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Accumbal dopamine function in postpartum rats that were raised without their mothers

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ABSTRACT

Postpartum rats that had been previously raised in an artificial rearing (AR) apparatus, without their mothers or siblings during the preweaning period, show altered maternal responses towards their own offspring in adulthood. In mother-reared (MR) rats, nucleus accumbens (NAC) dopamine (DA) responses to pups evoke a robust sustained rise during the postpartum period and following treatment with estrogen/progesterone parturient-like hormones (Afonso et al., 2009). These MR females had siblings that received AR rearing with varying amounts of preweaning tactile stimulation (ARmin; ARmax). The present study examined NACshell DA responses to pup and food stimuli in these AR rats, and statistically compared them to their MR siblings. Microdialysis samples were collected from adult (90 days postnatal) AR females in different parity states (cycling vs. postpartum, Exp. 1), or after ovariectomy with different hormone treatments (sham vs. hormone, Exp. 2. After basal sample collection, pup and then food stimuli were individually presented to the females in the dialysis chamber. As with their MR siblings, basal DA concentrations were lower and pup-evoked DA responses greater in hormonally-primed AR females than in non-primed AR controls. Compared to their postpartum MR sisters (Exp. 1), AR rats had increased basal DA levels, reduced pup related DA elevations, and disrupted maternal behavior. The postpartum AR impairment in pup-evoked DA was reversed by additional pre-weaning tactile stimulation. Exogenous hormones (Exp. 2) eliminated AR impairments on pup-evoked DA responses. Although MR and AR siblings had comparable DA responses to food stimuli, upon reanalyzing MR data it was found that only postpartum dams had DA responses to pups greater than to food. These data suggest that that the hormonally induced suppression of basal DA levels may reflect saliency of pups which was greater in MR than in AR dams. Preweaning tactile stimulation could partially reverse these effects only in naturally cycling or parturient animals.

Introduction

Variations in rodent maternal behavior affect many aspects of physiological and behavioral development of offspring. For example, the amount of licking that mothers exhibit towards their offspring following parturition is directly related to the levels of licking they received from their own mothers (Champagne and Meaney, 2001; Champagne et al., 2001). The amount of licking received in early development affects many aspects of the animals' physiology later in life (see Francis et al., 1999; Liu et al., 1997). During the pre-weaning period, periodic separations from the mother affect physiological and behavioral processes in developing pups that resemble the offspring of a low-licking mother (for reviews see Numan et al., 2006; Matthews and Robbins, 2003; Meaney, et al., 2002; Pryce and Feldon, 2003). The present research is a natural extension of the maternal deprivation work but utilizes a more pervasive form of maternal deprivation known as artificial rearing (AR) (see Hall, 1975), that involves complete isolation from mother and littermates commencing on postnatal day 4.

Fleming and colleagues have found that rats raised artificially show reduced maternal licking and crouching toward their own offspring in adulthood (Gonzalez et al., 2001; Melo et al., 2006; Lovic et al., 2011). AR rats also had altered locomotor activity, attention, impulsivity, response inhibition, cognitive function, and emotionality, which likely underlie the deficits observed in a number of the species-typical reproductive behaviors. Accompanying these behavioral deficits were alterations in physiology, neurochemistry and neurobiology (Akbari et al., 2007, 2008; Burton et al., 2007; Chatterjee et al., 2007; Chatterjee-Chakraborty and Chatterjee, 2010). In these studies, increasing the number of tactile stimulations (i.e. maternal licking-like stimulation) that AR pups received partially reversed many of these effects (see Akbari et al., 2007, 2008; Burton et al., 2006, 2007; Chatterjee et al., 2007; Fleming et al., 2002; Gonzalez and Fleming, 2002; Gonzalez et al., 2001; Lévy et al., 2003; Lovic and Fleming, 2004; Lovic et al., 2006, 2011; Melo et al., 2006; Novakov and Fleming, 2005; Rees et al., 2006).

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Early adversity results in impaired function of the mesolimbic dopamine (DA) system and DA mediated behaviors (see, Everitt and Robbins, 1997; Fone and Porkees, 2008). For example, elevated behavioral hyperactivity in response to DA agonists is observed following periodic isolation rearing (Brake et al., 2004;; Hall, 1998; Hall et al., 1999; Matthews and Robbins, 2003). Similarly, AR rats treated with DA agonists such as amphetamine show dose-dependent increases in locomotor activity above and beyond what is typically observed in maternally-reared (MR) rats (Lovic et al., 2006, also see Lomanowska et al., 2006). DA in mesolimbic pathway has been implicated in many of the functions necessary for optimal mothering. Using *in vivo* voltammetry to monitor changes in extracellular DA in the nucleus accumbens (NAC) shell of lactating rats interacting with pups, Champagne et al. (2004) found that the DA signal increased significantly with pup licking and that the magnitude of DA increases was greater in high- compared to low-licking dams.

In a microdialysis study (see Afonso et al., 2009), adult postpartum MR, but not cycling MR females showed elevated and sustained NAC DA responses to pups. In a follow-up experiment (Afonso et al., 2009), ovariectomized (OVX) females that had no previous experience with pups were given hormone treatments known to facilitate maternal behavior (see Bridges, 1984; Moltz et al., 1970). These hormone-treated MR females displayed the same DA profile as postpartum dams despite never having had experience with pups. OVX sham-treated females resembled cycling females with no prior maternal experience (Afonso et al., 2009). Together, DA alterations in adult rats have been shown to be modulated by preweaning maternal experiences and ovarian hormones associated with parity status. However, it is currently unknown how these factors interact.

To investigate how parity and hormones interact with early maternal experiences, the present study compared stimulus-evoked DA responses of rats reared in isolation from their mothers (AR) to their mother-reared (MR) siblings that were raised in a litter consisting of 4 male and 4 female pups (see Afonso et al., 2009). We examined pup-evoked DA responses in MR and AR female siblings following parturition (Parity, Experiment 1) or after hormone- treatments simulating parturition (Hormone Treatment, Experiment 2). As in our previous study with MR females, DA release was measured from the NAC shell in AR rats during interaction with donor pups and subsequently, during exposure to food stimuli. After the sampling procedure all rats were given a choice to interact with both pup and food stimuli presented simultaneously to provide information about the stimuli preferences in the different parity, treatment, and rearing groups.

Methods

General procedure

Subjects

Sprague–Dawley female rats (225–300 g) 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 standard 12:12 light cycle. Males (400–600 g) and pregnant females (300–425 g) obtained from the same breeder and housed as above, served for mating (Parity, Experiment 1) and for donor mothers (Hormone Treatment, Experiment 2), respectively. After mating (Experiment 1) or maternal induction tests (Experiment 2) females were placed into a colony room that contained other pregnant or nursing females. 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.

Artificial rearing

On postnatal day (PND) 4, two female pups were removed from each of 40 litters. These pups were randomly assigned to a rearing group: ARmin or ARmax. Female siblings were left with the mother (MR) in the culled litter that consisted of 4 male and 4 female pups. These MR rats were used in the Afonso et al. (2009) publication and were included in some statistical comparisons in the present study. The AR procedure was performed as in Novakov and Fleming (2005). After the pups were weighed on PND 4, a feeding tube was surgically placed into the right cheek of the pup with the use of a topical anesthetic (Eutectic Mixture of Local Anesthetics [EMLA], containing 2.5% lidocaine and 2.5% prilocaine). A leader wire (stainless steel, 0.25 mm in diameter) sheathed in silastic tubing (lubricated with mineral oil) and polyethylene (PE) 10 tubing was used to pierce the cheek. Once the flared end of the tubing contacted the inside of the cheek, the leader wire and silastic tubing were removed, and antibiotic ointment was applied topically to the site of penetration. Another leader wire was then used to insert a t-washer, which was secured in place with Superglue. MR pups had their cheeks pierced, but the PE 10 tubing was removed.

Following surgery, AR pups were individually placed into plastic cups (11 cm diameter × 15 cm deep) containing corn-cob bedding, and then placed inside another weighted cup. The cups floated in temperature controlled (34–37 °C) water directly below time-controlled infusion pumps (PHD 22/2000 syringe, Harvard Apparatus, Holliston, MA) to which they were connected by a cheek cannula tubing. The pups were fed via the infusion pump connected to the feeding tubes which delivered milk (Messer diet) for 10 min every hour, 24 h a day (for complete feeding procedure, see Novakov and Fleming, 2005). The amount of milk infused was calculated on the basis of mean pup body weight. Beginning on PND 4, pups received a volume of milk equal to 33% of the mean body weight, and this amount increased by 1% daily. Each morning, the pups were disconnected from the pumps, their weight was recorded, and all tubing was flushed with double distilled water. New syringes with fresh formula were prepared, and the new infusion rates were programmed based on the new weights.

Pre-weaning tactile stimulation

Each day, ARmin pups were stimulated twice (morning and night) for 30 s each with a wet camel hair paintbrush in the anogenital region to stimulate urination and defecation. ARmax pups received the same anogenital stimulation twice daily, as well as 1.5 min of dorsal stimulation with a dry camel hair paintbrush five times a day. Stimulations for both groups occurred daily from PND 4 through to PND 16. On PND 17–18, pups were removed from the pumps and given milk formula, rat chow, and a mixture of the two. On PND 21, all rats were weighed and paired with a social partner of the same sex from another litter that was not tested. Rats were left undisturbed until PND 90 when they were randomly assigned to one of 2 experiments.

In-vivo sampling

Prior to brain cannula surgery in adulthood (PND 100–120), all animals were habituated to the microdialysis cage. The caging apparatus consisted of an interactive system that allowed 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. All females were given 1-hour 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.

All females were then stereotaxically implanted with a unilateral guide cannula with a stylet (15 mm in length, BAS, West Lafayette, IN, USA) aimed above the NAC shell (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). During sampling, a probe (320 μm OD, 2 mm length, cut off 20,000 Da, BAS, West Lafayette, IN, USA) that extended 2 mm below the guide was attached

to tubing and a needle that dispensed the collection samples at a rate of 1 μl/min in 8 min bins into refrigerated vials (4 °C) containing a standard antioxidant (2.5 mM ascorbic acid in 0.9% saline, Sigma Uldridge, St. Louis, MO).

After 25 baseline samples were collected (200 min) in the undisturbed rat, 4 warm recently-fed donor pups (2 male, 2 females, PND 2–4) were placed on shredded paper towel (nesting material) in one of the dispensers for the duration of the three 8-min sample collection bins (i.e. pup stimuli for 24 min). Behavioral data during the pup stimuli exposure was recorded with a digital video camera attached to a tripod. After pup removal and 16 min (2 samples) with no stimuli, 4 FrootLoops® were placed into the center of the chamber. Food stimuli were given to the females in their homecage and during the habituation sessions to prevent neophobia. During sampling, 4 Frootloops® were always eaten within an 8-min period (1 sample). After food stimuli were eaten, the sampling procedure continued for 120 min in the absence of any stimuli.

High performance liquid chromatography (HPLC)

After collection of the samples, DA concentrations were determined with HPLC, as in Afonso et al. (2009). Briefly, 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 monochloroacetic 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 the detection limit was set at 500 nA. Of the 8 μl collected per sample, 5 μl was directly injected into the HPLC system 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 were approximately 3.32 min under the set conditions. Although we previously reported on these MR females (see Afonso et al., 2009), MR and AR siblings were yoked for sampling and HPLC assays.

Preference task

Following the sample collection procedure, probes were removed and the female was placed briefly into her homecage. In the dialysate chamber, one of the dispensers was filled to the top with Frootloops® and the other with four donor pups on shredded paper towel. The female was placed back into the chamber (untethered, no sampling) and her behavior was videotaped for 8 min. Time spent eating and/or engaged in maternal behaviors were measured during this 8-min preference 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.

Animal inclusion

After the experiments animals were perfused and probe placements were determined using histology. 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. 1 shows the anatomical placements of the "active" zones of the probe for both experiments.

Parity procedure (Experiment 1)

AR females (n = 20) were either placed with males for mating (postpartum) or left alone (cycling) in their homecages for 10 days. Cycling and pregnant (day 14–16 gestation) females were implanted with guide cannulae placed into the NAC shell. After delivery (PPD1), litters were removed from the homecage and the *in vivo* sampling procedure and the preference task were performed. The group sizes were as follows (see Fig. 1), 16 AR: postpartum dams (min, n = 4; max, n = 4) and cycling controls (min, n = 4; max, n = 4) and 11 MR rats: postpartum dams (n = 6) and cycling controls (n = 5). As indicated earlier, these MR females were raised with their mothers and sampled concurrently with the AR females. Sampling for postpartum females began 24 h after delivery of pups and cycling females were tested during random phases of their cycle.

Hormonal treatment procedure (Experiment 2)

To determine whether exogenous hormones could eliminate the AR effects on the DA profile, we performed a second study using OVXed, pup-naïve, virgin females given hormonal treatments known to facilitate the expression of maternal behavior (Bridges, 1984; Moltz et al., 1970) in OVX MR and AR animals (Novakov and Fleming, 2009). All virgin pup-naïve females (N = 20) were OVXed and randomly assigned to sham- or hormone- treatment groups. During the cannula surgery, hormone-treated females were implanted subcutaneously (s.c.) with a 17β-estradiol 3-benzoate (E) capsule in the dorsal region behind the neck. Two days later females were anesthetized briefly and three progesterone (P) capsules were implanted s.c.. For these pup-naïve females, P capsules were removed 10 days after implant and 24 h prior to the *in vivo* sampling procedure and preference task. Estradiol capsules were left in place (for more details see, Afonso et al., 2009). Sham-treated females were given empty capsules at the same time points. Included in statistical analysis were 19 OVX AR rats (see Fig. 1): hormone-treated (min, n = 5; max, n = 5), and sham-treated controls (min, n = 4; max, n = 5) and 10 MR siblings: hormone-treated (n = 5) and sham-treated controls (n = 5).

Results

Statistical procedures

All analyses of variance (ANOVAs) were followed by posthoc Tukey's HSD analysis ($P<0.05$, two-tailed) on individual means when appropriate.

Dopamine

For both experiments, baseline DA concentrations (pg/μl) were obtained by calculating the mean of three consecutive samples prior to stimulus presentation that differed from one another by no more than 10%. To assess baseline DA levels differences, we conducted 2 (for Exp. 1 Parity: cycling vs. postpartum; OR for Exp. 2 Hormone Treatment: sham vs. hormone) × 3 (Rearing: MR vs. ARmin vs. ARmax) ANOVAs.

To evaluate the stimulus-evoked DA responses for each experiment, a 2 (Parity OR Hormone Treatment) × 3 (Rearing) × 2 (Stimulus: pups vs. food) ANOVA was performed on DA responses (% of basal concentrations). For the stimulus variable, we averaged the three 8-min periods of pup-stimulation to contrast this data with the single 8-min period of food stimulus in which all rats consumed 4 Frootloops®.

Basal DA measurements differed with parity status (Exp. 1) and hormone treatments (Exp. 2). Thus in each experiment to further investigate the influence of basal DA levels on DA responses to pup-stimuli across time, we covaried the basal concentrations into a 2 (Parity OR Hormone Treatment) × 3 (Rearing) × 4 (Sample: basal and 3 sequential pup-exposure samples) ANOVA performed on DA responses (% over basal concentration).

Maternal behaviors

In each study, a 2 (Parity OR Hormone Treatment) × 3 (Rearing) ANOVA was performed on mean time (seconds) spent pup-sniffing, pup-licking, and exhibiting general locomotor activity. In Experiment

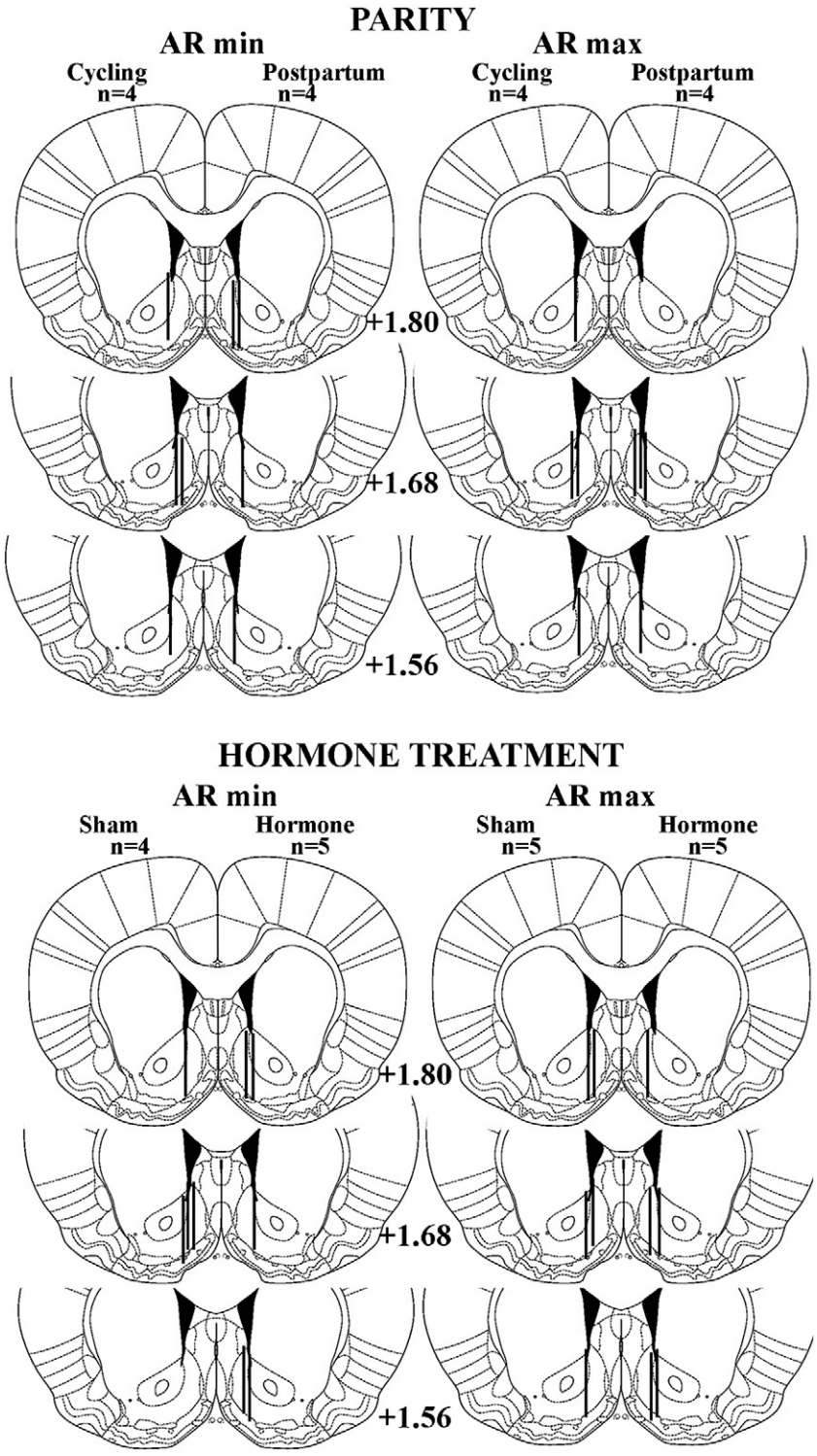

Fig. 1. Schematics of anatomical unilateral placements (anterior to Bregma, according to Paxinos and Watson, 1986) of the "active zone" (shaded lines) of microdialysis probes in the nucleus accumbens shell at three different locations anterior to Bregma, in artificially-reared (AR) females with minimal (min) or maximal (max) preweaning tactile stimulation that were intact cycling or postpartum (PARITY, Exp 1.) or OVX sham- or hormone- treated (HORMONE TREATMENT, Exp. 2.). To see similar depiction of probe placements for maternally-reared females see Afonso et al. (2009).

1, the number of pups retrieved during *in vivo* sampling procedure was also analyzed.

Preference task

After sampling, time (seconds) spent engaged with either pup or food stimuli during the preference test were analyzed using a 2 (Parity OR Hormone Treatment) × 3 (Rearing) × 2 (Behavior: maternal vs. eating) ANOVA.

Relation of DA to behavior

To assess the relationship between pup-induced DA responses with *ongoing* behaviors, partial correlations controlling for parity (Experiment 1) or hormone treatment (Experiment 2) and rearing

were performed on total DA responses (% of basal concentrations) during the 24-min of pup stimuli with total behavioral durations of pup-licking, pup-sniffing and general locomotor activity. Similar correlations were performed for basal DA levels with the behavioral measures.

Parity effects (Experiment 1)

Dopamine

Analysis of basal DA concentration showed significant main effects of Parity [$F(1, 21) = 17.46$, $P<0.001$] and Rearing [$F(2, 21) = 10.28$, $P = 0.001$], as well as a Parity × Rearing interaction [$F(2, 21) = 5.28$, $P = 0.014$]. Collectively, postpartum dams had significantly lower basal DA levels compared to cycling controls. Posthoc analysis revealed that cycling ARmin rats had greater baseline DA concentrations than MR rats, and postpartum AR (min and max) rats also had greater basal DA concentrations (see Fig. 2A).

For DA responses during access to pup vs. food stimuli, the ANOVA showed significant interactions between Parity × Rearing [$F(2, 21) = 3.50$, $P = 0.049$], Parity × Stimulus [$F(2, 21) = 14.63$, $P<0.001$]; and Rearing × Stimulus [$F(2, 21) = 3.56$, $P = 0.04$]. Relative to the cycling group, postpartum females had increased pup-evoked DA responses, with rearing differences observed in the postpartum group. Posthoc analyses indicated that MR and ARmax mothers had increased DA responses to pups compared to ARmin postpartum females. Postpartum MR females had increased DA responses during periods of pup interaction relative to periods of food consumption, a pattern that was not observed in the AR rats (Fig. 2B).

Parity and rearing group differences in pup-evoked DA responses remained significant when basal DA levels were covaried into the analyses. There was a main effect of Parity [$F(1, 20) = 10.08$, $P = 0.005$], and interactions of Parity × Sample [$F(3, 60) = 3.51$, $P = 0.02$] and Rearing × Sample [$F(3, 60) = 3.10$, $P = 0.01$] (see Fig. 8A). This additional analysis revealed that within the postpartum group there were rearing differences across the 24-minute period with pups. For the first 16 min of pup access, postpartum MR and ARmax females had increased DA responses to pups compared to postpartum ARmin rats, their cycling controls, and their own basal DA concentrations (i.e.100%). After 24 min with pups, only postpartum MR dams showed a sustained pup-evoked DA response, compared to all postpartum AR groups, their cycling controls, and their own basal DA concentrations (Fig. 8A). Finally, during the initial 8 minute exposure to pups, postpartum ARmax females showed the largest pup-evoked DA responses compared to the other postpartum groups.

Maternal behaviors

During sample collection, postpartum AR females performed many of the typical maternal behaviors observed in their postpartum MR siblings. These behaviors included pup-sniffing, retrieval, and licking of the body and/or anogenital region. See Fig. 3 for behaviors during *in vivo* sampling. However, there was one exception: postpartum AR females did not hover over pups as did postpartum MR females (see Afonso et al., 2009). Like cycling MR females, cycling AR females engaged in little to no pup related behaviors.

Postpartum females retrieved (Parity effect [$F(1, 21) = 48.53$, $P<0.001$], Fig. 3A) and sniffed pups more (Parity effect [$F(1, 21) = 7.35$, $P = 0.013$], Fig. 3B) compared to the cycling controls. For pup-licking duration, the ANOVA produced significant main effects of Parity [$F(1, 21) = 21.45$, $P<0.001$] and Rearing [$F(2, 21) = 8.16$, $P = 0.002$], as well as an interaction of Parity × Rearing [$F(2, 21) = 8.32$, $P = 0.002$]. Posthoc tests revealed that postpartum dams licked pups significantly longer compared to cycling females, however, the postpartum AR groups licked less compared to postpartum MR females (Fig. 3C). Finally, for locomotor activity, the ANOVA found a significant Rearing effect [$F(2, 21) = 3.786$, $P = 0.04$]. Postpartum AR females showed greater locomotor activity compared to postpartum MR females when pup stimuli were presented in the dialysis chamber (Fig. 3D).

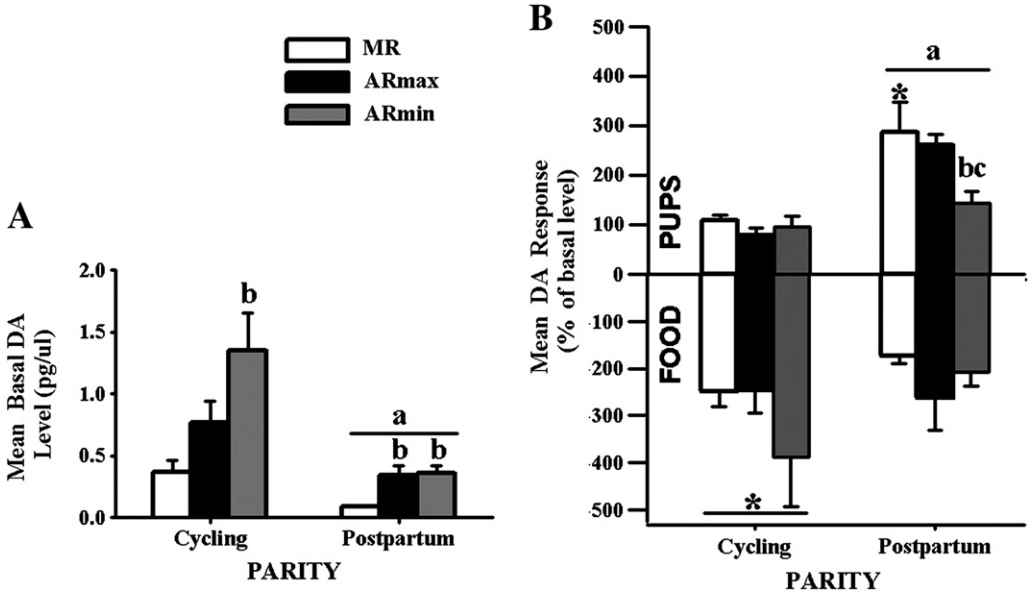

Fig. 2. Parity and rearing effects on dopamine (DA) (Experiment 1). *In vivo* DA sample collection for intact cycling or postpartum females that had been either mother- (MR) or artificially- (AR) reared with minimal (min) or maximal (max) preweaning tactile stimulation. (A) Mean (+ SEM) basal DA level data represent the average DA concentration of 3 samples collected prior to pup stimuli. Prior to any stimuli exposure, postpartum females had decreased basal DA concentrations compared to their cycling controls. This decrease was less robust in both postpartum AR groups. In the cycling females, ARmin had increased basal DA levels compared to MR females. (B) Mean (+ SEM) DA response data represent percent of basal concentration during access to pup (24-min, averaged across the 3 samples) and food (8-min, single sample) stimuli. Postpartum females had increased pup-evoked DA responses compared to the cycling group, although postpartum ARmin showed a less robust elevation compared to MR and ARmax dams. Only in the postpartum MR group was there increased pup- compared to food- evoked DA responses. All cycling females showed increased food- compared to pup- evoked responses. *a* $P<0.05$ cycling/postpartum parity difference when collapsed across rearing groups for a given stimulus; *b* $P<0.05$ individual rearing difference from MR for a given parity and stimulus; *c* $p<0.05$ min/max individual AR preweaning tactile stimulation difference for a given parity and stimulus; *$P<0.05$ pup/food individual stimulus difference for a given rearing group and stimulus (line under this symbol indicates data collapsed across those groups).

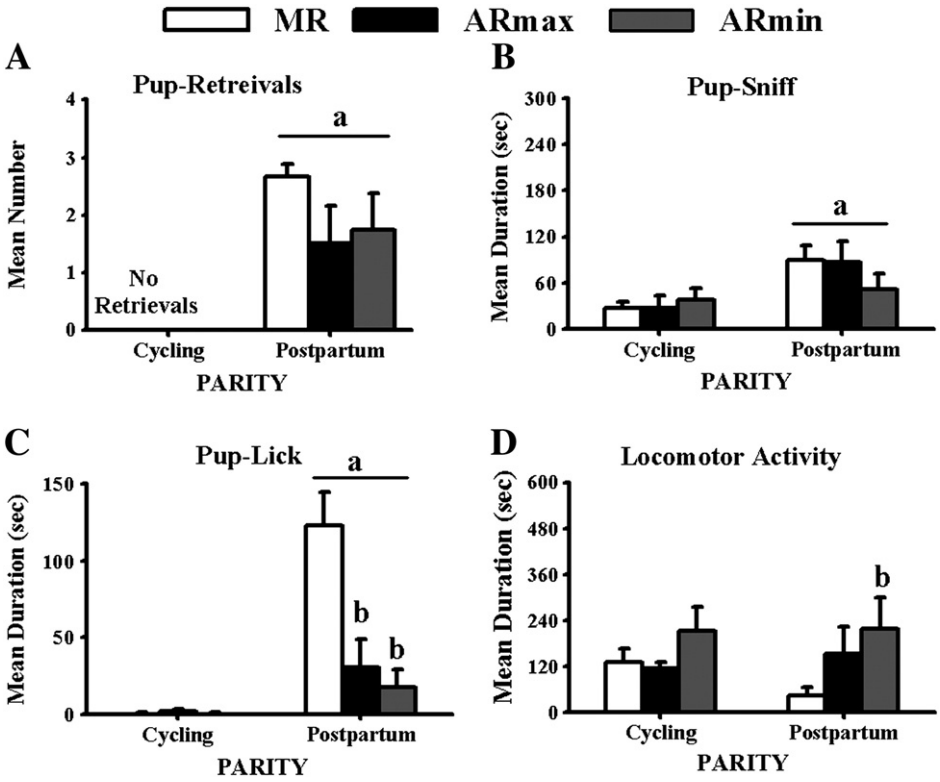

Fig. 3. Parity and rearing effects on maternal behavior (Experiment 1). Maternal behavior during *in vivo* sample collection for intact cycling or postpartum females that had been either mother- (MR) or artificially- (AR) reared with minimal (min) or maximal (max) preweaning tactile stimulation. (A) Pup-retrieval data represent the mean (± SEM) number of donor pups (total 4) retrieved. Other data represent mean (± SEM) of the total duration spent in (B) pup-sniffing, (C) pup-licking, and (D) general locomotor activity. Although all postpartum females displayed most of the typical maternal behaviors, compared to postpartum MR females, both AR groups licked pups less, with the ARmin group engaged in increased locomotor activity. *a* $P<0.05$ cycling/postpartum parity difference when collapsed across rearing groups; *b* $P<0.05$ individual rearing difference from MR for a given parity.

Preference task

When both types of stimuli were available, all cycling rats regardless of rearing experience only ate the Frootloops®. Conversely, postpartum MR females engaged exclusively in maternal behaviors (see Fig. 4). Unlike their cycling and postpartum MR siblings, postpartum AR females engaged in both eating and maternal behaviors (see Fig. 4). ANOVA results showed significant main effects of Rearing [$F(2, 21)=5.13$, $P=0.015$], Behavior [$F(1, 21)=44.16$, $P<0.001$], and interactions between Parity × Rearing × Behavior [$F(2, 21)=7.18$, $P=0.004$], Parity × Behavior [$F(1, 21)=32.23$, $P<0.001$], and Rearing × Behavior [$F(2, 21)=13.81$, $P<0.001$]. Compared to cycling controls, postpartum dams spent more time interacting with the pups. Posthoc tests revealed that among postpartum females, ARmin dams spent significantly less time with pups than did MR and ARmax dams. The ARmin females engaged in significantly more time eating relative to acting maternally, regardless of parity status (see Fig. 4). The postpartum MR dams spent the least amount of time eating compared to the other groups.

Relation of DA to behavior

Licking duration was correlated with pup-evoked DA responses ($df=23$, $r=.76$, $P<0.001$). Correlations between basal DA levels and other behavioral measures revealed no significant results.

Hormonal treatment effects (Experiment 2)

Dopamine

For baseline DA concentrations, we found significant main effects of Treatment [$F(1, 23)=36.62$, $P<0.001$] and Rearing [$F(2, 23)=33.00$, $P=0.001$], as well as a Treatment × Rearing interaction [$F(2, 23)=5.12$, $P=0.014$]. Hormone-treated females had significantly reduced basal DA levels compared to their sham controls. Within each

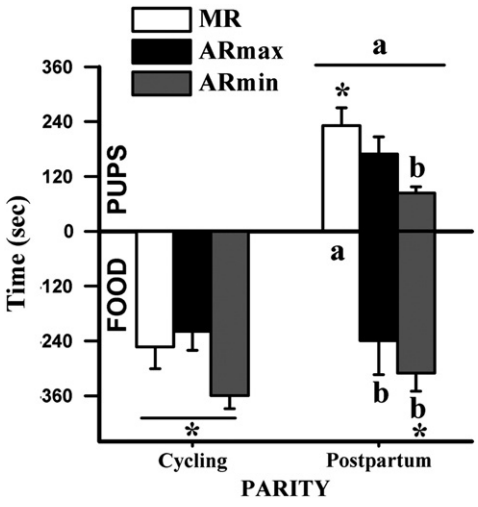

Fig. 4. Parity and rearing effects on preference task (Experiment 1). Choice task data represent mean (±SEM) duration engaged with pup and food stimuli. Unlike cycling females, postpartum females were responsive to pups during the choice task. Within the postpartum group, AR compared to MR females spent more time in food-related behavior and ARmin compared the MR females had decreased pup related behavior such that the ARmin females spent more time with food than pups. This was in contrast to postpartum MR females that ignored food stimuli when pups were available. All cycling groups only spent time in food-related behaviors during the choice task. *a* $P<0.05$ cycling/postpartum individual parity difference for a given rearing group and stimulus; *b* $P<0.05$ individual rearing difference from MR for a given parity and stimulus; *$P<0.05$ pup/food individual stimulus difference for a given parity and rearing group. Lines under letters and symbols indicate data collapsed across those groups.

treatment group, AR (min and max) females had increased DA basal levels compared to their MR siblings (see Fig. 5A).

For stimulus-evoked DA responses, the ANOVA found only main effects of Treatment [$F(1, 23) = 17.24$, $P<0.001$] and Stimulus [$F(1, 21) = 15.45$, $P = 0.001$]. The hormone-treated rats had increased DA responses to the stimuli compared to their sham controls, regardless of rearing experience or stimulus type. Collapsed across groups, the food-evoked DA response was greater than the pup-evoked DA response (Fig. 5B). When analyses of DA responses to pups were recomputed covarying basal DA levels, hormone-treated females no longer had significantly elevated DA responses to pups (Fig. 8B).

Maternal behaviors

OVX pup-naïve AR rats engaged in very little maternal behavior despite placement of pups on the dialysis cage floor, and hence without the need for active retrieval. For pup-sniffing duration, the ANOVA found significant main effects of Treatment [$F(1, 23) = 5.75$, $P = 0.025$] and Rearing [$F(2, 23) = 3.89$, $P = 0.035$], and a Treatment × Rearing interaction [$F(2, 23) = 6.13$, $P = 0.007$]. Sham-treated ARmin females sniffed pups significantly less compared to the other groups (Fig. 6A). For pup-licking duration, the ANOVA found significant main effects of Treatment [$F(1, 23) = 16.26$, $P = 0.001$] and Rearing $F(2, 23) = 10.323.89$, $P = 0.035$], and a Treatment × Rearing interaction [$F(2, 23) = 9.86$, $P = 0.001$]. Hormone-treated MR females showed significantly increased licking compared to all other groups (Fig. 6B). Finally, for locomotor activity duration, the ANOVA found a significant main effect of Rearing [$F(1, 23) = 4.22$, $P = 0.051$] and a Treatment × Rearing interaction [$F(2, 23) = 5.37$, $P = 0.012$]. Posthoc tests revealed ARmin sham-treated females had significantly increased locomotor activity compared to MR sham-treated and ARmin hormone-treated females (Fig. 6C).

Preference task

Prior to any pup experience, all pup-naïve females engaged in eating when both stimuli were available (Stimulus effect: $F(1, 23) = 662.58$, $P<0.001$). There were, however, significant interactions of Treatment × Stimulus [$F(1, 23) = 4.29$, $P = 0.050$] and Rearing × Stimulus [$F(2, 23) = 35.18$, $P<0.001$]. AR females spent more time eating compared to their MR siblings when both stimuli were present, regardless of treatment. Unlike hormone-treated MR females, hormone-treated AR females did not suppress eating when pups were in the neighboring dispenser (see Fig. 7).

After the sampling procedure and the preference task, all pup-naïve females were placed into their homecage with donor pups (2 males, 2 females, PND2-4, replaced daily). This procedure continued until the females reached the maternal criterion—defined as the retrieval of all pups to the nest site on two consecutive days, as well as observations of licking and crouching on at least one of those days. Crouching observations were made 4 times daily when the females were undisturbed. Hormone-treated female reached maternal criterion (range 2–6 days) more quickly than sham-treated females (range 10–22 days). No differences were observed between MR and AR females to reach maternal criterion after continuous exposure to pups in the homecage.

Only the hormone-treated females were tested twice on the preference task, before and after pup experience–pup experienced sham-treated females were not re-tested. During this second preference test, the hormone-treated MR siblings would retrieve pups and engage in other maternal behaviors (e.g. licking and nest building) but not eat FrootLoops ®. Unlike hormone-treated MR females, AR females continued to engage in eating behaviors following the pup experience. ARmin females engaged primarily in eating behaviors and ARmax females would retrieve the pups from their dispenser, bring them to the feeding dispenser, and eat while hovering over pups. Analyses confirmed these differences to be significant as the 3 (Rearing) × 2 (Behavior) × 2 (Pup Experience) ANOVA found all main effects and interactions to be significant (Ps<0.034). Posthoc tests revealed that following pup-experience MR females showed a shift towards maternal behavior and away from eating behavior. Unlike their MR siblings, AR females did not differ in time spent eating before and after pup experience. ARmin females spent less time exhibiting maternal behavior compared to both ARmax and MR females (see Fig. 7).

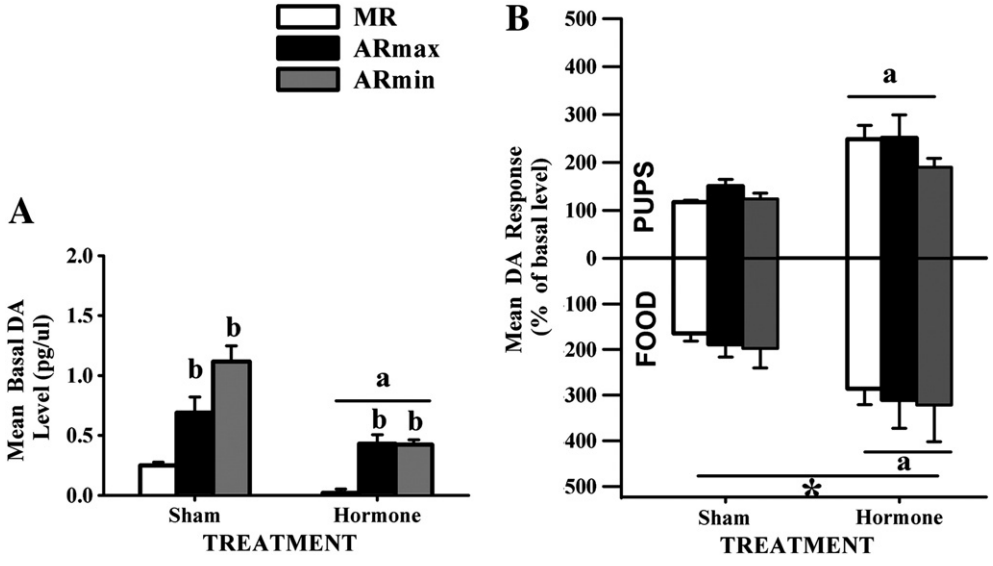

Fig. 5. Treatment and rearing effects on dopamine (DA) (Experiment 2). *In vivo* DA sample collection for OVX pup-naïve sham- or hormone- treated females that had been either mother- (MR) or artificially- (AR) reared with minimal (min) or maximal (max) preweaning tactile stimulation. (A) Mean (+ SEM) basal DA level data represent the average DA concentration of 3 samples collected prior to pup stimuli. Prior to any stimuli exposure, hormone-treated females had decreased basal DA concentrations compared to their sham controls. In a given treatment group, the AR females had increased basal DA levels compared to their MR siblings. (B) Mean (+ SEM) DA response data represent percent of basal DA concentration during access to pup (24-min, averaged across the 3 samples) and food (8-min, single sample) stimuli. The hormone- compared to sham- treated females had increased DA responses, regardless of the stimulus and rearing. Finally, food elicited more DA responses than did pups when collapsed across groups. *a* $P<0.05$ sham/hormone treatment difference when collapsed across rearing groups for a given stimulus; *b* $P<0.05$ individual rearing difference from MR for a given treatment and stimulus; *$P<0.05$ pup/food stimulus difference collapsed across treatment and rearing group.

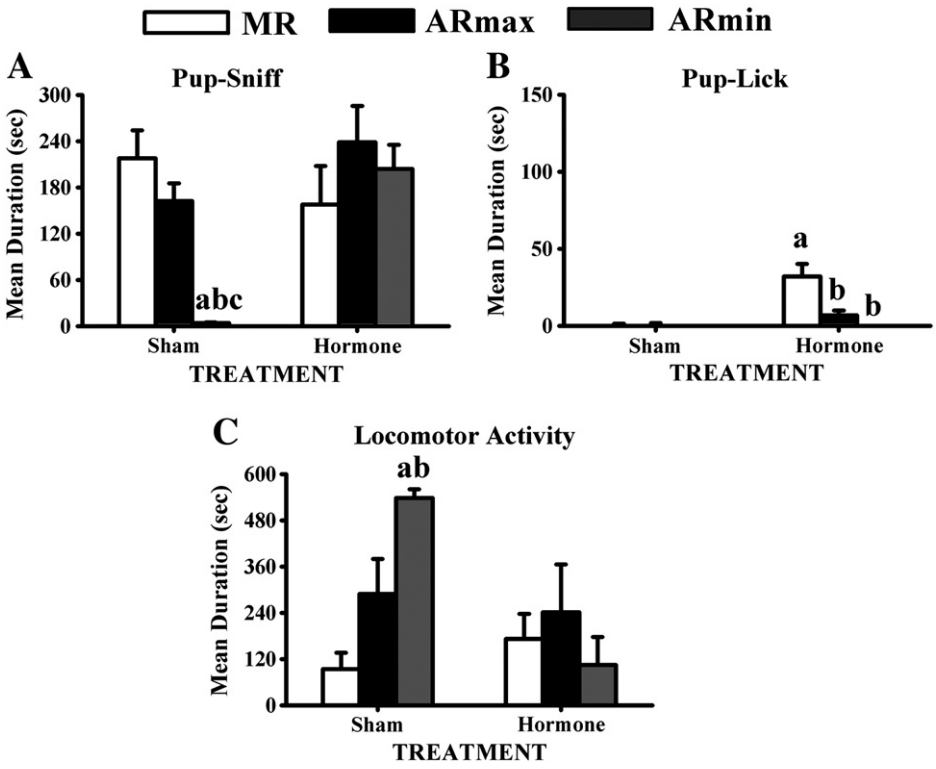

Fig. 6. Treatment and rearing effects on maternal behavior (Experiment 2). Maternal behavior during *in vivo* sample collection for OVX pup-naïve sham- or hormone- treated females that had been either mother- (MR) or artificially- (AR) reared with minimal (min) or maximal (max) preweaning tactile stimulation. Data represent mean (± SEM) of the total duration spent in (A) pup-sniffing, (B) pup-licking, and (C) general locomotor activity. Hormone-treated MR females licked pups more compared to other groups. In the sham-treated groups, ARmin sniffed pups less, compared to MR and ARmax females, and engaged in increased locomotor activity, compared to the sham-treated MR and hormone-treated control groups. *a* $P<0.05$ sham/hormone individual treatment difference for a given rearing group; *b* $P<0.05$ individual rearing difference from MR for a given treatment; *c* $P<0.05$ min/max individual AR preweaning tactile stimulation difference for a given treatment.

Relation of DA to behavior

Licking durations correlated positively to DA responses to pups ($r=0.614$, $P=0.001$). Basal DA concentrations correlated negatively with pup-sniffing durations ($df=25$, $r=-0.46$, $P=0.015$) and positively to locomotor activity ($df=25$, $r=.46$, $P=0.02$).

General discussion

Early adversity

In Experiment 1, postpartum dams displayed elevated pup-evoked DA responses against reduced basal DA levels, unlike the cycling females. The magnitude of these DA effects in postpartum dams was altered in AR when compared to MR females. Postpartum MR rats were the only group to demonstrate greater pup- compared to food -evoked DA responses. These postpartum rearing effects on DA were accompanied by rearing differences in pup-licking behavior during sampling and maternal responses during the preference task. Compared to AR dams, MR dams spent more time licking pups during sampling and engaged exclusively in maternal responses during the preference task.

Although pup-naive during the sampling session in Experiment 2, hormone-treated OVX females displayed elevated pup-evoked DA responses compared to sham-treated controls. Similar to postpartum dams, hormone-treated females demonstrated an elevated DA response in relation to reduced basal DA levels. Unlike the postpartum dams, only the magnitude of the basal DA suppression varied between MR and AR females. DA responses could be robustly provoked by either pup or food stimuli regardless of rearing experience, suggesting that treatment with exogenous hormones eliminated AR effects on pup-evoked DA responses. In contrast, after hormone treatment AR impairments persist in pup-related behavior. During sampling, only the hormone-treated MR females engaged in licking pups, and during the preference task these MR females demonstrated an increased sensitivity towards pups when food was available simultaneously. Before pup-experience, hormone-treated MR females had suppressed feeding behavior relative to their non-treated MR controls; however following experiences with pups, hormone-treated MR females ignored the food. AR rats did not show these behavioral effects.

In the present experiments, the experience of early neonatal isolation through AR modified the rats' adult responses to pups, both in terms of behavior and NAC DA function. Among hormonally primed conditions in both experiments, AR groups differed from MR siblings in basal DA levels and pup-licking duration, whereas ARmin and ARmax groups did not differ from each other. AR mothers not only showed impairment in DA functioning and ongoing maternal behavior, they also showed changes in their preference behavior and seemed to be easily distracted by other stimuli. Together these experiments demonstrated that compared to adult non-hormone primed controls, hormonally primed female rats (either shortly after parturition or as OVX pup-naïve virgins receiving hormone-treatment) showed decreased basal DA levels and increased pup-evoked DA responses in the NAC shell. Although this DA pattern in hormonally primed rats was seen in all early experience conditions, the motherless (AR) groups showed a different pattern than the MR animals, indicating that early preweaning experiences had long-term effects on later neurochemical regulation related to mothering behavior. Early adversity produced impairments to several DA mechanisms and licking behavior in postpartum females. While hormone-treatments in adulthood reversed AR deficits in pup-evoked DA responses, applying additional tactile stimulation during the AR preweaning experience

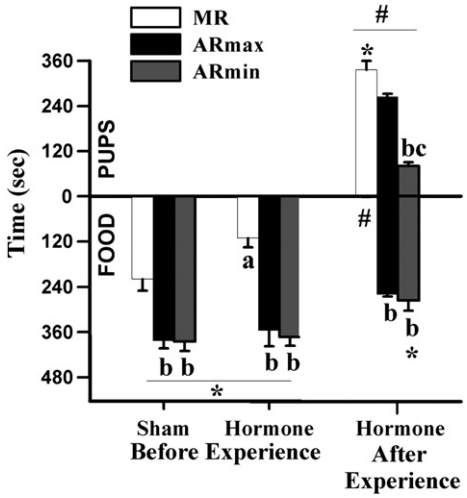

Fig. 7. Treatment and rearing effects on preference task (Experiment 2). Choice task data represent mean (± SEM) duration engaged in pup or food stimuli. Before pup experience, all OVX pup-naïve females only spent time with food when both stimuli were available, however AR groups showed increased food related behavior regardless of treatment. Also, when pup-naïve, hormone treated MR females spent less time with food than their untreated controls. After homecage pup experience (see text) when hormone-treated females became responsive to pups during the choice task, AR compared to MR females continued to spent more time in food-related behavior. This was in contrast to hormone-treated MR females that ignored food stimuli and primarily engaged with pup stimuli. ARmin compared the other two groups had decreased pup related behavior such that this group spent more time with food than pups. *a* $P<0.05$ sham/hormone individual treatment difference for a given rearing group; *b* $P<0.05$ individual rearing difference from MR for a given treatment and stimulus; *c* $P<0.05$ min/max AR preweaning tactile stimulation individual difference for a given treatment and stimulus; *$P<0.05$ pup/food stimulus individual difference for a given treatment and rearing group; #$P<0.05$ individual difference for MR hormone-treated females on time spent with a given stimulus after pup experience. Lines under symbols indicate data collapsed across those groups.

reduced impairments to pup-evoked DA responses in postpartum females.

Tactile stimulation

In Experiment 1, in comparison to both MR and ARmax siblings, ARmin females had (1) decreased pup-evoked DA responses and increased locomotor activity in the postpartum group, and (2) increased basal DA levels in the cycling group. In these cases ARmax and MR animals did not differ from one another, demonstrating that additional licking-like stimulation diminished some effects of isolation rearing. In contrast, tactile replacement had little effect in Experiment 2 on AR siblings. In terms of basal DA concentrations, while AR females did not differ from each other, these motherless rats compared to MR siblings had increased basal DA levels. All OVXed hormone-treated females had similar pup-evoked DA responses and locomotor activity regardless of the three rearing conditions. These data suggest that although the AR procedure had basal DA effects in females with no ovaries, hormone-treatment could eliminate robust AR alterations in pup-evoked DA.

It appears that normal ovarian function related to adult reproduction made the DA system susceptible to the effects of tactile stimulation. By eliminating ovarian function altogether, early adversity effects persisted in basal DA even after receiving additional tactile stimulation. Furthermore, removing variations in hormones related to parturition eliminated all early rearing effects on stimulus-evoked DA. Differences between postpartum and hormone-treated animals were likely derived from multiple endocrine inputs associated with parturition that were absent in hormonally-treated animals. These changes include adaptations that prepare the neuroendocrine systems to regulate the secretion of oxytocin at parturition and during lactation, and prolactin for postpartum milk production (for review, see Brunton and Russell, 2008).

It is unclear to what extent other aspects of the early rearing environment may have been relevant to both adult DA responses in the NAC shell and corresponding behavior. Any one of a number of sensory stimuli originating not only from mother, but also from peers (see Melo et al., 2006) such as olfactory, tactile, auditory, or a combination of these and other stimuli, may participate in the development of neurochemical systems that modulate later behavior. An obvious advantage of the AR paradigm is the ability to "add back" sources of sensory stimuli in order to test their importance for the development of specific neurobehavioral systems.

Increasing preweaning tactile stimulation may influence DA functions in the intact rat through alterations of gene expression related to ovarian hormones. In the context of natural variations in licking, rats display considerable individual differences in maternal behavior during the postpartum period (Champagne et al., 2003a) and these variations are associated with persistent changes in gene expression, physiology and behavior (Meaney, 2001). Studies in rodents suggest that variations in the quality of interactions between mother and infant during the postpartum period can induce brain site-specific changes in gene expression in the brain leading to differences in later adult maternal behavior (McGowan et al., 2011; Meaney, 2001, Weaver et al., 2004). It seems clear that licking is an important tactile stimulus from a mother for the development of neurochemical systems underlying later adult maternal behavior. Giving AR rats extra liking-like stimulation tended to reduce the differences between ARmin and MR groups such that ARmax groups showed behavioral and DA functions between ARmin and MR groups on the continuum.

Hormones

Our results suggest that factors associated with parity (e.g., hormonal states, pup experience) mediate DA functions, even in mothers who have had adverse experiences in their early postnatal lives in the form of isolation from mother and litter-mates (AR). The hormonally induced suppression of basal DA concentrations had a strong effect on stimulus-evoked DA responses in the NAC shell. In Experiment 2, rearing did not affect pup-evoked DA responses and hormone treatment differences were eliminated when basal DA levels were covaried. In Experiment 1, covarying basal DA concentrations did not eliminate parity or rearing effects on pup-evoked DA. The persistence of group differences was most likely due to robust differences in pup-experience and physiological states between cycling and postpartum females, thus exaggerating rearing differences amongst the postpartum females. Altered neurochemical regulation following the numerous adaptations that the maternal brain undergoes throughout pregnancy and parturition (see Brunton and Russell, 2008; Numan et al., 2006), along with the 24 h pup-experiences that postpartum dams experience, it is not surprising that rearing differences continue despite reduced basal DA levels.

In the absence of hormonally reduced basal DA level, DA-evoked responses to pups could be obtained from a cycling female that has had prior maternal experience through previous postpartum experiences or recent continuous pup exposure. These pup-evoked DA responses differ in a very important manner from DA responses of postpartum or hormone-treated rats (see Fig. 8) rats. Pup experienced cycling females had an acute (i.e., 1st 8-min period) pup-evoked DA response that did not remain elevated for the 24-min duration of pup stimuli accesses (see Afonso et al., 2008). Compared to the parturient and hormone-treated female, in the cycling or OVX females it takes significantly longer to acquire maternal responsiveness to pups (Bridges, 1984; Moltz et al., 1970; Rosenblatt, 1967). In the present study (data not shown) and in a previous study (Novakov and Fleming, 2005), motherless OVX rats with hormone treatments become responsive to pups as quickly as those raised with their mothers. Together, it appears that the hormonally induced reduction

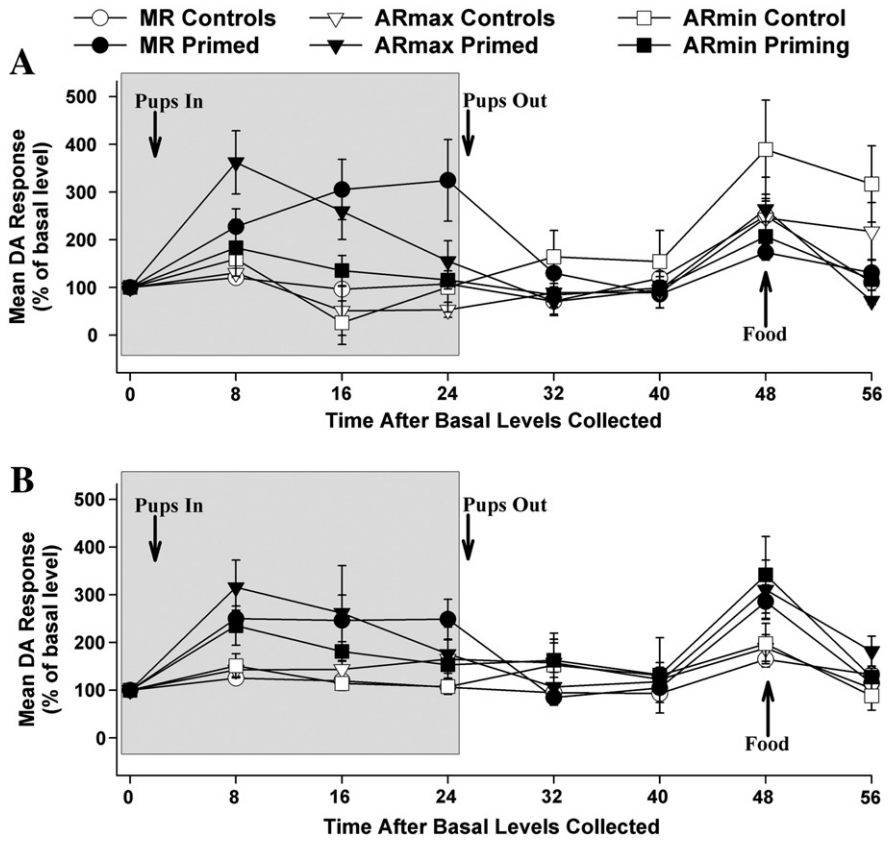

Fig. 8. Time course for dopamine (DA) sampling. *In vivo* DA response data represent mean (± SEM) percent of basal DA concentrations during access to pup and food stimuli in (A) intact, cycling (control) and postpartum (primed) females (Experiment 1), or, (B) OVX pup-naïve, sham- (control) and hormone- (primed) treated females (Experiment 2) that had been maternally-reared (MR) or artificially-reared (AR) with either minimal (min) or maximal (max) preweaning tactile stimulation. When basal level concentrations (see Figs. 2A and 5A) were partialled out of a mixed design ANOVA on pup-evoked DA responses (data included shaded grey area, see text) the results suggest that while there were no significant differences between any of the treatment or rearing groups in Experiment 2 (B), parity and rearing effects continue to exist in Experiment 1 (A). See text for significant differences.

of basal DA levels provides a very important mechanism for the rapid expression of maternal behavior in the postpartum rat.

Previous data show that gonadal steroid hormones affect the release and metabolism of biogenic amines, and that estrogens modulate dopaminergic activity at different stages of neurotransmission (see McEwen and Alves, 1999; McEwen and Parsons, 1982; Thompson, and Moss, 1994). In terms of estrogen's effects on basal DA release, there is evidence that prolonged hormone application or high concentrations of injected estrogen have suppressive effects on presynaptic non-stimulus-evoked (basal) DA release (Dupont et al., 1981). Interestingly, estrogen priming has been shown to increase NAC D_2 dopamine receptor and DA transporter (DAT) density, which could result in lower basal DA concentrations (Di Paolo et al., 1988, 1982a,b, 1985, 1992; Lammers et al., 1999). Given that previous literature demonstrates that stimulus-evoked DA release, DAT, D_2 dopamine receptor density, and D_2 dopamine sensitivity all increase with chronic application of estrogens, the enhanced salience of pups and the rapid expression of maternal behavior following hormonal priming may be mediated by any of these neural mechanisms (Chavez et al., 2010; Di Paolo et al., 1988, 1982a,b, 1985; 1992; Lammers et al., 1999; Lonstein et al., 2003; Zhou et al., 2002). Most likely, maternal behavior is mediated by the synergistic effects of these multiple dopaminergic adaptations following chronic estrogen.

One hypothesis regarding the function of NAC DA is that it promotes flexible approach responses in animals when they encounter environmental stimuli that have salience for them (e.g. food, sex, pups) (Ikemoto and Panksepp, 1999). When animals are presented with these salient stimuli, NAC DA is released, which in turn activates a state of 'incentive motivation' and exploratory arousal, leading to investigative activities. Damage to NAC neurons or disruption of NAC DA transmission blunts the ability of organisms to approach salient stimuli. Furthermore, it is suggested that animals with reduced DA transmission simply are not aroused enough to sustain attentional-investigatory patterns toward stimuli that are normally thought to have salience to that animal (Ikemoto and Panksepp, 1999). During the initial days after birthing, it would be useful for mothers to have dampened arousal states, thus remaining in a nest full of pups that provide passive reinforcing stimulation (e.g. suckling). Hormonally induced suppression of basal DA levels may aid rapid maternal expression through reduced DA noise (see Everitt and Robbins, 1997) in the absence of any stimulus and hence sharpens the DA signal to respond to pup stimuli, thereby enhancing the initial pup experience such that saliency develops. Impairments of this basal suppression, as seen in AR rats, would then set the stage for a reduction in the subsequent DA signal which reflects a reduction in the salience of pups, resulting in impaired interaction with pups. While pup saliency maybe reduced by early adversity, food saliency was enhanced within parity and treatment groups for AR females during the preference task. A recent study that utilized the AR paradigm in males found that inadequate early-life social experience enhanced the incentive salience of a reward-related cue (i.e. food) in adulthood (Lomanowska et al., 2011). The present data suggest that early adversity had varying affects on later adult responses to different salient stimuli under different hormonal priming conditions.

A new mother displays a dramatic change in behavior after parturition: she immediately cares for and defends her offspring. The expression of these essential components of maternal behavior is the culmination of changes, controlled by pregnancy hormones, in the activity of neural circuitry associated with this period. Under normal circumstances, the hormones of late pregnancy/parturition may enhance DA responding to

relevant stimuli by suppressing basal DA level, and thus, providing a mechanism for enhancing pup-saliency. Pups become so salient to lactating rats that they compete with self-administration of cocaine (Hecht et al., 1999; also see Mattson et al., 2001). During the early postpartum days in first time mothers, the development of salient qualities for pup stimuli is important for survival of the offspring.

Behavioral correlates to dopamine

In both experiments during pup availability, licking durations increased as a function of DA responses. Previous research has shown that pup-licking was correlated to NAC activation (Afonso et al., 2009; Champagne et al., 2004) as was suckling stimulation (Febo and Ferris, 2007; Ferris et al., 2005). Thus, it is unclear if the DA signal influences behavioral responses or the reverse, thus determination is yet to be made for pup-saliency being reflected in pup-evoked DA responses.

While pup-evoked DA signal may not reflect pup-saliency, the suppressed basal DA levels may influence the development of saliency for pups. Researchers (see Berridge, 2007; Wise 1986) suggest that salient attributions of stimuli influencing motivated behavior can be determined by the integration of two major inputs to dopaminergic mesolimbic mechanisms: 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), and learned reward associations. As follows, salient attributions of pups influencing maternally motivated behaviors can be determined by (1) hormonally induced reduction of the basal DA signal, and (2) experiences with pups. At the end of pregnancy, progesterone withdrawal combined with increasing estradiol levels activates reproductive neurons in the hypothalamus which is thought to override aversive neophobic behavioral responses to newborn odor and activate the mesolimbic dopaminergic reward circuitry (for review see Brunton and Russell, 2008). While the neural sites would be numerous for this process, a few critical sites are likely involved.

The estrogen receptor rich medial preoptic area (MPOA) has been shown to be essential for the expression of maternal behavior (see, Sheehan and Numan, 2002), as well as the ventral tegmental area (VTA), being the major source for DA release into the NAC (see Schultz, 1998). The NAC shell receives both direct and indirect connections from the MPOA via the VTA (Simon et al., 1979; Numan and Smith, 1984; Zahm and Heimer, 1993). Knife cuts severing the lateral projections from the MPOA to the VTA seriously disrupt maternal behavior (Numan and Smith, 1984). Research suggests that there is an oxytocinergic projection from the MPOA to the VTA (Pedersen et al., 1994; Numan and Sheehan 1997) that interacts with DA and influences ovarian functions (see Shahrokh et al., 2010). Infusion of an oxytocin receptor antagonist into the VTA disrupts maternal behavior (Pedersen et al. 1994). Estrogen increases oxytocin receptor expression in multiple brain regions (Breton et al., 1995; Breton and Zingg, 1997). Thus, increasing oxytocin receptors in the MPOA through rising estrogen levels during pregnancy activate an oxytocinergic MPOA-VTA projection, increasing the release of DA from VTA neurons projecting to the NAC to facilitate maternal behavior. These effects are further enhanced by differences in levels of DAT within the NAC shell, as well as by postsynaptic differences in DA receptor levels. Interestingly, high-licking females show increased estrogen receptor expression and higher levels of oxytocin receptor binding in the MPOA by comparison with low-licking dams (Champagne et al., 2001, 2003b; Francis et al., 2000). While variations in tactile stimulation may impact the MPOA-VTA projection (see Shahrokh et al., 2010), the AR paradigm itself may influence any number of the DA projections. For example, there is evidence that post-weaning social isolation reared rats have enhanced dopaminergic activity in various brain areas that include the accumbens (Jones et al., 1992), frontal cortex (Crespi et al., 1992), and the dorsal and ventral striatum (Jones et al., 1992; Hall et al., 1999).

Conclusion

Maternal deprivation can cause severe impairments that persist into adulthood. Research with animal neonates and human infants show that touch plays a critical role in early development. Research with preterm babies has found that tactile stimulation by nurses or mothers' stroking of the baby while it is in the incubator, can improve growth, neural activity and development (see Caulfield, 2000). Our data suggest that maternal care in animals, which we have simulated by AR and stroking, can serve as a mechanism for the transmission of individual differences in DA release.

The decreased basal DA levels following dramatic changes to ovarian hormone levels may serve to sharpen the DA signal, consequently affecting salient aspects of the various stimuli (e.g. pups, food) available in a dynamic environment. In the case of the postpartum and hormone-primed rat, suppressed basal DA is followed by a pup-induced enhancement of DA release—perhaps by altering the signal-to-noise ratio. This mechanism may serve to mediate the development of salient aspects attributed to pup stimuli when females are in the parturient physiological state. Although we cannot determine whether the DA response is necessary for behavior or just reflects a state of responsiveness, we are currently undertaking an investigation to separate the two possibilities.

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