13$, $p = .000$). The CON-FLU group used as a control for FLU displayed a significantly lower latency than did the FLU group ($U = 1.5$, $n = 10$, $p = .000$; see Figure 2).

Cumulative percentage of maternal rats across testing days. Figure 3 shows the cumulative percentages of rats in each treatment group (SUL, SCH, FLU, and VEH) to reach maternal criterion across testing days. Both the VEH and the CON FLU groups displayed the shortest latencies to become maternal (3 and 5 days, respectively, for all animals in each group). The FLU group took the longest to reach maternal criterion; 10 days of testing were required for the entire group of animals in this group to become maternal (see Figure 3).

Effect of Drug Treatment on the Quality of Maternal Behavior at RT2

Once subjects displayed the full onset of maternal behaviors on 2 consecutive days (RT2), the frequency and duration of various key maternal behaviors were analyzed to assess whether any of the treatments had any effect on the qual-
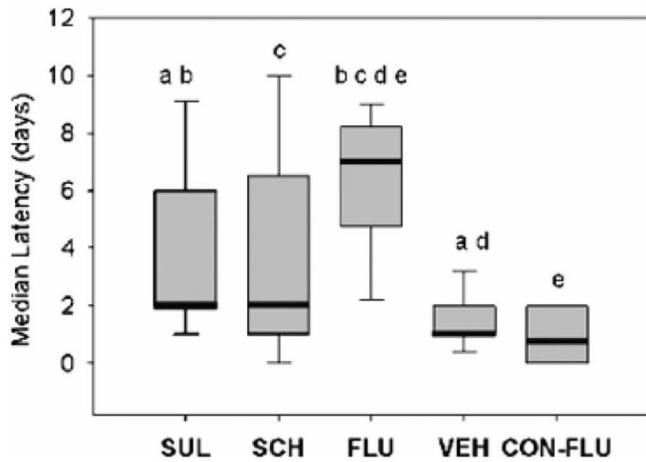
Figure 2. Median latency (in days) to exhibit full maternal behavior for sulpiride (SUL), flupenthixol (FLU), vehicle (VEH), and control flupenthixol (CON-FLU) groups. Sulpiride had a significantly longer latency than vehicle, $n = 10$, $U(1) = 40.0$, $p = .040$, and flupenthixol-infused animals had significantly longer latencies than those infused with SCH 23390 ($n = 6$, $U = 20.5$, $p = .081$), SUL ($n = 10$, $U = 24.0$, $p = .017$), and VEH ($n = 13$, $U = 4.0$, $p = .000$). The box represents the interquartile range. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. The line across the box indicates the median ($p < .05$) compared with the corresponding FLU and VEH control groups.

ity of maternal care. The behaviors considered important included pup retrieval, pup licking, crouching, and nest building. No significant differences were observed in the frequency or duration of these behaviors between the treatment groups once they reached criterion. All subjects displayed the same quality of care to the pups during this test (see Figure 4). Refer to Table 1 for the full onset of all behaviors displayed.
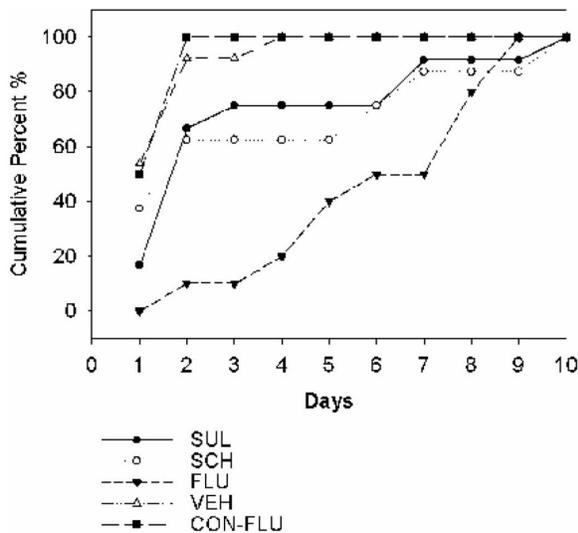

Figure 3. Cumulative percentage of rats in each group displaying maternal behavior on each of the 10 maternal retention testing days. The FLU-infused rats had the lowest percentage of maternal rats across the testing period. SUL = sulpiride; SCH = SCH-23390; FLU = flupenthixol; VEH = vehicle; CON-FLU = control flupenthixol.
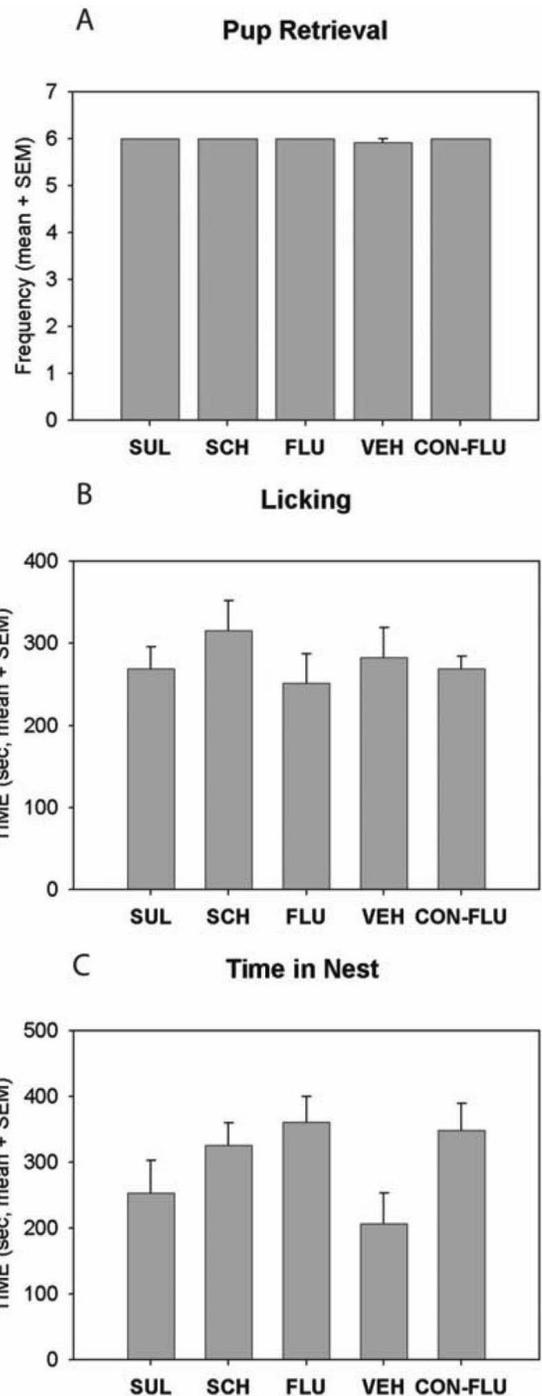
Figure 4. Mean frequency and duration of maternal behaviors once maternal, shown by rats in all drug and control groups once (M + SEM; SUL, $n = 10$; SCH, $n = 6$; FLU, $n = 10$; VEH, $n = 13$; CON-FLU, $n = 10$). SUL = sulpiride; SCH = SCH-23390; FLU = flupenthixol; VEH = vehicle; CON-FLU = control flupenthixol.

Discussion

The present study provides evidence that D_1 and D_2 receptors function in conjunction in the consolidation of maternal memory. We found that infusions of either a DA D_2 antagonist

Table 1. Effects of D_1/D_2 Receptor Antagonism on the Quality of Maternal Behaviors

Behavior	VEH	SUL	SCH	FLU	CON-FLU	$F_{(4, 44)}$	p
Maternal behavior at baseline (before drug infusion)							
Hover (seconds)	240.5575 ± 34.35528	276.81 ± 39.27794	329.7314 ± 36.53331	258.6036 ± 29.77888	374.6877 ± 29.58924	1.89	.129
Anogenital licking (seconds)	34.9759 ± 12.17230	27.1909 ± 7.11844	29.7878 ± 9.45788	12.9161 ± 5.65156	54.7720 ± 11.67452	1.848	.137
Body licking (seconds)	197.3168 ± 27.69214	221.5219 ± 23.94468	275.1392 ± 27.32984	247.9003 ± 29.85174	331.5398 ± 5.63773	3.048	.027
Mouthing (frequency)	5.8462 ± 1.78256	12.5 ± 1.85660	7.25 ± 4.62814	3.9 ± 1.33708	1.5 ± .95743	3.085	.025
Nest building (seconds)	17.7449 ± 11.75151	0.3043 ± 0.21282	9.5654 ± 6.29913	13.1855 ± 7.07963	6.8815 ± 5.22975	0.782	.543
Sniff pup (seconds)	48.9979 ± 16.33772	60.1138 ± 19.27191	14.1433 ± 4.11452	22.9981 ± 6.21531	25.8625 ± 7.21769	1.722	.162
Retrieval (frequency)	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	0.613	.656
Low crouch (seconds)	8.52169 ± 6.22496	2.93783 ± 2.778475	0 ± 0	15.56640 ± 15.566400	0 ± 0	0.586	.675
High crouch (seconds)	5.8293 ± 5.82931	0 ± 0	1.7846 ± 1.78463	13.7656 ± 13.76560	0 ± 0	0.637	.639
Behaviors on reaching maternal criterion (RT2)							
Hover (seconds)	185.8745 ± 45.35.35	251.0423 ± 48.71378	320.9668 ± 31.73518	247.6836 ± 21.24533	291.3835 ± 38.80547	1.371	.261
Genital licking (seconds)	85.7226 ± 16.89137	81.7560 ± 12.07420	68.8282 ± 22.42822	65.0644 ± 16.27631	27.3698 ± 22.31016	1.423	.244
Body licking (seconds)	169.7702 ± 22.19483	187.2621 ± 21.37414	246.6373 ± 18.25339	186.4393 ± 30.43006	255.0488 ± 21.96008	2.036	.107
Mouthing (frequency)	6.1538 ± 2.09959	7.3636 ± 1.3203	5.6667 ± 1.66667	2.7 ± 0.65064	3.8333 ± 2.88001	1.128	.357
Nest building (seconds)	14.6995 ± 6.85803	10.6304 ± 5.30157	36.0415 ± 20.71542	3.332 ± 1.80872	33.6913 ± 22.26161	1.657	.178
Sniff pup (seconds)	25.6369 ± 13.67545	26.9710 ± 16.39961	11.5822 ± 8.75736	7.2287 ± 4.41707	13.4115 ± 8.46212	0.499	.736
Retrieval (frequency)	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	0.613	.656
Low crouch (seconds)	3.8928 ± 2.86099	0 ± 0	47.5307 ± 47.53071	34.2344 ± 20.37247	51.6960 ± 9.44116	0.942	.449
High crouch (seconds)	4.0489 ± 2.84445	0 ± 0	4.0690 ± 4.0690	15.6602 ± 12.44900	0 ± 0	1.03	.403

VEH ± vehicle; SUL ± supiride; SCH ± SCH-23390; FLU ± flupenthixol; CON-FLU ± control flupenthixol

(sulpiride) or a combined DA D_1/D_2 antagonist (cis-Z-flupenthixol) impaired the consolidation of the maternal experience. The 3.0-µg/µl dose of SUL resulted in a 2-day latency to reach maternal criterion and a 10-µg/µl dose of FLU resulted in a 7-day latency to reach maternal criterion, both significantly longer than the VEH control group. Furthermore, 70% of females receiving 3.0 µg/µl of SUL reached maternal criterion and only 10% of females receiving 10 µg/µl of FLU reached maternal criterion by Day 3 of retention testing, compared with 100% of females in the VEH control group. Additionally, the CON-FLU group was used as a control for testing whether the D_1/D_2 neurotransmitter system within the NA shell was specific for this type of memory. Indeed, females receiving 10 µg/µl of FLU in the control region (dorsal hippocampus) resulted in a 1.5-day latency to reach maternal criterion compared with a 7-day latency in the NA shell FLU group. Of females in the CON-FLU group, 100% showed the full variation of maternal behaviors by Day 3 of retention testing compared with 10% of the NA shell FLU group. There was no difference between the CON-FLU group and the VEH group in the latency to reach maternal criterion.

Interestingly, there was only a marginal effect of SCH-23390 on maternal memory. Females receiving 5.0 µg/µl of DA D_1 antagonist SCH-23390 displayed a less than 2-day latency to reach maternal criterion. Matching the effect of VEH and CON-FLU, the SCH dose was significantly different than both the DA D_2 and DA D_1/D_2 antagonists. Our experiment showed an additive effect of combined DA D_1/D_2 antagonist FLU but only a marginal effect of DA D_1 antagonist SCH, which would suggest that both receptor subtypes could be working in tandem with one another to regulate behaviors that are mediated by the mesolimbic DA system, a hypothesis that has been suggested in previous work (Dall'Olio, Roncada, Vaccheri, Gandolfi, & Montanaro, 1989). Although we found that the effect of SCH on maternal memory was marginal, we note that there is an increase in variance in relation to the VEH group. This may have been a result of the drug or could be due to the fewer number of rats in this group compared with the other groups, obscuring the possible significant group differences (i.e., drug effect). This group consisted of two subgroups: 50% ($n = 3$) of the rats had long maternal latencies, and the remaining 50% had short latencies, whereas in other groups (e.g., VEH) all rats had short maternal latencies. Although nonparametric statistical tests show this group is no different from the VEH group, its increased variability may be a result of the dose of SCH used in this study. It is possible that an increased dose of SCH might have a stronger effect on maternal memory. It will be beneficial to examine dose responses to these antagonists to examine their effect on D_1/D_2 receptors.

Our findings suggest an interaction between both D_1 and D_2 receptor subtypes on maternal memory consolidation. Rats infused with either a D_2 or a D_1/D_2 combined DA antagonist following a brief maternal experience displayed a delayed onset of maternal behavior once reintroduced to a litter of pups. Because consolidation was not blocked with a D_1 antagonist alone, a primary role for D_2 receptors in this process is suggested. However, this does not exclude DA D_1 receptors from the bigger picture. There is a considerable amount of work that emphasizes the role of both DA receptors in the expression of maternal behavior (Keer & Stern, 1999; Miller & Lonstein, 2005; Numan, Numan, Pliakou, Stolzenberg, Mullins,

Murphy, & Smith, 2005). Our findings do in fact follow in accordance with other work on maternal memory (Byrnes et al., 2002), suggesting that D_1 receptor function is more important to the immediate expression of maternal behavior and does not substantially affect the formation of the memory for the maternal experience. Recent work by Stolzenberg et al. (in press) showed that administration of the D_1 receptor agonist SKF-38393 into the NA shell promoted the onset of maternal responsiveness in naïve dams with terminated pregnancies. The DA D_1 agonist was infused shortly after the termination of pregnancy, amplifying the activation of the D_1 receptor minus the experience of parturition. This increased activation reduced the latency to respond maternally when presented with pups. Interestingly, using the same process to activate DA D_2 receptors with Quinpirole, a D_2-specific agonist, in the same brain region did not promote maternal responsiveness. This finding is pivotal and complements the results of the present study by parsing out possible individual functions for each DA receptor. It is possible that differing doses of the drugs used in this study would show a stronger or weaker effect of D_1 or D_2 antagonism, helping to reveal whether blockade is truly due to receptor importance. However, the doses chosen were based on an active interruption of maternal behavior without long-lasting effects, which was tested previously in our lab. Future work should include multiple doses and examine how maternal memory is affected by the manipulation of DA D_1 and D_2 receptors.

Several studies have established that DA is released into the NA while a postpartum female interacts with her offspring (Champagne et al., 2004; Hansen, Bergvall, & Nyiredi, 1993). The neurochemical response in the NA is stronger in primiparous females than in nulliparous females that have not experienced parturition. However, there is still a response to pups regardless of previous reproductive experience demonstrated by the number of Fos-ir cells in the NA shell and not the core (Scanlan, Byrnes & Bridges, 2006). Therefore, although the NA shell helps to form the memory for the maternal response in either situation, the time after parturition is when this increased activation is more important and ecologically relevant. It is apparent that an interaction between the experience of gestational hormones and DA activation in the NA shell occurs in the promotion of maternal behavior.

In our study, maternal behavior was tested for at the end of a 10-day pup isolation period, at which point the hormonal profile necessary for maternal responsiveness that exists at the time of birth is no longer present in females. On the basis of this and previous findings, it would seem appropriate to suggest that DA D_1 receptors are activated at the time when mothers are in the presence of pups, promoting attentiveness and the saliency of pups to activate a maternal response in their direction. Furthermore, DA D_2 receptors are perhaps more important once maternal responsiveness has commenced, helping to store the response in long-term memory. The activation of both receptors in the NA shell region is very likely to occur for a short period of time because we know that lesioning this site at 24 hr following a female's first maternal experience has no effect on retrieval after 10 days of isolation (Li & Fleming, 2003b). Similarly, the use of cycloheximide, a protein synthesis inhibitor, in the same region using the same time points as the lesion work produced similar results, demonstrating that brain morphology has already been altered and that memory for this behavior has already been consolidated (Fleming, Cheung, & Barry, 1990).

The significant impairment on the retrieval of the maternal experience caused by D_2 receptor antagonism complements other findings that point to the D_2 receptor as having a major role in the formation of maternal memory (Byrnes et al., 2002). Furthermore, other forms of memory such as inhibitory avoidance and conditioned fear extinction have also been linked to D_2 receptor deactivation in other brain areas (Pakdel & Rashidy-Pour, 2007; Ponnusamy, Nissim, & Barad, 2005).

The specificity of D_1 and D_2 receptors acting on the NA shell has also been seen in studies on cocaine self-administration. D_1 and D_2 receptor antagonism in the core and shell of the NA produce different effects on both cocaine self-administration and food reinforcement. Antagonism of the shell in particular, and not the core, has an effect on cocaine self-administration only, leading us to believe that the shell region modulates the reinforcing efficacy of cocaine, whereas the core is thought to have a more general influence on motivated behaviors. From their lesion work, Li and Fleming (2003a) proposed previously that the NA shell is involved in maintaining the attention or motivation that is required for pup retrieval. Hence, it is more likely that the effect of D_1 and D_2 antagonism is to reduce the efficacy of the pups to activate the maternal responding in mothers. This idea is further strengthened by the fact that we observed no effect on the quality of maternal behaviors once maternal behavior commences, isolating the effect of DA antagonism to the more appetitive aspect of the maternal response and not what would be considered consummatory behaviors. This is similar to what we see in sexual behavior following DA antagonism (Liu, Sachs, & Salamone, 1998; Pfaus & Phillips, 1991). Still, NA DA has been found to be involved in various forms of learning and memory, not restricted to reward-based learning. For example, Fenu, Bassareo, and Di Chiara (2001) found that D_1 receptor blockade impairs conditioned taste aversion learning by disrupting the formation of a short-term memory trace of the gustatory conditioned stimulus (Fenu, Bassareo, & Di Chiara, 2001). Wadenberg, Ericson, Magnusson, and Ahlenius (1990) found that DA D_2 receptor blockade inhibits expression of active conditioned fear response. The exact role of NA DA in learning and memory is still under debate; however, it appears that NA DA is selectively involved in modulating the strength of a memory trace (Wise, 2004) as opposed to encoding specific information. This view is consistent with the current finding showing that NA DA is important for the consolidation of maternal memory.

Recent work has attempted to map the maternal circuit of the NA with the medial preoptic area (MPOA), VTA, and ventral pallidum (VP), all regions shown to be active participants in the expression of maternal behavior. It is proposed that connections from the MPOA either directly to the NA or indirectly via the VTA inhibit the NA shell, thereby disinhibiting the inhibitory connections (GABAergic) from the NA to the VP, resulting in maternal behavior. Numan, Numan, Schwarz, Neuner, Flood, & Smith (2005) proposed an indirect pathway through the VTA to the NA shell via dopaminergic projections from the VTA. DA is believed to suppress the inhibition of the NA shell. This would fit with other work because DA inhibition in the NA shell usually results in a deficit of maternal behavior. We take this a step further by suggesting that DA receptor inactivation in the NA shell might block the inhibitory action of the VTA to the NA shell, which continues GABA's inhibitory action to the VP, disrupting maternal behavior (see
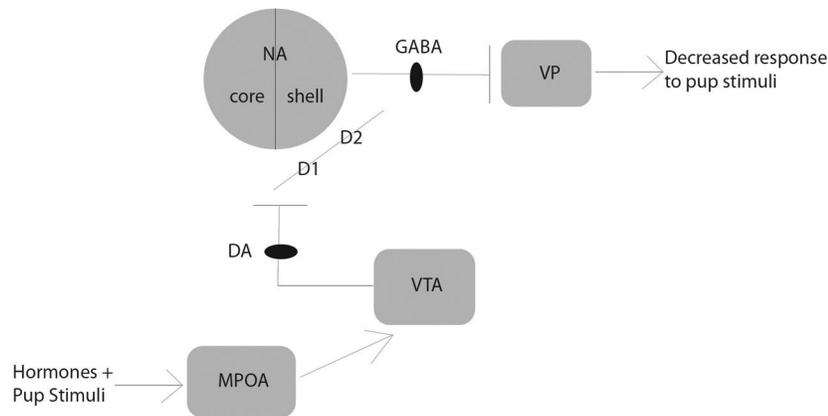
Figure 5. Neural model showing how the ventral tegmental area (VTA), nucleus accumbens shell (NA shell), nucleus accumbens core (NA core), and ventral pallidum (VP) might result in an inhibition of maternal behavior, with dopamine (DA) D_1 and D_2 receptor blockade. In a maternally experienced, hormone-primed female, D_1 and D_2 antagonism prevents the inhibitory actions of the DA projections from the VTA, which causes an increased activation of both the NA and VP. This increased activation in the NA and VP results in decreased responsiveness to pup stimuli. From "The Effects of D_1 or D_2 Dopamine Receptor Antagonism in the Medial Preoptic Area, Ventral Pallidum, or Nucleus Accumbens on the Maternal Retrieval Response and Other Aspects of Maternal Behavior in Rats," by M. Numan, M. J. Numan, N. Pliakou, D. S. Stolzenberg, O. J. Mullins, J. M. Murphy, et al., 2005, *Behavioral Neuroscience,* 119, p. 1601. Copyright 2005 by the American Psychological Association. Adapted with permission of the author. The use of APA information does not imply endorsement by APA.

Figure 5). Lesions of the NA shell (Li & Fleming, 2003b) after a brief maternal experience have been shown to delay maternal onset after a period of isolation. Maternal behavior, however, does become reinstated after some period of time, showing that the NA shell is necessary but not sufficient for maternal memory to form. As mentioned previously, it is likely that the NA in general is a site that promotes the reinforcing properties of pups during the expression of maternal behavior. In particular, during the initial expression of this behavior with a dam's first litter, the shell serves to reinforce and consolidate the memory for the rewarding value of pups. Hence, the consolidation process is stunted if deactivated periodically immediately after the first maternal experience, delaying the subsequent response to pups after a period of isolation.

That DA receptors in the NA shell may serve to promote the saliency of pups and enhance motivation to respond maternally is highly probable because D_1/D_2 antagonism in other areas like the MPOA seem to have different effects. DA D_1 and D_2 antagonism in the MPOA results in reduced oral-type behaviors such as licking, retrieval, and mouthing; however, it has little effect on the ability to initiate contact with pups (Miller & Lonstein, 2005). This suggests that the NA shell may affect the motivational component necessary for the onset of the behavior and, combined with our findings, the reward–saliency component for the consolidation of the behavior for the long term.

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