Hormones that increase maternal responsiveness affect accumbal dopaminergic responses to pup- and food-stimuli in the female rat

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ABSTRACT

The present study investigated hormonal mediation of maternal behavior and accumbal dopamine (DA) responses to pup-stimuli, as measured in microdialysis samples collected from the nucleus accumbens shell of female rats in non-homecage environment. In Experiment 1, samples were collected before and after continuous homecage pup experience from either intact postpartum or cycling females. In Experiment 2, samples were collected before and after responding maternally in homecage from ovariectomized females given either parturient-like hormone or sham treatments. After baseline sample collection in the dialysis chamber, pup and food stimuli were individually presented to females. Upon sampling completion, all animals were placed back into their homecage with donor pups for several days, and then the sample collection procedure was repeated. Prior to stimulus presentation, postpartum and hormone-treated females had decreased basal DA release compared to their controls. In response to pup stimuli, only postpartum and hormone-treated females had increased DA release compared to basal release (both sampling days). In response to food stimuli, all females had increased DA responses from basal; although there were group differences on the initial day of sampling. Findings suggest that hormones associated with inducing maternal behavior in the postpartum rat play a significant role in modifying accumbal dopaminergic responses on first exposure to pup stimuli in the rat. However, the postpartum experience provides further modifications to this brain region to promote DA responses to pup stimuli.

Introduction

The immediate onset of maternal responsiveness at or just before parturition is a result of the hormonal milieu associated with pregnancy and parturition (Bridges, 1990; Krehbiel and LeRoy, 1979; Mayer and Rosenblatt, 1979, 1980, 1987; Moltz et al., 1970). These hormonal effects can be mimicked in the ovariectomized virgin female by treating the female with hormones (e.g., estrogen and progesterone) normally associated with elevated maternal responsiveness at parturition (see Numan et al., 2006; Bridges, 1984; Novakov and Fleming, 2005; Rosenblatt et al., 1987; Stern and McDonald, 1989; Mayer et al., 1990a,b). In the postpartum rat, the onset of maternal behavior is mediated by hormones, but is sustained by recent sensory experiences interacting with the pups (for review, see Numan et al., 2006). Hormones exert their stimulatory effects on several brain areas to facilitate the rapid induction of maternal responsiveness (Mann and Bridges, 2001) and to reduce the initial neophobic responses to pups commonly seen in virgin rats (Fleming and Luebke, 1981; Hard and Hansen, 1985).

Hormones and experience act on the onset and maintenance of maternal behavior by acting on sites within the ‘maternal circuit’ (see Numan et al., 2006): comprised of an output projection pathway from the medial preoptic area to the ventral tegmental area, nucleus accumbens, and hindbrain; an input projection pathway to the medial preoptic area directly from the amygdala, bed nucleus of the stria terminals, nucleus accumbens, and cortex; and, indirect input from multiple sensory systems, especially the olfactory system (Numan and Insel, 2003; Numan, 2007; Numan et al., 2006). This report focuses on one component of the system, the nucleus accumbens (NAC) and the role of dopaminergic inputs into this site from the midbrain. Activity of the mesolimbic dopamine (DA) system, and in particular the NAC, has been shown to mediate many species-typical behaviors, including maternal behavior (see Fleming et al., 2008). For example, DA-depleting 6-OHDA lesions of the NAC disrupt many maternal behaviors and infusions of D1 or D2 receptor antagonists into the NAC reduce pup-retrieval and -licking (Parada et al., 2008, also see Numan et al., 2005). In contrast, infusions of the D1 receptor agonist into the NAC enhance pup-retrievals in pregnancy-terminated rats that would not normally be maternally responsive (Stolzenberg et al., 2007). Finally, in microdialysis studies, presentation of pups to a lactating dam increases extracellular DA release in this region (Hansen et al., 1993; but also see Champagne et al., 2004; Ferris et al., 2005; Hansen et al., 1991a,b; Olazábal et al., 2004).
The present study investigated hormonal mediation of NAC DA responses to pup- and food-stimuli, as measured from microdialysis samples collected from the female rat that were either: intact, postpartum, and lactating (Experiment 1); or, ovariectomized (OVX) and treated with hormones normally associated with elevated maternal responsiveness at parturition (Experiment 2).

Methods

General methods

Experimental design

Prior to the surgical implantation of guide cannulae above the NACshell (NACsh), female rats were assigned to one of two experiments. In Experiment 1, gonadally intact females had given birth (Postpartum) or had not (Cycling). In Experiment 2, OVX-pregnant females were either given parturition-like hormone (Hormone, via silastic capsules) or sham (Sham, via empty silastic capsules) treatment. On postpartum day 1 (PPD1) or 24 h after progesterone/sham capsule removal, females and their controls were sampled for DA and 3,4-dihydroxyphenylacetic acid (DOPAC) responses to donor pup- and food-stimuli. After the first collection procedure, females were placed in their homecage and given continuous access to donor pups. Prior to this, only postpartum females had some pup-experience. On PPD5 (Experiment 1) or after responding maternally in homecage (Experiment 2), all females were sampled again using the same sample collection procedure. Additionally, females were tested for their behavioral responses when given simultaneous choices of pup- and food-stimuli (choice task) immediately after each sample collection. This task provided information about pup-preference in the sampling chamber.

Subjects

Sprague-Dawley female rats (n = 40; 225–300 g) obtained from the colony bred at the University of Toronto at Mississauga, were housed individually in transparent Plexiglas cages (47 cm x 26 cm x 20 cm) and given food and water ad libitum on a standard 12:12 light cycle. Males (n = 16; 400–600 g) and pregnant females (n = 40; 300–425 g), obtained from the same breeder and housed as above, served for mating and donor (primiparous) mothers. All female rats were housed in colony rooms that contained no pregnant females. All females when mated or given donor pups were placed into a colony room that contained other pregnant or nursing females.

Cannula placement

All females, under general sodium pentobarbital anesthesia, (Somnotol, MTC Pharmaceuticals, 65 mg/kg, i.p.) with a pre-anesthetic (Atropine, 0.1 cc., s.c.), were stereotaxically (David Kopf Instruments, Tujunga, CA) implanted with a unilateral guide cannula with a stylet (15 mm in length, BAS, West Lafayette, IN, USA) aimed above the NACshell (flat skull; 1.7 mm caudal to bregma, 0.5–1.0 mm lateral to midline, 6.9–7.3 mm beneath the surface of the skull; Paxinos and Watson, 1986). The cannulae were secured in place with dental acrylic cement to three stainless steel screws (BAS, West Lafayette, IN, USA) inserted into the skull. All surgical procedures were in strict accordance with guidelines provided by the Canadian Council of Animal Care Committee and approved by the University of Toronto Animal Care Committee.

Dialysis chamber and habituation

During habituation females were moved into a room (illuminated with red light) that contained the caging system (chamber) (BAS, West Lafayette, IN, USA) in which dialysate samples were collected. The interactive caging system allowed freedom of movement in all directions. The dialysis chamber (clear circular plastic cage 188 cm², 24.77 cm diameter, 29.8 cm high), had two stainless steel dispensers. Once before surgery and a second time just prior to the first sampling, all females were given 4 consecutive 1-hour sessions in the dialysis chamber.

In vivo sampling procedure

Prior to probe placement, the inlet and outlet portions of the probe were attached to polyethylene (PE10 tubing (30 cm long). Attached at the other end of the: (a) inlet tubing was a syringe pump delivery system; and, (b) outlet tubing was a needle that dispensed the collection samples into vials kept refrigerated (4 °C). In addition, the vials contained a standard antioxidant (2.5 mM ascorbic acid in 0.9% saline, Sigma Uldridge, St. Louis, MO). Once females were tethered in the chamber, the stylet was removed from the guide and the probe (320 μm OD, 2 mm length, cut off 20,000 Da, BAS, West Lafayette, IN, USA) was placed into the guide cannula. The probe extended 2 mm below the guide. Samples were collected at a rate of 1 μl/min for 8 min.

Stimuli presentation during in vivo sampling procedure

After 25 baseline samples were collected (200 min), 4 warm recently-fed donor pups (2 male, 2 females, postnatal days 2–4) were placed on shredded paper towel (nesting material) in one of the dispensers for the duration of three 8-min sample collections (i.e., pup-stimuli for 24 min). Behavioral data during the pup-stimuli exposure was filmed with a digital video camera attached to a tripod. After pup removal and 16 min (2 samples) with no stimuli, 4 Fruitloops® were placed directly on the chamber floor. Previously we have piloted the procedure with the 4 of these food-stimuli placed in the dispensers on shredded paper. When sampling, females wulld pull the sampling lines out seeking food below the shredded towel. Food-stimuli were given to the present females in their homecage and during the habituation sessions. During sampling, food-stimuli were always eaten within an 8-min period (1 sample). After food-stimuli were eaten, the sampling procedure continued for 120 min with no stimuli.

Choice task (post-sampling)

Following sample collection procedure, probes were removed and the female was placed into her homecage. One of the dispensers was filled with Fruitloops® and the other with four donor pups on shredded paper towel. The female was placed back into the chamber (unattended, no sampling) and her behavior was video-taped for 8 min. Durations spent eating and occupied in maternal behaviors were summed for this 8-min choice task. Eating behavior was defined as any time the food-stimulus was in the animal’s mouth or forepaws (i.e. retrieving food from dispenser, biting off pieces, and chewing). Maternal behavior was defined as retrieving pups or nesting material from dispenser, licking pups, and hovering over pups.

Perfusion and histology

At the end of the experiments, females were sacrificed by an overdose of sodium pentobarbital (120 mg/kg, i.p.) and perfused intracardially with ice-cold phosphate-buffered saline (300 ml) followed by ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (300 ml). Brains were removed, postfixed in fresh 4% paraformaldehyde for 4 h, blocked, and stored overnight in 30% sucrose at 4 °C. The brains were frozen using dry ice and sliced into coronal sections (30 μm) using a cryostat. These sections were mounted on gel-coated slides, stained in cresyl violet, cover slipped, and examined under a microscope to confirm placements.

High performance liquid chromatography

After collection of the samples, the concentrations of DA and 3,4-dihydroxyphenylacetic acid (DOPAC) were determined. A BAS 460 HPLC system with electrochemical detection (BAS, West Lafayette, IN) was used together with a Uniget C-8 reverse phase column (BAS Cat no. 8949). The mobile phase consisted of buffer [0.1 M monochloro
acetic acid, 0.5 mM Na-EDTA, 0.15 g/L Na-octylsylfonate and 10 mM sodium chloride, pH 3.1], acetonitrile and tetrahydrofuran (Sigma) at a ratio 94:3.5:0.7. The flow rate was 0.5 ml/min and the working electrode (Iniget 3 mm glassy carbon, BAS P/N MF-1003) was set at 700 mV vs. Ag/Ag/Cl. Detection gain was 1.0 nA, filter was 0.2 Hz and detection limit was set at 500 nA. Of the 8 μl collected per sample, 5 μl was directly injected into the HPLC for analysis. The remaining 3 μl of the sample was used in combination with external standards of DA and DOPAC (Sigma) to quantify and identify the peaks on the chromatographs. The retention times for DA and DOPAC were approximately 3.32 and 5.56 min, respectively, under the set conditions.

Parity methods (Experiment 1)

Females (N=20) were either placed with males for mating (Postpartum) or left alone (Cycling) in their homecages for 10 days. Cycling and pregnant (days 14–16 gestation) females were given guide cannulae placed into the NACsh. On PPD 1, homecage litters were removed from the newly postpartum mothers and for all females the in vivo sampling procedure followed by the choice task was performed (see general methods for details). After PPD1 sampling, all females were placed into their homecage with well fed foster donor pups (2 male, 2 female, postnatal days 1–3). After 4–5 days with pups, females were sampled again (PPD5). Prior to PPD5 sampling, all pup-experienced females were tested for homecage maternal responding. Postpartum females were observed to retrieve, lick, and hover over pups with intermingled nest building bouts in this 10-min observation. Cycling females did not perform these behaviors in their 10-min homecage test prior to either sampling session.

Hormone treatment methods (Experiment 2)

Pup-naive Females (N=20) were randomly assigned to hormone or sham treatment groups. At 60–75 days of age, females were anesthetized with isoflurane gas (Aerrane brand) and both ovaries were removed (OVX). Silastic capsules (0.078 in. ID×0.124 in. OD; Dow Corning, Midland MI, sealed with wood bits and Silastic Medical Adhesive, full procedure see, Novakov and Fleming, 2005) were filled with either 17β-Estradiol 3-Benzoate (E, 20 mm length; 10 mm hormone, 10 mm wooden dowel endings), progesterone (P, 40 mm length; 30 mm hormone, 10 mm wooden dowel endings) (Sigma) [Hormone] or left empty (both lengths) [Sham]. During the cannula placement surgery (see above), hormone-treated females were given an E capsule.

FIG. 1. Days to reach homecage maternal criterion (see text for details). All hormone-treated females reached maternal criterion by day 6. Second day of sample collection occurred 3–7 days after progesterone removal in these animals. Only one sham-treated female reached maternal criterion, and thus no 2nd day samples were collected for these rats.

FIG. 2. Schematics of anatomical unilateral placements (anterior to Bregma, according to Paxinos and Watson, 1986) of the “active zone” (shaded lines) of microdialysis probes in the nucleus accumbens shell at three different locations anterior to Bregma, according to Paxinos and Watson (1986) for the cycling/postpartum and sham/hormone-treated females.
implant in the dorsal region behind the neck. Two days later females were anesthetized briefly and three P capsules were implanted (s.c.). For these pup-naïve females, P capsules were removed 10 days after implant and 24 h prior to in vivo sampling procedure (see General methods for details).

**Pup-experience [maternal criterion]**

After the initial sampling procedure and choice task, all pup-naïve females were placed into their homecage with donor pups (2 males, 2 females, postnatal days 2–4, replaced daily). This procedure continued until the females reached the maternal criterion; this involved the retrieval of all pups to the nest site on two consecutive days and observations on at least one of those days of licking and crouching. Crouching observations were made 4 times daily when the females were undisturbed. When the animal reached maternal criterion, the sampling procedure and choice task was performed again. If an animal failed to reach criterion within 8 days, the second sampling procedure was not performed. Only one OVX sham implanted female met the maternal criterion within 8 days, thus analysis from the second set of samples was performed only for hormone-treated females. All hormone-treated females met the criterion within 6 days (see Fig. 1).

We used a small pup litter size (i.e., n = 4) in the sensitization and sampling procedures for both experiments because of the space limitations in the dialysis chamber. The number of pups was adequate, however, to induce maternal behavior in postpartum and hormone-treated females through daily continuous contact, with latencies that were consistent with latencies typical of this strain.

**Animal inclusion**

For an animal to be included in the statistical analysis, the guide cannula had to end above the NAC and between 0.3 and 1.0 mm from midline. Fig. 2 shows the anatomical placements of the “active” zones of the probe. In addition, only Postpartum, Cycling and Hormone-treated (not Sham-treated as no maternal criterion was met) females for whom samples could be obtained from over the repeated days were considered for analysis. Included for statistical analysis were 21 animals: Experiment 1, intact postpartum lactating dams (n = 6, Postpartum), vs. intact cycling controls (n = 5, Cycling); and, Experiment 2, estrogen + progesterone treated OVX females (n = 5, Hormone) vs. OVX sham treated controls (n = 5, Sham).

**Parity effects: Results of Experiment 1**

**Maternal behavior during in vivo sample collection**

**Summary**

Postpartum females were observed to perform many of the typical homecage (data not shown) maternal behaviors (i.e. predominantly sniffing, nest building, retrievals, licking of body or anogenital region, and hovering over pups) in the dialysis chamber. On both sampling days, postpartum females were observed to retrieve pups from the dispensers to the floor of the dialysis chamber, then engage in licking and exploratory behaviors followed by hovering over the pups with more licking bouts. There were no nursing postures in the dialysis chamber.

**Fig. 3. Parity in vivo (A) maternal behaviors and (B) corresponding dopamine (DA) measurements.** Intact postpartum (black) and cycling (white) females were sampled (every 8 min; 1 μl/min) at postpartum day 1 (PPD1, circles) and 5 (PPD5, triangles). (A) Maternal behavior data represent mean (±SEM) duration spent pup-sniffing, -licking, and -hovering. Pup-retrieval data represent the mean (±SEM) number of donor pups (total 4) retrieved from one of the dispensers. Similar to their homecage maternal behavior, during sampling cycling females only displayed pup-sniffing and postpartum females displayed all maternal behaviors. (B) DA basal release (bar graph) data represent the average DA concentration of 3 samples prior to the 24-min period of pup-stimuli exposure (shaded area on x-axis). Line graph data represent mean (±SEM) percent of basal DA release during stimuli (pups and food) presentation and removal during the collection procedure (see text). Compared to baseline, postpartum females had increased DA responses to both types of stimuli and cycling females, even after some homecage pup-experience (see text), had only increased responses to food consumption. a P<0.05 parity differences; b P<0.05 PPD differences; a∗P<0.05 for (A) behavioral data differences from 8-min; (B) DA response differences from basal release.
Partial correlations (controlling for parity) relating dopamine responses to pup-stimuli and maternal behaviors exhibited during PPD1 sample collection.

* p < 0.05, two-tailed.
** p < 0.005.

in the dispenser. Parity comparisons were made between cycling and postpartum animals for time (sec) spent pup-sniffing, pup-licking, and hovering over pups during in vivo sampling procedure. The number of pups retrieved was also scored.

### Pup-retrievals

A 2 (Parity) × 2 (Day) analysis of variance (ANOVA) revealed a significant interaction of Parity × Day [F(1, 9) = 32.73, P < 0.001] and main effects of Parity [F(1, 9) = 8.1802, P < 0.001] and Day [F(1, 9) = 32.73, P < 0.001]. Posthoc Tukey’s HSD analysis (P < 0.05), performed on individual means with the appropriate variance corrections, revealed that postpartum females showed significant increased retrievals compared to cycling controls (both days); and increased pup-retrievals from PPD1 to PPD5 (see Fig. 3A).

### Pup-sniffing

A 2 (Parity) × 2 (Day) × 3 (Sample; three 8-min pup-stimuli periods) ANOVA revealed a significant interaction of Parity × Sample [F(2, 18) = 3.944, P = 0.04] and Parity effect [F(1, 9) = 7.34, P = 0.02] and Sample effect [F(2, 18) = 31.74, P < 0.001]. Posthoc Tukey’s HSD analysis (P < 0.05), performed on individual means with the appropriate variance corrections, revealed that postpartum females sniffed pups longer than cycling females on the initial 8-min access to pup-stimuli across days. Pup-sniffing revealed that postpartum females showed significantly increased responses to food-stimuli, however, on PPD1, postpartum females had increased DA responses (% of basal) compared to cycling females during all three time points of sample collection during the pup-stimuli, independent of pup-experience (i.e., Day). These DA increases in postpartum females were significant over basal release for the duration of the pup-stimuli on both days (see Fig. 3B).

### Food-stimuli

A 2 (Parity) × 2 (Day) × 2 (Sample, sample collected just prior to and during food-stimuli) ANOVA was performed on percentages of basal DA concentrations (see Fig. 3B bar graph for mean ± SEM). The ANOVA revealed a significant interaction of Parity × Sample [F(3, 27) = 12.24, P < 0.001] and main effects of Parity [F(1, 9) = 14.165, P = 0.004] and Sample [F(3, 27) = 12.63, P < 0.001]. Posthoc Tukey’s HSD analysis (P < 0.05), performed on individual means with the appropriate variance corrections, revealed that postpartum females had increased DA responses (% of basal) compared to cycling females during all three time points of sample collection during the pup-stimuli, independent of pup-experience (i.e., Day). These DA increases in postpartum females were significant over basal release for the duration of the pup-stimuli on both days (see Fig. 3B).

### Dopamine assessments

#### Basal release

Prior to the pup-stimuli exposure, basal DA concentrations were obtained from means of three consecutive samples that differed from one another by no more than 10%. A 2 (Parity) × 2 (Day) ANOVA performed on DA concentrations (pg/μl) revealed a significant interaction [F(1, 9) = 11.27, P = 0.008] and main effects of Parity [F(1, 9) = 7.75, P = 0.02] and Day [F(1, 9) = 17.25, P = 0.002]. Postpartum dams had significantly reduced basal DA release compared to cycling females; however, the reduced postpartum basal release increased from PPD1 to PPD5 (see Fig. 3B, bar graph).

### Pup-stimuli

DA responses to pup-stimuli were analyzed with a 2 (Parity) × 2 (Day) × 4 (Sample; basal and 3 pup-stimuli periods) ANOVA performed on percentages of basal DA concentrations (see Fig. 3B bar graph for mean ± SEM). The ANOVA revealed a significant interaction of Parity × Sample [F(3, 27) = 12.24, P < 0.001]; and main effects of Parity [F(1, 9) = 14.165, P = 0.004] and Sample [F(3, 27) = 12.63, P < 0.001]. Posthoc Tukey’s HSD analysis (P < 0.05), performed on individual means with the appropriate variance corrections, revealed that postpartum females had increased DA responses (% of basal) compared to cycling females during all three time points of sample collection during the pup-stimuli, independent of pup-experience (i.e., Day). These DA increases in postpartum females were significant over basal release for the duration of the pup-stimuli on both days (see Fig. 3B).

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**Table 1**

Correlations between online maternal behaviors and dopamine assessments on postpartum day 1 (PPD1).

<table>
<thead>
<tr>
<th>Dopamine response % of basal</th>
<th>At 8-min pup-stimuli (df = 8)</th>
<th>At 16-min pup-stimuli (df = 8)</th>
<th>At 24-min pup-stimuli (df = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Licking duration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 8-min pup-stimuli</td>
<td>r = .82**</td>
<td>r = .69*</td>
<td>r = .57*</td>
</tr>
<tr>
<td>p = .004</td>
<td>p = .007</td>
<td>p = .035</td>
<td></td>
</tr>
<tr>
<td>At 16-min pup-stimuli</td>
<td>r = .55</td>
<td>r = .72*</td>
<td>r = .52</td>
</tr>
<tr>
<td>NS</td>
<td>p = .020</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>At 24-min pup-stimuli</td>
<td>r = -.14</td>
<td>r = -.20</td>
<td>r = -.02</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Hovering duration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 24-min pup-stimuli</td>
<td>r = -.40</td>
<td>r = -.18</td>
<td>r = -.49</td>
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<tr>
<td>NS</td>
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Partial correlations (controlling for parity) relating dopamine responses to pup-stimuli and maternal behaviors exhibited during PPD1 sample collection.

* p < 0.05, two-tailed.
** p < 0.005.

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**Table 2**

Correlations between online maternal behaviors and dopamine assessments on postpartum day 5 (PPD5).

<table>
<thead>
<tr>
<th>Dopamine response % of basal</th>
<th>At 8-min pup-stimuli (df = 8)</th>
<th>At 16-min pup-stimuli (df = 8)</th>
<th>At 24-min pup-stimuli (df = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Licking duration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 8-min pup-stimuli</td>
<td>r = .75*</td>
<td>r = .87**</td>
<td>r = .51</td>
</tr>
<tr>
<td>p = .01</td>
<td>NS</td>
<td>p = .001</td>
<td></td>
</tr>
<tr>
<td>At 16-min pup-stimuli</td>
<td>r = .90**</td>
<td>r = .88**</td>
<td>r = .45</td>
</tr>
<tr>
<td>p &lt; .001</td>
<td>p &lt; .001</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>At 24-min pup-stimuli</td>
<td>r = .73</td>
<td>r = .85**</td>
<td>r = .01</td>
</tr>
<tr>
<td>p = .02</td>
<td>p = .002</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Hovering duration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 24-min pup-stimuli</td>
<td>r = -.04</td>
<td>r = .06</td>
<td>r = .81**</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>p = .005</td>
<td></td>
</tr>
</tbody>
</table>

Partial correlations (controlling for parity) relating dopamine responses to pup-stimuli and maternal behaviors exhibited during PPD5 sample collection.

* p < 0.05, two-tailed.
** p < 0.005.
Online maternal behaviors and dopamine assessments

To assess the relationship between stimulus-induced DA release with ongoing maternal behavior (i.e., licking, hovering), partial correlations controlling for parity were performed for these variables at each sampling session (i.e., PPD1 and PPD5). For PPD1, significant partial correlations were found between DA responses with early licking durations at 8- and 16-min of pup-stimuli (see Table 1 for data). For PPD5, similar analysis found significant correlations during early and late sample collections (see Table 2 for data).

Choice task

A 2 (Parity) × 2 (Day) × 2 (Behavior, maternal vs. eating) ANOVA on durations engaged in behavior revealed a significant interaction of Parity × Behavior [F(1, 9) = 222.27, P < 0.001]. On both days, postpartum dams engaged only in maternal behaviors and cycling females only ate food when both types of stimuli were available (see Fig. 4A). Because of the consistency of behavioral choices made by cycling and postpartum females, we assessed how consistent DA responses to the individual stimuli were across the two sampling days. The average DA released across the 3 samples during pup-stimuli was correlated (partially, controlling for parity) across the sampling sessions (see Fig. 4B). This was not true for DA responses to food-stimuli across sessions.

Metabolism

DOPAC

Basal values of DOPAC were obtained from the mean concentrations of three consecutive samples prior to the pup-exposure phase. For the 56-min sample collection period, during which stimuli (pups and food) were given to experimental females, the DOPAC concentrations were averaged across the stimulus procedure. A 2 (Parity) × 2 (Sample, basal and average during pup-stimuli exposure) ANOVA was performed on DOPAC concentrations (pg/μl). There were no significant differences in DOPAC levels (see Fig. 5A).

Turnover

DA to DOPAC ratios were assessed using the basal and stimulus procedure (across the 56-min period) averages. A 2 (Parity) × 2 (Day) × 2 (Sample, basal and sample average during pup-stimuli exposure) ANOVA revealed a significant Parity × Sample interaction [F(1, 9) = 13.08, P = 0.006; Day × Sample interaction [F(1, 9) = 5.908, P = 0.038]; and Sample effect [F(1, 9) = 11.55, P = 0.008]. Tukey’s HSD analysis (P < 0.05), revealed that postpartum females on PPD1 had significantly decreased basal DA turnover rates compared to cycling females on the same day and during later sampling on PPD5 (see Fig. 5B).

Hormone treatment: Results of Experiment 2

Maternal behavior during in vivo sample collection

Summary

On the 1st sampling day (Experiment 2 only) pups were placed in the center of the dialysis chamber (previous pilot no pups were retrieved from dispensers). When pup-naive, typically hormone-treated females initially sniffed and then licked the pups. When hormone-treated females met the maternal criterion (>7 days, see Fig. 2), all pups were retrieved from dispensers. Retrievals were intermingled with licking bouts, followed by nest building and eventually hovering behavior was observed. No crouching postures were observed in the dialysis chamber. In contrast, OVX sham
implanted females only sniffed pups, although one rat engaged in a single (<3 s) licking bout. In addition, only one sham control met the homecage maternal criterion within 8 days. Thus, repeated days analysis was performed only on data from hormone-treated females.

**Hormone treatment comparisons when pup-naive**

To investigate the hormone effect, the following analysis was performed on pup-naive (i.e., day 1) females only. A 2 (Treatment) × 3 (Sample; three 8-min pup-stimuli periods) ANOVA was performed on the time (s) spent pup-sniffing and -licking. The ANOVA for pup-sniffing revealed only a significant Sample effect \( F(2, 16) = 7.79, P = 0.004 \). Pup-sniffing significantly decreased over time when pup-naïve (see Fig. 6A). For pup-licking, the ANOVA found a significant interaction of Treatment × Sample \( F(2, 16) = 5.30, P = 0.02 \) and main effects of Treatment \( F(1, 8) = 14.42, P = 0.005 \) and Sample \( F(2, 16) = 4.32, P = 0.03 \). Posthoc Tukey’s HSD analysis \( P < 0.05 \) revealed that hormone-treated females licked pups longer than sham females on the later samples (at 16- and 24-min) when pup-naïve. In addition, the licking-bout durations at 16-min was significantly increased compared to the initial 8-min period of licking for hormone-treated female (see Fig. 6A). No hovering was observed by either pup-naive group.

**Experience comparisons when hormone-treated**

To investigate the pup-experience effect, the following analysis was performed on hormone-treated females only. A 2 (Experience) × 3 (Sample; three 8-min pup-stimuli periods) repeated measures ANOVA was preformed on behavioral durations. For pup-sniffing -licking, the ANOVA revealed significant interactions of Experience × Sample \( F(2, 8) = 7.50, P = 0.02; F(2, 8) = 12.91, P = 0.003 \, \text{respectively} \). Posthoc Tukey’s HSD analysis \( P < 0.05 \) revealed that sniffing significantly decreased over time when pup-naïve; however, after pup-experience sniffing did not change over sample collections (see Fig. 6A). After pup-experience, licking bouts were increased during the initial sample and decreased over sampling. During later sampling (at 16- and 24-min), pup-experienced females licked pups less than when pup-naïve at the same time points (see Fig. 6A). Similar ANOVA and post hoc analysis (all \( P < 0.005 \) on hovering durations revealed that pup-experienced, hormone-treated females hovered over pups for significantly greater durations at 24-min compared to at any time point and compared to when pup-naïve.

**Dopamine assessments**

**Basal release**

Basal values of DA and DOPAC were obtained from the means of three consecutive samples prior to the pup-exposure phase that differed from one another by no more than 10%. Two t-tests were performed on basal release: (1) comparing the treatment groups when pup-naïve \( t(8) = 8.57, P < 0.001 \); and, (2) comparing the hormone-treated females across the sampling days \( t(8) = -6.90, P = 0.002 \). As shown in Fig. 6B (bar graph), hormone-treated females had significantly: (1) decreased DA basal release compared to sham controls when pup-naïve; and, (2) increased DA basal release from the initial to second day of sampling when pup-experienced.
To investigate the hormone effect, the following analysis was performed on pup-naïve (i.e., day 1) females only. DA responses to pup-stimuli were analyzed with a 2 (Treatment)×4 (Sample, basal and 3 during pup-stimuli exposure) ANOVA, performed on percentages of basal DA concentrations (see Fig. 6B bar graph for mean±SEM). The ANOVA revealed a significant interaction of Treatment×Sample \( F(3, 24)=4.16, P=0.02 \); a Treatment effect \( F(1, 6)=20.36, P=0.002 \), and Sample effect \( F(3, 24)=6.56, P=0.002 \). Posthoc Tukey’s HSD analysis \( P<0.05 \) revealed that hormone-treated females had increased DA responses (% of basal) compared to sham controls at each pup-stimuli period. This DA increase in hormone-treated naïve females was significant over basal release for the duration of the pup-stimuli (see Fig. 6B).

**Pup-stimuli across maternal experiences**

For pup-experience comparisons in hormone-treated females, a 2 (Experience)×4 (Sample, basal and 3 during pup-stimuli exposure) ANOVA was performed on percentages of basal DA concentrations (see Fig. 6B bar graph for mean±SEM). The ANOVA revealed significant main effects of Experience \( F(1, 4)=8.89, P=0.04 \) and Sample \( F(3, 12)=10.59, P=0.001 \). Posthoc Tukey’s HSD analysis \( P<0.05 \) revealed that hormone-treated, pup-experienced, females had increased DA responses (% of basal) to pup-stimuli when collapsed across the samples, however, these elevations in DA release were less than the pup-induced elevations in DA found on the first assessment when females were pup-naïve (see Fig. 6B).

**Food-stimuli**

For the first day of sample collection (when pup-naïve), DA responses to food-stimuli were analyzed with a 2 (Treatment)×2 (Sample, collected just prior to and during food-stimuli) ANOVA was performed on percentages of basal DA concentrations (see Fig. 6B bar graph for mean±SEM). The ANOVA revealed a significant interaction \( F(1, 8)=14.24, P=0.005 \) and a Sample effect \( F(1, 8)=76.89, P<0.001 \). Posthoc Tukey’s HSD analysis \( P<0.05 \), performed on individual means with the appropriate variance corrections, revealed that all females had increased DA responses during food consumption, however, hormone-treated females had increased DA elevations in response to food-stimuli compared to Sham controls (see Fig. 6B).

### Pup-stimuli when pup-naïve

To investigate the hormone effect, the following analysis was performed on pup-naïve (i.e., day 1) females only. DA responses to pup-stimuli were analyzed with a 2 (Treatment)×4 (Sample, basal and 3 during pup-stimuli exposure) ANOVA, performed on percentages of basal DA concentrations (see Fig. 6B bar graph for mean±SEM). The ANOVA revealed a significant interaction of Treatment×Sample \( F(3, 24)=4.16, P=0.02 \); a Treatment effect \( F(1, 6)=20.36, P=0.002 \), and Sample effect \( F(3, 24)=6.56, P=0.002 \). Posthoc Tukey’s HSD analysis \( P<0.05 \) revealed that hormone-treated females had increased DA responses (% of basal) compared to sham controls at each pup-stimuli period. This DA increase in hormone-treated naïve females was significant over basal release for the duration of the pup-stimuli (see Fig. 6B).

### Table 3

Correlations between online maternal behaviors and dopamine assessments.

<table>
<thead>
<tr>
<th>Dopamine response ( % of basal )</th>
<th>At 8-min pup-stimuli</th>
<th>At 16-min pup-stimuli</th>
<th>At 24-min pup-stimuli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pup-licking duration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 8-min pup-stimuli</td>
<td>( r = -0.20 )</td>
<td>( r = 0.42 )</td>
<td>( r = -0.16 )</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>At 16-min pup-stimuli</td>
<td>( r = 0.68^* )</td>
<td>( r = 0.51 )</td>
<td>( r = -0.34 )</td>
</tr>
<tr>
<td></td>
<td>( p = 0.044 )</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>At 24-min pup-stimuli</td>
<td>( r = 0.80^* )</td>
<td>( r = 0.90^{**} )</td>
<td>( r = 0.09 )</td>
</tr>
<tr>
<td></td>
<td>( p = 0.01 )</td>
<td>( p = 0.001 )</td>
<td>NS</td>
</tr>
</tbody>
</table>

Partial correlations (controlling for hormone treatment) relating dopamine responses to pup-stimuli and maternal behaviors exhibited on day 1 after progesterone/empty capsules removal in pup naive females.

* \( P<0.05 \), two-tailed.

** \( P<0.005 \).

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**Fig. 7.** In hormone-treated females, there was a shift in maternal responsiveness after pup-experience. This was evidenced by the findings that after pup-experience, maternal behavior rather than eating was observed during the choice task (A). Thus, to analyze this shift in responsiveness several correlations were performed on data from the hormone-treated females: (B) choice task durations and DA responses to pup-stimuli; (C) days to reach maternal criterion (or days after progesterone removal) and DA responses to pup-stimuli; and, (D) DA responses to pup- and food-stimuli across days. In hormone-treated females, (B) behavioral choice responses correlated with DA responses to pup-stimuli (on any given sampling day); (C) hormonally-induced maternal responding correlated with DA responses to pup-stimuli; (D) DA responses to pup- and food-stimuli correlated when pup-naive. a \( P<0.05 \) hormone treatment differences; b \( P<0.05 \) behavior differences; c \( P<0.05 \) experience differences.
For analysis across the sampling days, in hormone-treated females DA response to food-stimuli was analyzed with a 2 (Days)×2 (Sample, collected just prior to during food-stimuli) ANOVA, performed on percentages of basal DA concentrations (see Fig. 6B bar graph for mean ± SEM). The ANOVA revealed only a significant Sample effect \( F(1, 4) = 19.35, P = 0.01 \). Across the two tests hormone-treated females had similar increased DA responses to food-stimuli (see Fig. 6B).

Online maternal behaviors and dopamine assessments

To assess the relationship between stimulus-induced DA release with ongoing maternal behavior in pup-naïve females, partial correlations (controlling for hormone) were performed on data collected on day 1 between DA responses during each time point of pup-stimuli exposure and the duration engaged in licking behavior (at 8-, 16-, 24-min of pup-stimuli). The DA responses to pups were significantly correlated to later pup-licking durations (see Table 3 for values).

Choice task

When given a choice between interacting with pup- or food-stimuli, all pup-naïve females ate food only. However after pup-experience, hormone-treated females engaged only in maternal behaviors when pup-naïve had decreased basal DOPAC levels. A similar increased DA responses to food-stimuli (see Fig. 6B).

Metabolism

DOPAC

Basal values of DOPAC were obtained from the mean concentrations of three consecutive samples prior to the pup-exposure phase. Following basal collections, for the 56-min sample collection period, during which stimuli (pups and food) were given to experimental females, the DOPAC concentrations were averaged across the stimulus procedure. For treatment comparisons when pup-naïve, a 2 (Treatment)×2 (Sample, basal and average during pup-stimuli exposure) ANOVA performed on DOPAC concentrations (pg/μl) revealed Treatment×Sample interaction \( F(1, 8) = 5.00, P = 0.054 \), Treatment effect \( F(1, 8) = 6.26, P = 0.007 \), and sample effect \( F(1, 8) = 20.83, P = 0.002 \). Tukey’s HSD analysis \( P < 0.05 \) revealed that hormone-treated females when pup-naïve had decreased basal DOPAC levels. For day comparisons in hormone-treated females, a 2 (Day)×2 (Sample, basal and average during pup-stimuli exposure) ANOVA was performed on DOPAC concentrations (pg/μl). The ANOVA performed on DOPAC concentrations (pg/μl) revealed no significant differences across days for hormone-treated female (see Fig. 8A).

Turnover

DA to DOPAC ratios were assessed using the basal and stimulus procedure (across the 56-min period) averages. For treatment comparisons when pup-naïve, a 2 (Treatment)×2 (Sample, basal and average during pup-stimuli exposure) ANOVA revealed a significant Treatment effect \( F(1, 8) = 11.90, P = 0.009 \). Hormone-treated females had decreased turnover rates compared to Sham females, on sampling day 1 (pup-naïve) (see Fig. 8B). For day comparisons in hormone-treated females, a 2 (Day)×2 (Sample, basal and average during pup-stimuli exposure) ANOVA revealed a significant Day effect \( F(1, 4) = 41.75, P = 0.003 \). By the second day of sampling, hormone-treated (pup-experienced) females had increased DA turnover rates.

Reassessing dopamine responses to pup-stimuli (covariate analysis)

DA measurements from both recently postpartum (>24 h after birthing) and OVX hormone-treated groups (>24 h after progesterone removal) were significantly decreased in comparison to their cycling and sham treated controls, respectively. To further investigate the influence of DA basal release on group (i.e., Parity and Hormone Treatment) differences in DA responses to pup-stimuli, we performed analyses of covariance by partialling out the basal concentrations from two analyses. For each experiment on the first day of sampling, a 2 (Group: cycling vs. postpartum; or sham vs. hormone-treated) ×4 (Sample, basal and 3 during pup-stimuli exposure) ANOVAs were performed on DA responses (% over basal release). Only for cycling/postpartum females did groups differences in pup-related DA responses continue to exist when the basal concentrations were partialled out of the analysis: Parity effect, \( F(1, 8) = 11.79, P = 0.009 \) and Parity×Sample interaction \( F(3, 24) = 8.52, P < 0.001 \).
Discussion

The present study found that adaptations in the brain follow the sudden withdrawal of pregnancy hormones to permit the activation of maternal behaviors. Basally, postpartum and hormone-treated females had significantly decreased DA release in the NACsh compared to their controls. After several days when progesterone levels declined (naturally or artificially with progesterone removal), the postpartum (although not significant) and hormone-treated (significant) females displayed increased DA release compared to their previous basal assessments. In response to pup-stimuli, only postpartum and hormone-treated females had significant increased DA responses from basal release. This finding was observed even in pup-naïve, hormone-treated females. In our previous study (Afonso et al., 2008) cycling females with prior postpartum experiences (2 previous litters) or recent continuous pup interactions (until highly maternal in homecage) displayed increased accumual DA responses to pup-stimuli; however, this increase was only observed during the first 8-min of a 24-min pup-exposure period (Afonso et al., 2008). Unlike the previous study with cycling rats, postpartum and hormone-treated females displayed increased DA responses to pup-stimuli that remained elevated until pups were removed. In response to food-stimuli, all females had increased DA responses above basal release on both sampling days; although there were parity and hormone treatment differences on day 1 of sampling. During the choice task, postpartum (PPD1 and PPD5) females were observed to only interact with pup-stimuli when both stimuli were available. For hormone-treated females there was a shift in eating to maternal behavior after pup-experience. All control females only ate during availability of both stimuli, regardless of pup-experience.

These results support earlier studies that show DA activity in the NAC in response to pups is important for new mothers (Hansen et al., 1993; also see Champagne et al., 2004; Ferris et al., 2005; Hansen et al., 1991a,b). The results extend the existing literature by showing that DA responses to pups in females hormonally primed to be maternal, but with no previous pup-experience, are similar to those DA responses in postpartum females (i.e., elevated DA for the duration of pup-stimuli). Thus, previous experience with maternal behavior itself is not necessary for DA release in this brain region. Hormone treatments associated with parturition/early postpartum period also enhanced DA responses to non-pup-related cues, suggesting that the DA responsiveness is not exclusive to pup-stimuli with application of this estrogen/progesterone hormone profile.

Basal dopaminergic functioning

Studies have shown that gonadal steroid hormones affect the basal release and metabolism of biogenic amines (see, McEwen and Parsons, 1982; Thompson, and Moss, 1994). Estrogen and progesterone released during the estrous cycle affect basal DA function in the striatum (see, Becker and Beer, 1986; Castner et al., 1993; Di Paolo et al., 1985; Fernandez-Ruiz et al., 1991). Prolonged estrogen treatment reduces the concentrations of DA (in situ) in several structures including the NAC (Dupont et al., 1981). This treatment was without effect on DA turnover in all brain areas studied (Dupont et al., 1981). With chronic estrogen treatment, or high concentrations of injected estrogen, presynaptic DA activity is decreased, and D2 DA receptors become supersensitive (Di Paolo et al., 1988, 1982a,b). Progesterone also has effects on basal dopaminergic functioning. Catecholamine content (brain homogenate) was decreased after a single injection 75 h prior to decapitation (Loüström, 1978). The mechanism(s) through which the effects of prolonged hormone treatment affect DA activity in the NAC have not been investigated to a great extent.

The present study found that in the intact recently postpartum (24 h after birthing) and OVX hormone-treated rat (24 h after progesterone removal), DA basal release in the NACsh was significantly decreased compared to their controls. This suggests that the hormone profile has some role in the basal firing rate of DA neurons. There were no basal DOPAC level parity differences; however, DA turnover was decreased in PPD 1 lactating dams compared to similar females at PPD 5 and cycling females. The administration of the parturition-like hormones to OVX females had a suppressive effect on DOPAC levels and DA turnover rates 24 h after progesterone removal. While the turnover ratio increased by the second sampling day, DOPAC levels remained low in hormone-treated females. Together the experiments suggest that basal DA functioning in the NACsh is mediated by the hormones associated with increasing maternal responsiveness, however, this hormone profile did not account for all DA changes that occurred during the postpartum period.

Dopamine responses to stimuli

While there was an apparent inhibition of basal DA release in the NACsh of females after parturition or hormone treatments, in comparison to their controls, there was increased DA release in response to pup-stimuli. There is research on prolonged hormone treatment and its effects on DA responses to stimuli (see, Becker, 1990a,b; Becker and Cha, 1989; Becker, and Ramirez, 1980; Becker et al., 1984; Dluzen and Ramirez, 1990; Fernandez-Ruiz et al., 1991; Xiao and Becker, 1998). Literature suggests that the enhancement of pharmaceutically-induced DA is greater following repeated treatments than with a single acute treatment, suggesting that prolonged doses of estrogen, with or without progesterone treatment, produce both acute and long-term effects on DA functioning in striatal tissue (Becker and Rudick, 1999; also see Dluzen and Ramirez, 1990). In addition DA responses to amphetamine (AMPH) can vary with the time after cessation of hormone treatments. For example, 24 h after 4 daily estrogen treatments, AMPH-induced DA in dialystat and stereotyped behaviors were significantly greater than that seen in OVX untreated controls, but significantly lower than the responses of rats that are tested 30 min after the last estrogen treatment (Becker and Rudick, 1999). In regards to progesterone, only after prior exposure to estrogen is the enhanced effect on AMPH-stimulated DA observed in the dorsolateral striatum (Becker and Rudick, 1999).

Like AMPH-induced DA responses, pup-stimuli may have similar effects on DA activity in postpartum or hormonally-treated females. The current study demonstrated that in the dialystat of rats treated with the estrogen/progesterone hormone profile associated with elevating maternal responsiveness, the DA release was increased in response to both stimulus types. This was true for even the hormone-treated females that had never before experienced pup-stimuli. In addition, the hormone-treated females, 24 h after progesterone removal, had increased elevated DA responses to the previously given food-stimuli—a response not observed in postpartum rats. Thus, the estrogen/progesterone hormone profile mediates some aspects of DA responses to pup-stimuli. However, postpartum alterations in NAC DA functions require further modifications that the intact postpartum period provides.

In the presence of the increased DA release during stimulation, there were few significant changes in metabolic activity, as measured by DOPAC levels and turnover rates. Extracellular DOPAC is thought to reflect changes in the intracellular pool of DA (Zetterstrom et al., 1986), and the increased extracellular DOPAC level theoretically implies a variety of factors such as increased DA synthesis, decrease of active uptake process, or an increase in DA release. In the postpartum or hormone-treated rat, there were significant decreases in basal DA release. Thus, the variety of factors mediating DOPAC levels may also be affected by hormones that increase maternal responsiveness. For example, DOPAC levels may decrease when less DA synthesis for intracellular DA is required, as would be the case for the suppressed basal DA release observed in postpartum and hormone-treated females. An absence of notable DOPAC changes following increased DA release to stimuli may be observed in such a case. A proposed mechanism for the absence of increased DA metabolism would be difficult to make with the present study.
Dopamine and pup-saliency

Researchers have suggested that DA signals between neurons are an important link in the neural chain that promotes reward learning (for review see Berridge, 2007). Neurobiologically, it has been proposed that DA signals modulate synaptic plasticity in target neurons or adjust synaptic efficacy in the learning networks, especially in the neostriatum and NAC. Psychologically, it has been suggested that DA acts to associatively reinforce new links between stimulus-response events. Recent molecular biology studies suggest that DA modulates cellular and molecular plasticity mechanisms of long-term potentiation and long-term depression inside neurons in ways possibly relevant to memory (Berke and Hyman 2000; Kelley 2004a,b; Wickens et al. 2003).

Evidence for associative DA modulatory roles include demonstrations that DA manipulations performed soon after a learning trial can alter the consolidation or reconsolidation of memories, similar in respect, to other memory consolidation phenomena (Dalley et al. 2005; Everitt and Robbins 2005; Fenu and Di Chiara 2003; Lee et al., 1999; Li and Fleming, 2003a, Hernandez et al. 2005; McGaugh 2002; Robertson and Cohen 2006). One such memory consolidation modification after suspected DA destruction is the maternal experience effect (MEE). Long-term enhancement in maternal behavior as a result of experience interacting with pups during the postpartum period is referred to as the MEE (Orpen and Fleming, 1987). While lesions to the NACsh do not have a major disruptive effect on ongoing maternal behavior, these lesions have a substantial disrupting effect on the development of the MEE (Li and Fleming, 2003a,b). Thus, DA in the NACsh maybe involved in responding to previous cues that were involved in the development of the MEE. Presumably during early postpartum days in first time mothers, the development of salient qualities for pup-stimuli is not only important for the MEE, but also for survival of offspring. Salient attributions of stimuli are normally determined by the integration of two major inputs to these mesocorticolimbic mechanisms: 1) learned reward associations, and 2) current physiological states relevant to the biological reward that influence mesolimbic neurobiological function (e.g., states of caloric hunger, satiety, thirst, salt appetite, and drug-induced mesolimbic activation and sensitization).

During early postpartum days, postpartum females had suckling experience prior to each of the sample collection days. Suckling stimulation in lactating dams is a robust stimulus for activating the mesocorticolimbic system (Ferris et al., 2005). This pathway appears to be a critical neurochemical pathway in the anticipatory and consummatory aspects of maternal behavior in the lactating rat (Champagne et al. 2004; Hansen et al., 1993; Ferris et al., 2005). Postpartum females, only after the extensive homecage experience (i.e., PPD5), had DA responses to pup-stimuli that correlated to hovering over pups (creating potential for sucking). Continuous experience with pup-stimuli reward (e.g., physiological responses associated with sucking) may serve to reduce DA responses to other stimuli when the NAC has low basal DA release. Thus, the estrogen/progesterone hormone profile may increase DA responses to many types of stimuli, however, experience with pup-related reward states in the intact postpartum dam may reduce DA responses to non-pup-related stimuli. These findings suggest that the generation of robust salient values for pups, which draws on both preexisting reward related associations and current neurobiological states, requires accumbal DA signaling. This maybe one manner that physiological results of postpartum hormones interact with stimuli to influence aspects of maternal motivation.

Dopamine and shifts in maternal responsiveness

Previously we have found that lesions to NAC (Li et al., 2004) or the administration of DA receptor antagonists to the NAC (Numan et al., 2005; Parada et al., 2008) in postpartum dams have disruptive effects on: (1) retrievals during the first days of maternal behavior; and, (2) retention latencies in a MEE paradigm. This suggests a role for DA in approach/appetitive responses and in the ‘reinforcing’ value of the pups necessary for learning to occur. In hormone-treated females, there was an obvious shift in maternal responsiveness after pup-experience. This was evidenced by the findings that after pup-experience: the full complement of maternal behaviors was performed in homecage; hovering over pups and retrieval patterns were observed in the sampling chamber; and, maternal behavior, rather than eating, was observed during the availability of both stimuli (choice task). In contrast, when treated with empty capsules, only one sham female reached the homecage maternal criterion, while the remaining did not even engage in retrieval patterns in the homecage. Although the OVX females given some pup-experience, were not tested on the choice task, presumably these rats would not engage in pup-related behavior—given that even cycling females from Experiment 1 did not engage in maternal behaviors in the dialysis chamber during the sampling procedure or choice task.

There was also a shift in maternal responsiveness in hormone-treated females. During day 1 sampling, hormone-treated females had significantly greater licking durations compared to sham-treated females. Eating durations were significantly decreased compared to sham controls when both stimulus types were available. The hormonally-induced shifts in maternal responsiveness were accompanied by DA responses that appeared biased towards pup-stimuli (see Figs. 7B–D). It was found that when pup-naive, hormone-treated females with greater DA responses during pup-stimuli presentation had shorter eating durations when both stimuli were available (i.e., choice task). However after pup-experience, those hormone-treated females with greater DA responses during pup-stimuli presentation had longer maternal behavior durations during the choice task. Food related DA responses did not correlate with any of the durations in the choice task (data not shown). Similarly, the latency to become fully maternal in the homecage correlated selectively to pup-related DA responses. Intact postpartum females also demonstrated a robust bias for DA responses toward pup-stimuli. The DA signaling during the availability of pup-stimuli correlated across the postpartum days. This was not true of DA responses to food-stimuli. There has been previous evidence for the idea that pups can obtain robust saliency as reflected in DA activity (Ferris et al., 2005; Fleming et al., 1994; Fleming et al., 2008; Hansen et al., 1993; Hansen et al., 1991a,b; Hecht et al., 1999; Li and Fleming, 2003b; Lee et al., 2000). In lactating rats, the pups become so salient that they can compete with self-administration of cocaine (Hecht et al., 1999) and suckling stimulation activates the mesocorticolimbic system (Ferris et al., 2005). Although the correlations imply a pup-biased DA response, DA responses to pup- and food-stimuli correlated with each other prior to any experience with pups. While these findings are exciting, without further investigations we can simply conclude here that hormones that increase maternal responsiveness play a significant role in modifying accumbal dopaminergic responses to pup-stimuli in the rat. Currently we are performing studies to investigate the NAC’s selectivity of DA responses to stimuli after prolonged treatments of hormones.

Conclusions

Given the consistent finding of the reduced basal DA release in maternally sensitive females (i.e., postpartum and hormone-treated), low basal DA activity may set the physiological stage for relevant DA responses to pup-stimuli. Partially out basal DA concentrations from the analysis of pup-related DA responses (percent increase over basal) did not eliminate parity difference. The same analysis eliminated all significant differences in hormone-treated females. Despite hormonal manipulations and their effects in the NAC, the postpartum experience provides further modifications in this brain region to promote pup-related DA responses. While prolonged estrogen/progesterone treatments with sudden progesterone withdrawal interact with stimuli to influence aspects of maternal motivation, multiple adaptations in
maternal neuroendocrine systems are necessary for the complete postpartum adaptations to dopaminergic functioning in the NAC.

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