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Research Report

Previous maternal experience affects accumbal dopaminergic responses to pup-stimuli

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ABSTRACT

The present study investigated the release of dopamine from the nucleus accumbens (shell) in response to pup-stimuli in the absence of lactation and maternal behaviors at time of sample collection. Subjects were female rats given maternal experiences through prior parturitions, recent pup-induced sensitization, or a combination of both. Nulliparous (N) or multiparous (M, had 2 prior litters but cycling) female rats either received pup-sensitization (S^+) until they responded maternally in their homecage or no pup-sensitization (S^-), thus, there were four groups: NS^- ($n=5$), NS^+ ($n=6$), MS^- ($n=5$), and MS^+ ($n=8$). Four hours after removal of pups (from homecage for S^+ groups), all females were placed into the microdialysis chamber for sample collection. After baseline collection, four foster pups were given to the females. In this paradigm females show little to no maternal behavior in the test chamber. Samples (collected every 8 min) were analyzed for dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) with electrochemical detection using HPLC. Relative to the inexperienced NS^- females, the experienced NS^+, MS^- and MS^+ females displayed significantly increased DA levels only during the first 8 min of pup-exposure. The more experience a female had with pups, the greater was the DA response ($p<.05$). The results suggest that enhanced responding to pups following previous maternal experiences may be mediated through accumbal DA.

1. Introduction

In the postpartum rat, the onset of maternal behavior is mediated by hormones and sustained by recent sensory experience but the long-term retention of maternal responsiveness is dependent upon prior maternal experience (for review, see Numan et al., 2006). Once a female has weaned her first litter (primiparous), she retains an enhanced responsiveness to pups and will show a reduced latency to behave maternally in future tests (Bridges, 1975, 1977; Cohen and Bridges, 1981; Orpen and Fleming, 1987). Furthermore, the experienced multiparous rat is less dependent on hormones to express responses to pup cues (Moltz and Wiener, 1966; Moltz et al., 1969). When given continuous pup-exposure, virgin and post-parturient (cycling) female rats will eventually show maternal behavior (4–10 days) to the foster pups with post-parturient females requiring fewer days compared to virgin females (Rosenblatt, 1967; Fleming and Rosenblatt, 1974; Orpen and Fleming, 1987).

Like the postpartum rat, the maternal behavior of pup-sensitized females can be sustained by the consolidation of a maternal experience (Bridges and Scanlan, 2005). Unlike the recently postpartum rat (lactating) the onset of maternal behavior in the pup-sensitized female is not mediated by large fluctuations in hormones associated with gestation and parturition, although experiences acquired under hormonal priming

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(e.g. pregnancy, hormonal treatments) seem to be more 'robust' than those acquired as untreated virgins (Scanlan et al., 2006). Enhanced responsiveness towards pups attained through either, prior pup-experiences under maternal hormones, recent experience with donor pups under cyclic hormones, or a combination of the experiences, has not been measured neurochemically in the freely moving non-lactating rat.

In vivo studies demonstrate that lactating rats engaged in pup-licking and nursing behaviors have increased DA activity in the nucleus accumbens shell (NACsh, Champagne et al., 2004) and the ventral striatum (Hansen et al., 1993), suggesting that the consummatory components of maternal behavior may have rewarding properties. Interestingly, lesions to the NACsh produce only subtle delays in retrieval responses to pups within a test (Li et al., 2004), while having a substantial depressing effect on the formation of a maternal memory (Li and Fleming, 2003). Hence, the NACsh may be important in the development of saliency to pup-associated cues during the postpartum period much like the role the NACsh plays in the development of saliency to drug-taking cues (for review see Di Chiara et. al., 2004). Alterations to NACsh functions following maternal experiences may be necessary for the later ability of pup-cues to promote maternal responding in cycling rats.

The present study investigated in the non-lactating female rat, how enhanced maternal responsiveness, acquired through experiences during prior parities (i.e., 2 litters) or through recent pup-sensitization, affects later dopaminergic mesolimbic responses to pup-stimuli in the absence of ongoing maternal behavior. After several short habituation sessions with donor pups in the sampling chamber (a non-homecage environment), DA measurements were made from dialysates collected from the NACsh of cycling female rats exposed to donor pup-stimuli. Prior to sampling, females in the homecage were given one of the following experiences engaging in maternal behavior: (1) no experience; (2) two previous parturition and lactational experiences with own pups; (3) recent sensitization to donor pups; or (4) a combination of the two maternal experiences. Previously we have observed that while these maternal experiences enhanced maternal responding in homecage tests of maternal behavior, in non-homecage tests (e.g., the sampling chamber) these cycling rats were unlikely to display maternal behaviors. This observation provides a unique opportunity to investigate neurotransmission in response to pup-stimuli in the absence of ongoing maternal behavior.

We hypothesized that DA levels would increase in response to pup-stimuli for females given previous experience with their own pups under the influence of maternal hormones, and recent experiences with donor pups in the absence of maternal hormones. In females given the combined experiences (i.e., under prior maternal hormones and with recent pup-induced sensitization), DA responses to pup-stimuli may be additive. These DA responses, in the absence of ongoing maternal behavior, most likely increase during the initial exposure to pup-stimuli, rather than the duration of the exposure.

2. Results

2.1. *Histology*

For an animal to be included in the statistical analysis, the guide cannula had to end above the NAC and between 0.3–1.0 mm from midline. Fig. 1 shows the anatomical placements of the "active" zones of the probe. Included for statistical analysis were 25 animals: NS^- ($n=5$), NS^+ ($n=6$), MS^- ($n=6$), and MS^+ ($n=8$).

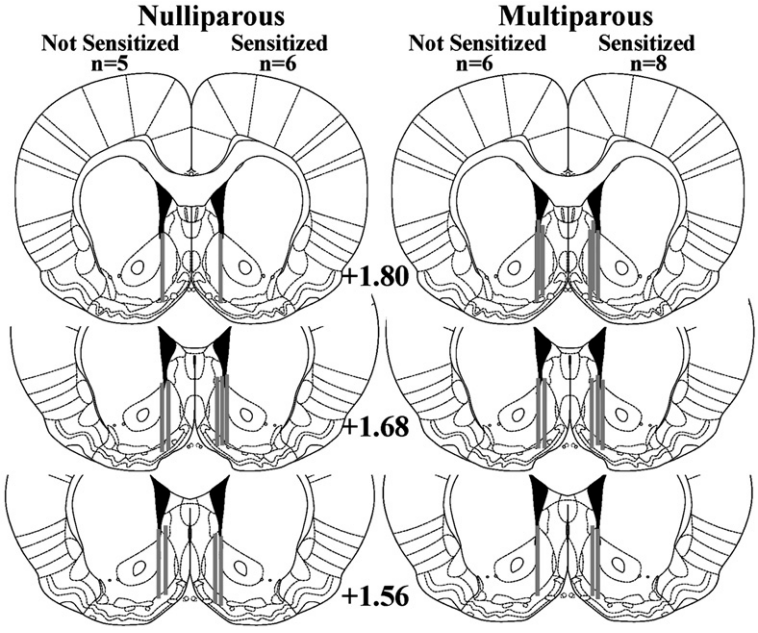

Fig. 1 – Schematics of anatomical unilateral placements (anterior to Bregma, according to Paxinos and Watson, 1986) of the "active zone" (shaded lines) of microdialysis probes in the nucleus accumbens shell at three different locations anterior to Bregma, according to Paxinos and Watson (1986) for the four maternally experienced groups.

Table 1 – Frequency and duration of licking bouts

Maternal experience		Nulliparous not sensitized		Nulliparous sensitized		Multiparous not sensitized		Multiparous sensitized	
Licking	Frequency	0.00	±0.00	0.50	±0.34	0.40	±0.24	1.17	±0.58
	Duration (s)	0.00	±0.00	0.91	±0.64	1.49	±0.91	3.97	±2.61

Mean (±SEM) frequency and duration (s) of licking bouts during the first 8-min of pup-exposure. No maternal behaviors (i.e., licking, nursing postures, mouthing) were observed after the initial sample collection during the pup-exposure phase.

2.2. *Maternal behavior during sampling*

There were no group differences in licking behavior during initial pup-exposure, as determined by a two-way (Parity X Recent maternal experiences) analysis of variance (ANOVA) [$P>0.10$]. For the full duration of the pup-exposure phase, no females retrieved pups to a new location in the chamber nor engaged in nursing or hovering behavior. A few females with pup-experience were observed to lick the pups during sample collection, although at very low frequencies and durations (see Table 1). After the first 8 min with the pups, licking behavior was no longer observed in any of the females.

A 2 (Parity) X 2 (Recent Experience) X 3 (Time) ANOVA revealed that the duration spent pup-sniffing decreased across time for all females [$F(2, 38)=91.81$, $P<0.001$] and that recent pup-experiences increased pup-sniffing durations compared to females not given this manipulation [$F(1, 19)=17.96$, $P<0.001$] (see Fig. 2, top left). There were also significant interactions of Parity X Time [$F(2, 38)=4.46$, $P=0.01$] and Recent X Time [$F(2, 38)=11.36$, $P<0.001$]). Posthoc Tukey's HSD analysis ($P<0.05$), performed on individual means with the appropriate variance corrections, revealed that for females given pup-experience (i.e., NS^+, MS^-, MS^+) the duration spent pup-sniffing was increased during the initial 8-min period of pup-stimuli exposure compared to all other time periods (i.e., 2nd and 3rd 8-min period).

2.3. *Parity and recent maternal experiences did not affect basal levels of DA and DOPAC*

Basal values of DA and DOPAC were obtained from the means of three consecutive samples just prior to the pup-exposure phase that differed from one another by no more than 10%. Levels of DA and DOPAC did not differ between the groups, as assessed by a two-way (Parity X Recent Experience) ANOVA [$P>0.10$]. Data were expressed as percentage of basal values (see Table 2 for mean±SEM basal values).

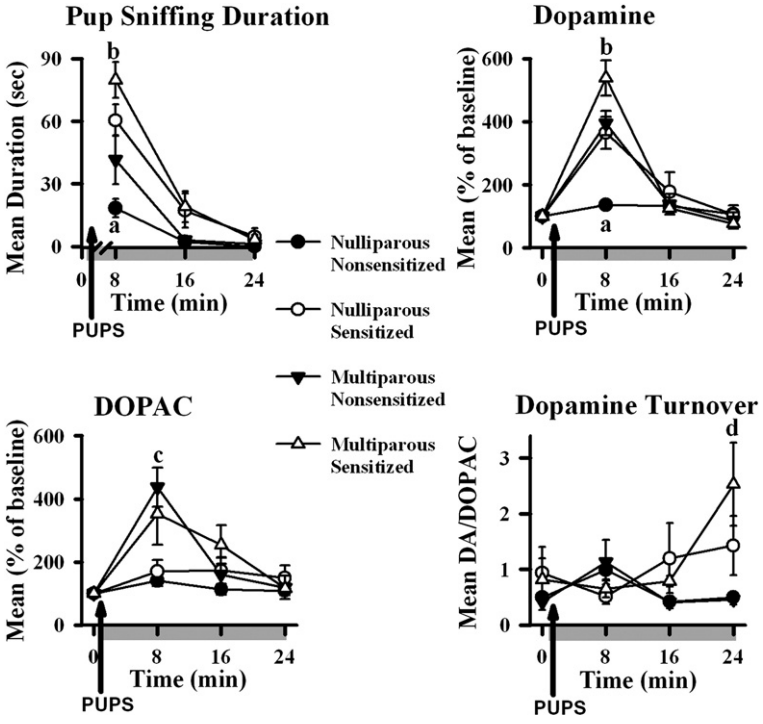

Fig. 2 – After different parity or recent maternal experiences with pups, dopamine and DOPAC levels in the nucleus accumbens shell increased in response to presentation of 4 foster pups. Data represent mean (±SEM) of the: duration of pup-sniffing (top left); percentage of baseline dopamine (top right) and DOPAC (bottom left) levels; and, dopamine to DOPAC ratio (bottom right). Dopamine and DOPAC levels were collected every 8 min (1 μl/min); baseline represents an average of 3 samples prior to the 24 min of exposure to pup-stimuli (shaded area on x-axis). (a) $p<0.05$ The maternally inexperienced group (i.e., nulliparous nonsensitized) differed with the other maternally experienced groups; (b) $p<0.05$ combined maternal experiences (i.e., multiparous sensitized) differed from all other groups; (c) $p<0.05$ Parity Effect; (d) $p<0.05$ Recency Effect.

Table 2 – Basal concentrations of DA and DOPAC

Maternal experience	Nulliparous not sensitized	Nulliparous sensitized	Multiparopus not sensitized	Multiparopus sensitized
DA (pg/μl)	0.29±0.05	0.30±0.04	0.35±0.04	0.36±0.09
DOPAC (pg/μl)	37.56±9.45	41.27±15.45	39.48±15.87	57.56±17.44

Mean basal concentrations of analytes corresponding to 100% baseline (uncorrected for probe recovery). DA, dopamine; DOPAC, dihydroxyphenlacetic acid.

2.4. *Parity and recent maternal experiences affect DA responses to pup-stimuli*

Characteristically, in females with maternal experience there was an initial increase of DA in response to pups that returned to baseline levels within 16–24 min of pup-exposure (see Fig. 2, top right).

A 2 (Parity) X 2 (Recent Experience) X 4 (Sample, baseline sample and 3 samples collected during pup-exposure) ANOVA was performed on DA concentrations. The ANOVA revealed significant interactions: Sample X Parity [$F(3, 60)=14.04$, $P<0.001$]; and Sample X Recent Experience [$F(3, 60)=8.70$, $P<0.001$]. To investigate the interactions, Posthoc Tukey's HSD analysis ($P<0.05$) was performed on individual means with the appropriate variance corrections. Posthoc analysis revealed that for females given pup-experience (i.e., NS^+, MS^-, MS^+), independent of the recency of that experience, DA levels significantly increased in response to initial pup-exposure compared to the inexperienced group (NS^-) and basal levels (see Fig. 2, top right). The DA levels returned to baseline levels by 16 min of pup-exposure for the NS^+ and MS^+ females and by 24 min for the MS^- females. Posthoc analysis also revealed that the MS^+ females, compared to the NS^+ and MS^- had significantly greater DA levels in the first 8 min of pup-exposure.

In addition to the significant interactions, the ANOVA also revealed significant main effects of Sample [$F(3, 60)=95.16$, $P<0.001$]; Parity [$F(1, 20)=4.97$, $P=0.037$]; and Recent Experience [$F(1, 20)=6.76$, $P=0.017$]. Collapsed across the samples the maternally experienced groups had increased levels of DA during pup-exposure compared to the inexperienced females (see Fig. 2, top right).

2.5. *Parity maternal experiences affect DOPAC levels in response to pup-stimuli*

A 2 (Parity) X 2 (Recent Experience) X 4 (Sample, baseline sample and 3 samples collected during pup-exposure) ANOVA was performed on DOPAC concentrations. The ANOVA revealed a significant interaction of Sample X Parity [$F(3, 60)=7.26$, $P<0.001$], and main effects of Sample [$F(3, 60)=28.12$, $P<0.001$] and Parity [$F(1, 18)=5.95$, $P=0.024$]. To investigate the significant interaction, Posthoc Tukey's HSD analysis ($P<0.05$) was performed on the individual means with the appropriate variance corrections. The analysis revealed that only during the first 8-min pup-exposure period, multiparous females had significantly greater levels of DOPAC compared to nulliparous females, independent of recent pup-sensitization (see Fig. 2, bottom left).

Table 3 – Correlations between dopamine levels and pup-sniffing behavior across time

Time after pup-exposure	8-min	16-min	24-min
R=	.5722	.5239	.4048
P (one-tailed)=	.0035	.0075	.0035

Significant ($p<0.05$)) partial correlations (controlling for parity and recent factors) between dopamine levels and pup-sniffing behavior were found across the 3 time points of sample collection during the pup-exposure period.

2.6. *Recent maternal experiences affect DA turnover in response to pup-stimuli*

To investigate the effects that maternal experiences had on DA metabolic rate, the DOPAC:DA concentration ratio was analyzed with a 2 (Parity) X 2 (Recent) X 4 (Sample, baseline sample and 3 samples collected during pup-exposure) ANOVA. The ANOVA revealed a significant Sample X Recent Experience [$F(3, 60)=2.66$, $P=0.054$]. To investigate the interaction the groups were collapsed on Parity and Posthoc Tukey's HSD analysis ($P<0.05$) was performed on the individual means with the appropriate variance corrections. The analysis revealed that the recently pup-sensitized females, independent of parity, had significantly greater DA turnover by the last 8 min of pup-exposure compared to non pup-sensitized females (see Fig. 2, bottom right).

2.7. *DA release correlates with pup sniff*

A partial correlation, controlling for maternal experience (parity and recent experience) was performed on the DA levels (% of baseline) and the duration spent pup-sniffing for the first three samples collected after pup-exposure. For all three time points the DA was significantly correlated to pup-sniffing (all p values <0.05, see Table 3).

3. Discussion

The present study found that experience with maternal behavior in homecage environments augmented later dopaminergic mesolimbic responses to pup-stimuli in the non-homecage environment (i.e., sampling chamber). The non-maternal interactions provided by the short habituation sessions with donor pups to the sampling chamber did not increase DA responses to pups above basal levels. The DA increases observed in maternally experienced females were independent of whether the experiences were acquired through prior interactions with the dam's own pups under hormonal priming or recent interactions with donor pups in the absence of lactational hormones. Moreover, the combination of the two

experiences significantly increased DA activity compared to any single type of maternal experience, suggesting an additive effect of different experiences. In the absence of ongoing maternal behavior these increased DA responses in experienced females were observed during the initial 8-min period of pup-stimuli exposure and were no longer different from basal level by 24 min of exposure to pup-stimuli for all females. Finally, the DA activity in the NACsh was significantly correlated to approach behavior (i.e., the duration spent pup-sniffing) at all time points.

3.1. *Dopamine and pup-saliency*

Many studies have shown that pups become rewarding to female rats. For example, nulliparous females can acquire a place preference following pup-sensitization and conditioning trials (Fleming et al., 1994). With recent pup-experience, multiparous (but cycling) rats will press a lever to gain access to pups (Lee et al., 2000). In addition, lactating dams will cross a floor with electric shock to retrieve pups (Fahrbach and Pfaff, 1982), develop conditioned place preferences for pups (Mattson et al., 2001, 2003; Mattson and Morrell, 2005), and choose pups over food rewards (Fahrbach and Pfaff, 1982). In fact in lactating rats, the pups become so salient that they can compete with self-administration of cocaine (Hecht et al., 1999). Together these studies demonstrate that pups become reinforcing to postpartum lactating or recently pup-responsive (cycling) female rats.

The mesolimbic dopamine system and its forebrain targets are part of the motivational system that regulates responses to many reinforcers such as food, drink, sex, social interactions and drugs (for review see, ; Kelley and Berridge, 2002; Everitt, 1990; Robinson and Berridge, 2000, 2001). Activation of dopaminergic mesolimbic system or reward pathway (for review see, Robinson and Becker, 1986; Robinson and Berridge, 2000, 2001; Robbins and Everitt, 1996; Robinson and Kolb, 1997) has been hypothesized to contribute to the reinforcing property of pups (Champagne et al., 2004; Fleming et al., 1994; Hansen et al., 1991a,b; Lonstein et al., 2003; Lee et al., 2000; Magnusson and Fleming, 1995; Numan, 2007; Rosenblatt and Mayer, 1995). For example, lesioning the ventral tegmental area (VTA) (Gaffori and LeMoal, 1979) and depleting DA levels in the terminals of the VTA to the NAC (Hansen et al., 1991a,b), reduce maternal behaviors. The reinforcing properties of pups during the postpartum period can be blocked with a DA receptor antagonist (Fleming et al., 1994). Infusions of DA_1, but not DA_2, receptor antagonists into the NAC disrupt maternal retrievals in postpartum animals (Numan et al., 2005). When partially hormone-primed (hysterectomy at day 16 of gestation) pup-naive female rats are intracerebrally injected with a D1 receptor agonist into the NAC, maternal behavior is facilitated (Stolzenberg et al., 2007). Previous *in vivo* studies evaluating the mesolimbic DA system in sustaining ongoing maternal behavior have shown that DA is important for appetitive and consummatory aspects of maternal responding in the lactating rat (Champagne et al., 2004; Hansen et al., 1993). Together, these studies suggest that NAC DA seems important for approach behaviors within the maternal context.

Given that nulliparous pup-sensitized females were not engaged in active maternal behaviors during sampling, increased DA responses and approach behavior associated with the initial presentation of pup-stimuli, rather than the duration, may indicate that pups acquire salient properties important for approach behavior. Furthermore, maternal responding seems important for the formation of pup-saliency, as without recent maternal responding nulliparous females do not demonstrate the increased DA or approach responses to pups, despite having received pup-stimuli (30 min for 5 days) during the habituation process. This finding is not surprising, as lactating females prevented from interacting with pups during conditioning sessions do not develop place preferences for pups (Magnusson and Fleming, 1995). It is important to note here that DA responding to other rewards (e.g., food) was not contrasted to DA responding to pups. Consequently it is unknown if the effects on DA release are specific to pups. Currently we are addressing this question in the lab.

3.2. *Dopamine and maternal memory*

Once the parturitional hormones have exerted their effects and maternal behavior has been displayed (see Numan et al., 2006) in postpartum rats, a different set of neuroendocrine and brain mechanisms are activated to maintain responsiveness throughout the lactational period (Fleming et al., 1996). The latter mechanisms are the ones that most likely overlap with the ability of pup-stimuli to elicit significant DA increases, in the absence of recent pup-experiences in multiparous rats. Interactive experience with pups during the peripartum hormonal environment enhances the long-term retention of maternal behavior. Long-term change in behavior as a result of experience interacting with pups during the postpartum period is referred to as the maternal experience effect (MEE, Orpen and Fleming, 1987). Lesions to the NACsh do not have a major disruptive effect on ongoing maternal behavior but have a substantial disrupting effect on the development of the MEE (Li and Fleming, 2003). Thus, DA in the NACsh maybe involved in responding to previous cues that were involved in the development of the MEE.

Engaging in maternal crouching and stimulation by moving and suckling pups are probably essential features of the maternal experience in rats (Morgan et al., 1992; and see, Ferris et al., 2005). Suckling stimulation in lactating dams is a robust stimulus for activating the mesocorticolimbic system (Ferris et al., 2005). For example, pup suckling stimulates the same mesocorticolimbic dopaminergic system as cocaine in virgin females, providing evidence that both reinforcing stimuli elicit similar brain mechanisms regarding motivation and reward (Ferris et al., 2005). This pathway appears to be a critical neurochemical pathway in the anticipatory and consummatory aspects in maternal behavior of the lactating rat (Champagne et al. 2004; Hansen et al., 1993; Ferris et al., 2005). However, this dopaminergic pathway from the VTA to the accumbens and prefrontal cortex system involved in reward seeking may help to strengthen the pup–dam bond by mediating aspects of the MEE.

3.3. *Metabolic activity*

In addition to assessing DA, metabolic activity was also measured after maternal experiences. DOPAC levels significantly

increased initially in response to pup-stimuli for females given prior parities, independent from the recency of donor pup-experience. 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 multiparous rat, changes in the NACsh that alter dopaminergic functions may be a result of physiological changes (e.g., hormone profile) associated with pregnancy, the birthing process, and/or the parturient experiences with pups.

The DA turnover rate (i.e., DA/DOPAC) significantly increased during later interactions with pup-stimuli in females given recent donor pup-experience, independent of prior parities. Recent maternal responding with pups altered DA activity such that DOPAC increased while DA decreased. Thus, DA metabolism was accelerated during pup-exposure. One possible explanation for this finding may be that pup-stimuli increases DA release from axon terminals and might simultaneously accelerate DA reuptake via a proportional increase in the activity of DA transporters, thereby maintaining a steady extracellular concentration of DA and increasing the production of DA metabolites (see, Fink-Jensen et al., 1994). Although an increase in dopaminergic neuronal activity increases DOPAC, the increase in concentrations of DA metabolites that occur during pup-exposure may be independent of DA release (see, Adachi et al., 2000).

3.4. *Nucleus accumbens and maternal experiences*

The present study demonstrated that both prior parity and recent donor pup-experience had similar effects on NACsh DA response to pups. However, the finding that the combination of the two experiences had greater effect than either experience alone suggests that the experiences have different effects on the NACsh. Recent pup-experiences may temporarily alter mechanisms associated with NAC DA responding, while prior parity experiences may provide long-lasting modifications in the NAC. Recently, we found that the NACsh changes significantly after postpartum pup-experiences (Akbari et al., 2007). Compared to maternally inexperienced females, experienced females (30 days after parturition and separation from pups) showed higher levels of surviving new neurons in the NACsh (Akbari et al., 2007). While these morphological changes within the NACsh that result from postpartum pup interactions may be one mechanism that mediates the pup-induced DA activity, this conclusion awaits further investigation.

4. Experimental procedures

4.1. *Animals and housing*

Sprague–Dawley female rats (225–300 g, $n=30$) obtained from the colony bred at the University of Toronto at Mississauga, were housed individually in transparent Plexiglas cages (47 cm × 26 cm × 20 cm) and given food and water ad libitum on a 12:12 light cycle. Sixteen male (400–600 g) and ten pregnant female (300–425 g) obtained from the same breeder and housed as above served for mating (Parity factor) and donor mothers for the pup-sensitization (Recent factor) procedure, respectively. All female rats were housed and mated in colony rooms that contained no pregnant females. Once mated or given donor pups, females were placed into a colony room that contained other pregnant or nursing females. Thus, if a female was in a group that did not require maternal experience, she did not receive pup cues from other cages.

4.2. *Procedure prior to sampling*

4.2.1. *Summary*

Briefly, the females were randomly assigned to one of four groups: (1) nulliparous (no previous litters) (NS^-); (2) nulliparous and recently sensitized to pups (NS^+); (3) multiparous (two previous litters) (MS^-); and, (4) multiparous and recently sensitized to pups (MS^+). Females in the multiparous groups were mated twice and allowed to nurse and wean their litters. Nulliparous females remained individually housed during this time. When no longer lactating, all females were surgically implanted with guide cannulae above the NACsh. Then, females in the pup-sensitization groups were given pups until they behaved maternally in their home cage. All females were given habituation trials with donor pups in the microdialysis collection chamber. A previous pilot study in our laboratory has shown that under the habituation conditions used here the maternally experienced females that show high levels of all maternal behavior in their homecage (either previously with their own litter or recently with donor pups), show moderate levels of pup-sniffing, very low levels of pup mouthing and licking, and no instances of nursing postures, pup retrieval, or nest building in the dialysate chamber. Maternally inexperienced females show low levels of pup-sniffing and no maternal behaviors.

4.2.2. *Post-partum maternal experience with own pups (Parity factor)*

Half of the females were mated in their homecage for 10 days with a male. These females gave birth (range 18–23 days after separation from male) and were allowed complete access to their culled litter (3 males, 3 females) until postpartum day 20 at which time pups were removed permanently. This procedure was repeated a second time (hence, two births) three weeks after the pup removal of the first litter. Cannula implant surgery took place 30–45 days after the second litter was removed from the dam. The females that were not mated remained alone in their homecage during this time but were intermittently handled by the experimenters until the surgery.

4.2.3. *Surgery*

All females 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 NACsh (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. The surgery was carried out under general sodium pentobarbital

anesthesia, (Somnotol, MTC Pharmaceuticals, 65 mg/kg. i.p.) with a pre-anesthetic (Atropine, 0.1 cm^3., s.c). All surgical procedures were in strict accordance with the Canadian Council Animal Care Committee.

4.2.4. *Pup-induced maternal experience with donor pups (Recent factor)*

One week after the cannula implant surgery, half of the females from each Parity group (i.e., Nulliparous, Multiparous) were placed into a colony room with pregnant and postpartum mothers with their pups. Daily, these rats were given 6 well-fed donor foster pups (3 males, 3 females, postnatal days 2–4) from a donor lactating mother. Pups remained with experimental females for 24 h. This procedure continued until the females were observed consecutively for 2 days to build a nest, retrieve all of the new pups to the nest site within 5 min, lick the pups on the body and anogenital region, and adopt nursing postures (i.e., high and low couch). The assessments of the retrieval and licking criterion were made daily when placing the new pups into the homecage; and nursing postures observations were made 4 times daily when the females were undisturbed. Females given donor pups took 3 to 21 days to meet the above maternal criterion with the multiparous females (mean ± SEM = 4.3 ± 1.9) acquiring maternal behavior more quickly (assessed with a *t*-test, $p < .05$) than nulliparous females (mean ± SEM = 9.3 ± 3.3). Donor pups were removed from the homecage 5 h prior to sampling. The females not receiving donor pups remained alone in their homecage during this time.

4.2.5. *Habituation*

During habituation females were moved into a room (illuminated with red light) that contained the caging system BASi Raturn™, (BAS, West Lafayette, IN, USA). The caging system is an interactive system that allows freedom of movement in all directions, while maintaining the integrity of infusion lines. A counterbalanced arm and tethering system kept test lines out of the animal's reach, while permitting the animal to rise and stretch at will. During the pup-sensitization procedure, all females were given 1-h sessions in the clear circular plastic cage (188 cm^2, 24.77 cm diameter, 29.8 cm high, with two stainless steel food dispensers) for 5 consecutive days. After 30 min in the chamber, 4 pups (2 male, 2 female) were given to females. During the habituation trials, females were never observed to retrieve, adopt nursing positions, nor mouth the pups. While two females, given prior parities and sensitization (MS^+), were observed to lick the pups briefly (<3 licking bouts at <2 s/bout) during the later habituation sessions, no other females engaged in maternal responding.

4.3. *Procedure during in vivo sampling*

4.3.1. *Apparatus*

Prior to probe placement, the inlet and outlet portion 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 placed in the refrigerated (4 °C) BASi HoneyComb fraction collection system (BAS, West Lafayette, IN, USA). In addition, the vials contained a standard antioxidant (2.5 mM ascorbic acid in 0.9% saline, Sigma Aldridge, St. Louis, MO). The probe was flushed at a rate of 2 ul/min for 30 min with artificial cerebral spinal fluid (BAS, West Lafayette, IN, USA).

4.3.2. *Procedure*

Females were tethered in the caging system. 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 ul/min for 8 min. After 25 samples were collected (200 min), 4 warm recently-fed donor pups (2 male, 2 females, postnatal days 2–4) were placed in the chamber opposite to the feeding dispensers for the duration of 3 sample collections (24-min pup-exposure phase). Behavioral data during the pup-exposure phase was filmed with a digital video camera attached to a tripod. The pups were then removed and the female remained in the chamber for two more hours of sample collection.

4.3.3. *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 (Bioanalytical Systems, 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 nM 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 2.93 and 4.39 min, respectively, under the set conditions.

4.3.4. *Perfusion and histology*

After sample collection, 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.

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