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Use of fast scan cyclic voltammetry to assess phasic dopamine release in rat models of early postpartum maternal behavior and neglect

Tatiana A. Shnitko^{1,*}, Kyla D. Mace^{1,*}, Kaitlin M. Sullivan², W. Kyle Martin¹, Elizabeth H. Andersen², Sarah K. Williams Avram², Josephine M. Johns^{1,2}, and Donita L. Robinson^{1,2,#}

¹Bowles Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC, USA

²Department of Psychiatry, University of North Carolina, Chapel Hill, NC, USA

Abstract

Maternal behavior (MB) is a complex response to infant cues, orchestrated by postpartum neurophysiology. While mesolimbic dopamine contributes to MB, little is known about real-time dopamine fluctuations during the postpartum period. Thus, we used fast-scan cyclic voltammetry (FSCV) to measure individual dopamine transients in the nucleus accumbens of early postpartum rats, and compared them to dopamine transients in virgins and in postpartum females exposed to cocaine during pregnancy, which is known to disrupt MB. We hypothesized that dopamine transients are normally enhanced postpartum, and support MB. In anesthetized rats, electrically-evoked dopamine release was larger and clearance was faster in postpartum females than in virgins, and gestational cocaine exposure blocked the change in clearance. In awake rats, control mothers showed more dopamine transients than cocaine-exposed mothers during MB. Salient pup-produced stimuli may contribute to differences in maternal phasic dopamine by evoking dopamine transients; supporting the feasibility of this hypothesis, urine composition (glucose, ketones and leukocytes) differed between unexposed and cocaine-exposed infants. These data, resulting from the novel application of FSCV to models of MB, support the hypothesis that phasic dopamine signaling is enhanced postpartum. Future studies with additional controls can delineate which aspects of gestational cocaine reduce dopamine clearance and transient frequency.

Keywords

Mother; infant; dopamine; cocaine; maternal behavior; nucleus accumbens; rat

Introduction

The mother and infant form a critical dyad to promote the survival of the offspring. The mother exhibits maternal behavior (MB), a complex response to infant cues that is orchestrated by the hormonal and neuropeptide milieu that arises at parturition and continues

[#]Corresponding author: Donita L. Robinson, PhD, Bowles Center for Alcohol Studies, CB #7178, University of North Carolina, Chapel Hill, NC 27599–7178; DLR@unc.edu; Phone: 919–966–9178; Fax: 919–966–5679.

^{*}Authors contributed equally

into the postpartum period. The infant, in turn, displays stimuli that elicit MB. For example, maternal care in humans is elicited by a variety of infant stimuli, from distress stimuli such as cries, to physical features like big eyes, small noses, chubby cheeks, or baby smiles. These infant-produced stimuli are salient to the mother and activate brain systems known to be involved in motivated behavior, such as the mesolimbic dopamine system (Lorberbaum et al., 2002). In animal studies, mesolimbic dopamine is generally increased during focused maternal care of pups, such as licking and grooming (Champagne et al., 2004; Hansen et al., 1993; Lavi-Avnon et al., 2008) and is involved in motivated choice of pup-related environments (Seip and Morrell 2009). Moreover, disruption of mesolimbic dopamine transmission disrupts MB (Numan et al., 2005; Numan et al., 2009; Pereira and Morrell 2011). The ventral tegmental area (VTA) contains mesolimbic dopamine cell bodies and receives reciprocal innervation from the medial preoptic area and the paraventricular nucleus of the hypothalamus, which regulate MB (Numan et al., 2009; Simerly and Swanson 1988; Tobiansky et al., 2016). Therefore, by acting on the GABA and dopaminergic neurons in the VTA, hypothalamic nuclei engaged in MB may trigger dopamine release in VTA projection targets such as the nucleus accumbens (NAc) to produce motivated behavior directed toward the infant.

Dopamine release is known to occur on both tonic and phasic time frames, with phasic release consisting of fast fluctuations of high dopamine concentration that can activate low-affinity dopamine receptors (Wightman and Robinson 2002). Importantly, phasic dopamine release events, or dopamine transients, in the NAc encode reward-associated stimuli, facilitate approach behavior, and accompany social and sexual behaviors in rats (Day et al., 2007; Robinson et al., 2001, 2002, 2011a,b; Roitman et al., 2004). In rodents, pup cries (ultrasonic vocalizations, USV), olfactory stimuli, and pup temperature are among the most salient stimuli to elicit MB (Rosenblatt 1975; Kinsley and Bridges 1990; Stern and Johnson 1990; Brouette-Lahlou et al., 1992; Fleming and Walsh 1994; Lonstein and Stern 1997; Okabe et al., 2013). Thus, it is reasonable to predict that these pup-produced stimuli could evoke dopamine transients in rat mothers, facilitating maternal attention and approach to the pups, consistent with theoretical roles of dopamine in reward prediction, salience detection, and approach (Ikemoto and Panksepp 1999; Redgrave et al., 2008; Schultz 2007). While previous studies have used other techniques to tie dopamine release to MB, those techniques lacked either temporal resolution (microdialysis; Hansen et al., 1993; Lavi-Avnon et al., 2008; Afonso et al., 2008, 2009) or chemical selectivity (chronoamperometry; Champagne et al., 2004; Shahrokh et al., 2010) to detect dopamine transients. Thus, the assessment of phasic dopamine in MB requires the use of fast-scan cyclic voltammetry (FSCV), an electrochemical detection method with the spatial, temporal and chemical resolution to resolve sub-second dopamine release events *in vivo* (Robinson et al., 2008).

Maternal drug use can impair parenting behavior and contribute to maternal neglect of the offspring (for review, see Nephew and Febo 2012; Strathearn and Mayes 2010). The 2013 National Survey on Drug Use and Health found that 5.4% of pregnant women in the US were using illicit drugs, including cocaine (SAMHSA 2014). Affecting both mother and fetus, prenatal cocaine use has linked to deficits in infant development and maternal care in humans (Burns et al., 1991; Kelley et al., 1991; Murphy et al., 1991; Williams-Petersen et al., 1994; Mayes et al., 1997; Strathearn and Mayes 2010; Rutherford et al., 2011; Cain et

al., 2013; Liu et al., 2013; Chiriboga et al., 2014; Grewen et al., 2014) and in rats (Johns et al., 2005; McMurray et al., 2013; Zeskind et al., 2014; McMurray et al., 2015; Lippard et al., 2015). As cocaine targets monoamine transporters, including the dopamine transporter, phasic dopamine transmission in cocaine-exposed mothers may be compromised. Indeed, researchers have proposed that maternal cocaine exposure can both reduce both the infant's ability to elicit maternal care and the mother's ability to respond appropriately, perhaps through blunted phasic dopamine signaling (Morrell et al., 2011; Robinson et al., 2011b; Rutherford et al., 2011; Pereira and Morrell 2011).

For this study, we hypothesized that phasic dopamine release is enhanced postpartum and during interaction with pups, but might be dampened by maternal cocaine exposure. To test this, we used FSCV to assess phasic dopamine activity in the NAc of postpartum female rats at baseline and during MB, characterizing endogenous dopamine release and uptake kinetics as well as the frequency of spontaneous dopamine transients. We compared phasic release in control rat mothers to virgin females and to rat mothers exposed to cocaine during pregnancy, a manipulation that is known to disrupt MB. Finally, we tested the feasibility that pup-produced stimuli are altered by prenatal cocaine and, thus, could contribute to differences in dopamine transients observed during MB and the gestational cocaine model of maternal neglect.

Methods

Subjects and Breeding

Nulliparous Sprague-Dawley female rats were purchased from Charles River (Raleigh, NC) at approximately 200g and single-housed under 12h light /12h dark reversed light cycle (lights off at 9:00AM) with free access to food and water. In Experiment 1, rats were divided into three groups: virgin females (n=6, Virgin), handled but untreated postpartum rats (n=8, UN), and cocaine-exposed postpartum rats (n=5, CC). In Experiment 2, there were two groups of rats: handled but untreated (n=6, UN) and cocaine-exposed (n=5, CC) postpartum animals. In Experiment 3, there were two groups of rats: handled but untreated (n=39, UN) and cocaine-exposed (n=40, CC). One week after arrival to the animal facility, the females were housed with male rats until conception was confirmed by the presence of a vaginal plug (gestation day [GD] 0). Then, females were weighed and singly housed, and cocaine or handling began the following day (GD 1). On GD 7, rats were moved to a room with a normal 12h light /12h dark cycle (lights on at 07:00h) until the end of the experiment, as the light cycle switch generally results in deliveries of litters during daylight hours (Mayer and Rosenblatt 1998; Johns et al., 2005). For all experiments, postpartum day 1 is defined as the 24-h period following parturition. For virgin females in Experiment 1, experimental dopamine measurements were acquired 1–2 weeks after arrival to the animal facility.

Gestational Exposure

Pregnant rats were randomly assigned to experimental groups and weighed daily before treatment. Rats in the CC group were given twice daily injections of 15 mg/kg (s.c.) cocaine hydrochloride (Sigma, St. Louis, MO; dissolved in normal saline) at approximately 09:00

and 16:00 h, while UN rats were given no injections but were weighed and handled daily, as previously described (McMurray et al., 2015).

Experiment 1. Effects of parity status and cocaine exposure on phasic dopamine release in the nucleus accumbens of female rats

Surgery and FSCV recording—The surgical and voltammetric procedure was as previously described (Robinson et al., 2005, Shnitko et al., 2016a). On postpartum day 1–3, rats were anesthetized (1.5 g/kg urethane, i.p.) and placed in a stereotaxic frame. Electrode placements (from bregma) were AP –5.0mm and ML –1.2 mm for the stimulating electrode and AP +1.7 mm and ML +1.8 mm for the carbon fiber electrode. A reference electrode (Ag/AgCl) was placed in the left hemisphere and secured with a stainless-steel screw and dental cement. The stimulating electrode (bipolar, parallel, stainless steel, 0.2 mm diameter/tip; Plastics One, Roanoke, VA) was initially placed in the VTA at DV –8.0 mm, and the carbon fiber electrode (length 60–115 μ m) was initially placed in the NAc core at DV –6.0 mm. Both electrodes were lowered to optimize dopamine signal, with final placements ranging from –8.1 mm to –9.0 mm for the stimulating electrode and –6.0 mm to –6.3 mm for the carbon fiber electrode.

The potential applied to the carbon fiber electrode was ramped from –0.4 V to +1.3 V and back to –0.4 V every 100 ms, and the resulting current was recorded. Phasic dopamine signals were elicited by electrical stimulation of the dopamine cell bodies in the VTA (stimulating current: 300 μ A, 24 biphasic square-wave pulses, 2 ms per phase, 60 Hz). Three evoked signals were collected 5 minutes apart. In order to convert current to estimated dopamine concentration, electrodes were calibrated *in vitro* post-experiment, as previously described (Robinson et al., 2009). In short, current was recorded from each electrode while submerged in a flow of TRIS buffer and then in a buffer with a known concentration of dopamine (1 μ M). Note that post-experiment electrode calibration is more likely to underestimate than overestimate *in vivo* concentrations (Logman et al., 2000). Electrodes from 14 animals were preserved for calibration, and calibration factors (μ M/nA) for the remaining electrodes were estimated from these calculations.

Data analysis—Electrochemical data were analyzed as previously described (Robinson et al., 2005, Shnitko et al., 2016a). Changes in current due to dopamine oxidation and reduction were identified in the voltammetric data by using color plots and cyclic voltammograms. An average background voltammogram was taken from the 5s preceding stimulation and was subtracted from voltammograms taken at stimulation to create background-subtracted voltammograms. No two-dimensional smoothing was applied to the data. Current-versus-time traces were used to determine peak current (nA) for each stimulation, and these were converted to maximal concentration (μ M), or $[DA]_{\max}$ by using the *in vitro* calibration estimates. The three baseline measurements of each rat were averaged to one mean measurement. We required the average signal-to-noise ratio throughout the experiment to be ≥ 5.0 ; one UN and two CC rats were eliminated from all analyses using this criterion.

To investigate whether dopamine release and uptake parameters differed among virgin and postpartum groups, dopamine release and clearance were modeled as previously described (Shnitko et al., 2016a) using a Michaelis-Menten kinetic equation (Wu et al., 2001). This equation models electrically stimulated dopamine release as a function of the concentration of dopamine released per pulse of stimulation ($[DA]_p$, nM), the frequency of electrical stimulation (f , Hz), the velocity of dopamine transporters (V_{max} , nM/sec), and the affinity of those transporters (K_m , nM). For each baseline recording, a model of the current-versus-time plot was generated using this equation. K_m was held at 200 nM and $[DA]_p$ and V_{max} were modified until the model resembled the experimental data, with a correlation coefficient of 0.9. For each animal, average $[DA]_p$ and V_{max} measurements were calculated from the three baseline files and were then compared among virgin, UN postpartum, and CC postpartum groups. To examine how postpartum status and gestational cocaine exposure affected $[DA]_{max}$, $[DA]_p$, and V_{max} , one-way ANOVA was performed for three groups (Virgin, UN, CC). Data were transformed by rank before analysis when non-normal distribution of the data were revealed with the Shapiro-Wilk test.

Experiment 2. Phasic dopamine release events associated with maternal behavior early postpartum and effects of cocaine exposure

Surgery—In preparation for FSCV, pregnant rats were anesthetized with isoflurane (4% induction, 1.5% maintenance) on GD 15 and placed in a stereotaxic frame on a heated pad. GD 15 was chosen because surgery at this point was less likely to produce abortions by the female in previous experiments (Lubin et al., 2003). A guide cannula for later insertion of the carbon fiber electrode was placed above the NAc with the coordinates AP +1.7 mm and ML +1.7 mm. An electrode for electrical stimulation was lowered with a 6° angle (toward midline) to the VTA at AP –5.2 mm, ML 1.2 mm and DV –8.8 mm. All coordinates were taken from bregma. An Ag/AgCl reference electrode was implanted in the left hemisphere. All electrodes were secured in place with stainless-steel screws and dental acrylic. Rats were given ibuprofen at a dose of 15 mg/kg for three days after surgery. Rats from the CC group were given no morning injection on the day of surgery.

Fast scan cyclic voltammetry—FSCV with carbon-fiber microelectrodes was used to measure spontaneous dopamine transients in the NAc core of rats as described previously (Robinson et al., 2003, Shnitko et al., 2016b). A microelectrode was lowered acutely to the NAc core and potential was applied as in Experiment 1. Electrically-evoked or spontaneous dopamine release was detected in the NAc to verify that the carbon-fiber electrode was positioned near active dopamine terminals. To elicit dopamine release, a current of 125 μ A was applied to the VTA stimulating electrode at 16–24 pulses, 30–60 Hz. The experiment began when the carbon-fiber electrode was positioned in the NAc where dopaminergic terminals were present.

Experimental design—All rats were habituated to the tether and to the experimental chamber on GD 18 or 19. On postpartum day 1 (approximately 18–24 h after delivery), the mothers and their pups were placed in the experimental chamber (a sound-attenuated Plexiglas box equipped with a house light and white noise), along with a small amount of bedding from the home cage. Rat mothers were tethered during the voltammetric

experiments to connect the carbon-fiber electrode with instrumentation. Baseline “pre-separation” behavior and electrochemistry of mothers were recorded during 15 minutes, then the door to the chamber was opened and the pups were carefully removed by the experimenter and placed in a warmed cage in an adjacent room. After 30 min the pups were returned and distributed across the chamber. Next, the 30-min “post-separation” period was recorded.

Data Analysis—The mothers’ behavior and accompanying dopamine activity were measured and analyzed in this study. A camera situated on the wall opposite to the door recorded the behavior during experimental sessions. Video records were coded for MB using Observer software (Noldus Information Technology, Wageningen, the Netherlands) by experimenters blinded to group. The behavioral parameters recorded and analyzed were the duration of crouching (active nursing), lying flat on top of pups but not nursing, resting in the nest (i.e., where the pups were gathered), touching pups with nose or paw, licking pups, approaching pups, resting away from the nest without touching pups, locomotion (not toward pups), self-grooming, rearing on hind legs, and sniffing the cage or air outside the nest. These parameters were collapsed into two categories: pup-directed (crouching/nursing, lying flat on pups, resting in the nest, touching pups, licking pups, approaching pup) and self-directed behavior (resting away from the nest, locomotion, self-grooming, rearing, and sniffing). Statistical data analysis was conducted using repeated measures (RM) two-way ANOVA on the collapsed behavioral data with group (CC and UN) used as a between-subject factor and behavioral category as a within-subject factor, with the Holm-Sidak method for multiple comparisons. Data were transformed by rank before analysis when non-normal distribution of the data were revealed with the Shapiro-Wilk test.

The dopamine transients were analyzed using TarHeel software (Department of Chemistry, University of North Carolina at Chapel Hill) as described previously (Robinson et al., 2003, 2009). Briefly, voltammetric currents resulting from oxidation and reduction of electroactive compounds were plotted versus applied potential and presented as background-subtracted cyclic voltammograms, with the background consisting of 10 consecutive scans within 3 seconds of the target. The voltammograms were then compared to a known cyclic voltammogram of electrically-evoked dopamine (i.e., template). Voltammograms with a correlation of $r > 0.866$ to the template were identified as dopamine transients. The timing of transients during specific behaviors of rat mothers was determined to produce transient frequencies during various behavioral events. Transient frequency was analyzed with two-way, RM ANOVA with the Holm-Sidak method for multiple comparisons. Data were transformed by rank before analysis when non-normal distribution of the data were revealed with the Shapiro-Wilk test. All statistical analysis was performed in SigmaPlot 11.0 (Systat Software Inc, San Jose CA).

Experiment 3. Analysis of rat infant urine

Urine collection—As rat infants require tactile stimulation to release excretions through a reflex, CC and UN pup urine was collected by trained experimenters who gently held pups and stroked the anogenital region with a soft paintbrush (similar to licking they receive from their mother) to elicit excretion. When urine was excreted in the absence of feces, a pipette

tip was used to collect the sample. Urine samples were collected separately for each sex and from at least 2 male and 2 female pups per litter (pooled by sex) on postnatal day 1. Urine samples were frozen and stored at -80°C until assays were performed.

Urinalysis and data analysis—Pup urine was thawed and tested with Seimens Multistix 10 SG Reagent strips for urinalysis (Tarrytown, NY), per manufacturers' instructions. These strips measure amounts in mg/dl unless otherwise noted: glucose, bilirubin, ketone, specific gravity, blood, pH, protein, urobilinogen, nitrite, and leukocytes (5–15 white blood cells/high power field). Three μl of urine was applied to each measurement field in the order listed above until all fields were complete or urine was completely used for that sample; this procedure resulted in fewer measurements in later variables. Components measured as categorical variables were tested using Fisher's exact tests. Valued measurements were analyzed with Mann-Whitney U tests due to non-normal distributions. Each variable was first tested for sex differences, and as no significant differences were observed, male and female samples were averaged, resulting in a single score for each litter tested.

Results

Experiment 1. Effects of parity status and cocaine exposure on phasic dopamine release in the nucleus accumbens of female rats

To determine how parity and gestational cocaine exposure affect VTA-evoked dopamine release at baseline (i.e., not during behavior), we examined $[\text{DA}]_{\text{max}}$ in anesthetized virgin, UN postpartum, and CC postpartum rats (Figure 1). Figure 1A demonstrates dopaminergic signals obtained in the NAc of individual rats with FSCV. $[\text{DA}]_{\text{max}}$ is represented by amplitudes of evoked signals in the current-versus-time traces. The background-subtracted cyclic voltammograms (left) depict changes in current at all applied potentials (-0.4 V to 1.3 V), confirming the characteristic oxidation and reduction peaks associated with catecholamine electrochemistry. Figure 1B shows $[\text{DA}]_{\text{max}}$ collapsed across groups. The smallest dopamine signal was detected in virgin rats at $121 \pm 14\text{ nM}$, while in postpartum rats $[\text{DA}]_{\text{max}}$ was $786 \pm 146\text{ nM}$ and $384 \pm 90\text{ nM}$ in UN and CC rats, respectively. A one-way ANOVA revealed that the $[\text{DA}]_{\text{max}}$ values were significantly different across groups ($F_{2,13} = 9.2$, $p < 0.01$). Post-hoc comparisons confirmed that UN postpartum mothers exhibited over 6-fold higher concentrations of evoked dopamine release than virgin animals ($t = 4.2$, $p < 0.01$). $[\text{DA}]_{\text{max}}$ of CC mothers was intermediate between control mothers and virgins ($p > 0.05$).

As $[\text{DA}]_{\text{max}}$ is a compilation of dopamine release and simultaneous clearance by the dopamine transporter, we estimated these parameters via modeling and compared them across the three groups. $[\text{DA}]_{\text{p}}$, or the concentration of dopamine release per pulse of electrical stimulation, was smallest in the group of virgin rats ($10.7 \pm 1.7\text{ nM}$) and largest in UN postpartum females ($57.8 \pm 9.6\text{ nM}$; Figure 1C). One-way ANOVA on ranks yielded a significant effect of group ($H = 9.7$, $\text{df} = 2$, $p < 0.01$), and post-hoc comparisons revealed that UN mothers showed higher $[\text{DA}]_{\text{p}}$ than virgin animals ($p < 0.01$), while $[\text{DA}]_{\text{p}}$ in CC mothers was intermediate between control mothers and virgins ($p > 0.05$). In contrast, V_{max} , or the maximal velocity of uptake via the dopamine transporter, was faster in UN postpartum

rats than virgin or CC postpartum rats (1657 ± 213 nM/s versus 774 ± 146 nM/s and 639 ± 161 nM/s, respectively). One-way ANOVA revealed a main effect of group ($F_{2,13} = 8.4$, $p < 0.01$), and post-hoc comparisons confirmed that $V_{\max}$ in UN postpartum rats was significantly different from virgin and CC postpartum rats (both $t > 3.3$ and $p < 0.05$).

Experiment 2. Phasic dopamine release events associated with maternal behavior early postpartum and effects of cocaine exposure

This experiment evaluated pup-directed and self-directed behavior of CC and UN rat mothers on postpartum day 1. This evaluation took place over a 15-min “pre-separation” period and a 30-min “post-separation” period, when pups were returned after a 30-min separation. Table 1 describes the proportion of UN and CC females expressing specific self-directed or pup-directed behaviors across experimental phases. Prior to separation, rearing/sniffing the air (observed in 60% of CC and 50% of UN rats) and locomotor activity away from pups (40% of CC and 33% of UN rats) were the self-directed behaviors observed in most rats. As expected, crouching (active nursing) was the most common pup-directed behavior and was observed in 80% of CC and 60% of UN rats, followed by either lying flat on pups (80% of CC and 17% of UN rats) or resting in the nest with pups without nursing (20% of CC and 50% of UN rats).

A wider variety of behaviors was observed in most rats post-separation, as the rats responded to the return of their pups to the chamber. More than 80% of rats demonstrated a variety of self-directed behaviors like locomotion, self-grooming or resting away from pups. Similar to the pre-separation phase, locomotion (100% of CC and 67% of UN rats) and rearing/sniffing (80% of CC and 100% of UN rats) were the most observed types of non-pup directed behavior in rat mothers after the separation. The majority of rats (80% of CC and 100% of UN) exhibited four pup-directed behaviors: crouching, resting in the nest, touching/sniffing pups and approaching pups. As these behaviors were the most observed and therefore representative across the groups, their parameters were used to evaluate differences in general MB between groups.

The duration of these specific behaviors was used as a main parameter to compare maternal care between the groups (Johns et al., 1994, 2005). As expected during the early postpartum period, crouching occurred more than other behaviors during both pre- and post-separation phases. CC and UN mothers spent similar amounts of time crouching during pre-separation (Figure 2A; CC: 346 ± 162 sec, 42% of phase time; UN: 401 ± 169 sec, 49% of phase time), with additional time resting in the nest, touching or sniffing pups. During post-separation (Figure 2B), CC rats spent half as much time crouching as UN rats (CC: 572 ± 220 sec, 32% of the phase time; UN: 1177 ± 245 sec, 64% of the phase time); CC rats' time crouching was proportionally less than their previous crouching behavior during the pre-separation phase, while UN rats spent proportionally more time crouching.

For statistical analysis, we collapsed the duration of all self-directed behaviors and compared them to that of all pup-directed behaviors. During the pre-separation phase (Figure 2C) both groups spent most of their time doing pup-directed behaviors (749 ± 82 sec in CC and 664 ± 133 sec in UN). RM ANOVA on ranks revealed a significant main effect of behavior type ($F_{1,18} = 15.5$, $p < 0.001$), with no effect of group or group by behavior

interaction. During the post-separation phase (Figure 2D), CC-exposed rats spent somewhat more time doing pup-directed behaviors (822 ± 164 sec) than non-pup-directed behavior (557 ± 198 sec). However, UN rats spent considerably more time performing pup-directed behavior (1523 ± 106 sec) than CC rats and displayed shorter amounts of self-directed or non-pup-related activity (101 ± 32 sec). RM ANOVA revealed a significant main effect of behavior type ($F_{1,18} = 41.2$, $p < 0.001$) and group by behavior interaction ($F_{1,18} = 19.3$, $p < 0.001$). Post-hoc comparisons showed that CC rats spent significantly more time in self-directed behaviors and less time exhibiting pup-directed behaviors compared to UN rats, and that UN rats spent significantly more time in pup-directed than self-directed behaviors (Holm-Sidak method, all t 's > 2.4 , p 's < 0.05).

We used FSCV to record phasic dopamine transients in these same rat mothers during the behavior reported above. Figure 3 demonstrates an example of the phasic dopamine activity recorded in the NAc core of an individual UN rat during the post-separation phase. The color plot displays current in color across all applied potentials (y-axis) and over time (x-axis). Dopamine is identified as oxidative current at ~ 0.65 V versus the reference (indicated by the dotted line) and reductive current at ~ -0.2 V. To be identified as a dopamine transient, the electrochemical signal is positively identified via the cyclic voltammogram.

We calculated the frequency of dopamine transients during each experimental phase (pre- and post-separation), as shown in Figure 4A. Overall, the number of transients recorded in CC rats was small during both pre- and post-separation (1.0 ± 0.3 and 0.8 ± 0.4 transient/min, respectively). More dopamine activity was recorded in the UN rats (pre-separation: 4.4 ± 1.5 , post-separation 1.7 ± 0.8 transient/min). RM ANOVA yielded significant main effects of group ($F_{1,9} = 13.4$, $p < 0.005$) and time ($F_{1,9} = 10.8$, $p < 0.01$), but no significant interaction. Thus, dopamine transient frequency was blunted in CC-exposed mothers compared to UN controls. Moreover, dopamine transient frequency declined over the entire recording session, regardless of group.

As pup-directed behavior was different between CC and UN mothers in the post-separation phase, we further assessed the frequency of dopamine transients during pup-directed behaviors (Figure 4B). Similar to the pattern observed across the entire session, transients during pup-directed behaviors were more frequent in UN than CC rat mothers, and declined from pre-separation to post-separation. RM ANOVA revealed a significant group by time interaction ($F_{1,9} = 5.8$, $p < 0.05$) as well as a main effect of time ($F_{1,9} = 7.4$, $p < 0.05$), but no main effect of group. Post-hoc comparisons showed that the rate of transients in UN rats during the pre-separation period was significantly higher than during the post-separation phase ($t = 3.8$, $p < 0.005$) and was marginally higher from the rate in CC rats during the pre-separation period ($t = 2.8$, $p = 0.051$). In contrast, when dopamine transient rates during self-directed behaviors were compared between groups and across phases (Figure 4C), no main effects or interactions of group and time were found, although these data carry the caveat that UN rats displayed little self-directed behavior.

To investigate whether the decline in frequency of dopamine transients observed in UN rats was specific for postpartum day 1, we repeated the experiment in a subset of the UN rats ($n = 2$) 24 hours later. On postpartum day 2, the frequency of dopamine transients did not

decline across the session as on day 1. Specifically, the rats exhibited 9.2 ± 3.2 transients/min during the pre-separation phase and 9.3 ± 0.7 transients/min during the post-separation phase. While preliminary, these data suggest that the reduction in transient frequency observed on day 1 was specific to that day.

Experiment 3. Analysis of rat infant urine

To assess the feasibility that pup-produced stimuli contributed to the difference in MB and dopamine transients observed in unexposed and cocaine-exposed rat mothers, we analyzed the composition of infant urine on postnatal day 1 (Figure 5). We tested urine from 39 UN and 40 CC litters, although not all samples were successfully analyzed for each component due to the amount of available urine (see methods). Glucose was only observed in samples from CC litters; thus, CC infants were significantly more likely to have glucose in the urine compared to UN pups (Fisher exact test, $p < 0.001$). CC infants were less likely than UN infants to have leukocytes in their urine (Fisher exact test, $p < 0.05$). Finally, CC pups were significantly more likely to have measureable ketones in urine compared to UN pups (Fisher exact test, $p < 0.01$), and the levels when detected were also higher (Mann-Whitney $U = 341.0$, $p < 0.01$). No exposure effects were observed in measures of blood, protein, bilirubin, nitrites, specific gravity, or pH.

Discussion

Mesolimbic dopamine is critical to motivated behavior and has long been considered an important mechanism in MB. As phasic dopamine is particularly involved in reward prediction and stimulus salience, we hypothesized that phasic dopamine release is enhanced postpartum and during interaction with pups. Previous reports used microdialysis, a method to sample dopamine *in vivo* on the time scale of minutes, to find that tonic dopamine concentrations rise during maternal interactions with pups (Hansen et al., 1993, Lavi-Avnon et al., 2008) or in response to pup stimuli (Afonso et al., 2008, Afonso et al., 2009). Additional studies found similar results with chronoamperometry (Champagne et al., 2004, Shahrokh et al., 2010), although this electrochemical method lacks the chemical selectivity to confirm that changes observed *in vivo* are primarily due to dopamine as opposed to pH or another analyte (Gerhardt and Hoffman 2001, Robinson et al., 2008, Wightman and Robinson 2002). Thus, to address our hypothesis, we employed FSCV, an electrochemical technique that has both the selectivity and sensitivity to detect sub-second fluctuations in dopamine release during behavior. We report here that both dopamine release per impulse and the frequency of spontaneous dopamine transients increase postpartum, despite more efficient uptake via the dopamine transporter. Moreover, in a well-characterized model of maternal neglect – chronic cocaine exposure throughout gestation – these aspects of phasic dopamine transmission are reduced. To our knowledge, this is the first study to compare dopamine release and clearance in virgin and postpartum rats and to measure dopamine transients in postpartum rats with and without a history of cocaine exposure. Together, the results support our hypotheses, but also lead to additional research questions: What is the time course of postpartum dopamine dynamics? What aspect of gestational cocaine exposure dampens dopamine clearance and transient frequency? What is the role of pup-produced

stimuli in maternal dopamine dynamics? Future studies are needed to extend the present results and address these questions.

To evaluate dopamine release and clearance kinetics in early postpartum, we assessed electrically-evoked transients in anesthetized rats, which offered several advantages. First, by evoking dopamine release via uniform stimulation of the VTA, the kinetics of the signals can be more easily compared across rats, as it eliminates variability due to environmental stimuli, interoceptive stimuli, or movement artifacts. Second, as the dopamine signals are evoked by known stimulation parameters and possess a high signal-to-noise ratio, they can be modeled to estimate the dopamine release and clearance parameters contributing to the overall signal. We used urethane anesthesia, which is the preferred anesthetic for dopamine measurements for many reasons, including a lack of effect on dopamine clearance (for discussion, see Robinson et al., 2005). The resulting data revealed that evoked dopamine transients are substantially larger in postpartum versus virgin rats, consistent with previous reports of higher dopamine tissue content (Byrnes et al., 2001). Larger evoked dopamine signals could result from either more dopamine released per stimulation impulse or from slower uptake via the dopamine transporter. In this case, modeling revealed that the differential signal was driven by greater dopamine release. Indeed, the dopamine transporter was more efficient postpartum than in virgin rats, as shown by the increase in $V_{\max}$; in fact, increased $V_{\max}$ would limit the signal rather than amplify it. Thus, the data support the conclusion that dopamine release is enhanced and the dopamine transporter is upregulated as a compensatory measure in the early postpartum period. Previous microdialysis studies reported that lower tonic, or steady-state, concentrations of dopamine were measured under basal conditions in early postpartum rat mothers as compared to virgin females (Afonso et al., 2009). Reduced tonic dopamine concentrations could result from upregulation of the dopamine transporter, and this would allow phasic release events (transients) to achieve greater signal-to-background.

Interestingly, similar postpartum adaptations in uptake kinetics were not observed in cocaine-exposed postpartum rats. In rat mothers exposed to cocaine during gestation, evoked dopamine signals were intermediate between control mothers and virgin rats, and were not significantly different from either. Moreover, through modeling we found that while dopamine release per pulse followed the same pattern as the overall signal ($[DA]_{\max}$), $V_{\max}$ in CC mothers was no different than virgin rats. Considering the moderate increase in $[DA]_p$ in tandem with no change in $V_{\max}$, it is possible that cocaine-exposed rat mothers would show greater tonic dopamine concentrations under basal conditions, as opposed to lower tonic dopamine reported for non-exposed rat mothers (Afonso et al., 2009). As a consequence, the signal-to-background ratio of dopamine transients may be reduced, which may be one reason that fewer spontaneous dopamine transients were detected (a finding discussed in more detail below). Thus, maternal cocaine appears to dampen at least some of the adaptations in the dopamine system that would normally occur in the early postpartum period.

To assess how these differences in dopamine release and clearance kinetics might manifest during behavior, we measured MB and dopamine transients in awake rat mothers. For this experiment, we did not use virgin females, as pup-produced stimuli would be unlikely to

elicit MB in virgin females, at least upon initial presentation (Rosenblatt et al., 1988). Consistent with previous reports (e.g., Johns et al., 1994, 2005; Kinsley et al., 1994), we saw that cocaine-exposed rat mothers exhibited less MB than control mothers. Specifically, we removed the pups for 30 min and then returned them, which typically induces MB such as gathering pups and nursing (Johns et al., 2005), and cocaine-exposed rat mothers were less attentive to pups after separation (Johns et al., 1998, and present study). During the post-separation period, control mothers spent twice as much time in pup-directed behavior than did cocaine-exposed mothers. This effect was largely driven by differences in duration of active nursing or crouching, which are known to be affected by maternal cocaine history (e.g., Johns et al., 1994; Johns et al., 1997a).

Spontaneous dopamine transients were also affected by gestational cocaine exposure. While there was individual variability, the average frequency of dopamine transients in the pre-separation period was four times greater in control than in cocaine-exposed mothers. This differential dropped during the course of the experiment, and dopamine transients were only twice as frequent in the post-separation period in unexposed versus cocaine-exposed rats. The cause of the reduction in transient rate, which was especially apparent in control rats, is unknown, although multiple possibilities arise. First, FSCV studies sometimes (although not always) report reductions in dopamine signals across an awake-rat recording session, both for evoked $[DA]_{\max}$ (Budygin et al., 2001) and for dopamine transient frequency (Robinson et al., 2009), potentially due to cumulative damage at the electrode tip due to vigorous movement. The reduction observed here may be similar, and it would be observed more readily in control than cocaine-exposed rats due to the elevated baseline of control rats. However, another possibility is that the elevated dopamine signaling observed in control mothers is simply dynamic during the hours following parturition, or is more vulnerable to some aspect of electrochemical recording such as insertion of the electrode, tethering or the novel environment. This explanation is supported by our preliminary recordings in two control rats 24 hours later, in which dopamine transients did not decline in frequency. Additional studies can address this by recording over multiple postpartum days, perhaps by using chronically implanted electrodes (Clark et al., 2010). This approach would also reveal how long the increase in spontaneous dopamine transients observed in control rats and the reduction observed in cocaine-exposed rat mothers persist; note that these recordings were made at least 48 hours after the final cocaine injection.

A major limitation of the effects reported in present study is the comparison of untreated rat mothers with cocaine-exposed mothers without the full complement of control groups. As this study was primarily used to evaluate the method of FSCV to assess postpartum maternal response, we used gestational cocaine exposure as a tool to manipulate maternal response, given its known effects to blunt MB (e.g., Johns et al., 2005; Rutherford et al., 2011). This initial study limited the number of experimental groups to focus on viability of the experimental approach. Moreover, as the primary purpose of the study was to measure phasic dopamine release in the early postpartum period, it was critical at this early point to compare untreated female rats to virgins. These controls were handled like the cocaine-exposed rats, but not injected daily or matched for food intake, both of which might alter dopamine in addition to the cocaine. Saline injections, another common control for this maternal cocaine model, have been shown to induce higher corticosterone levels in mothers

and fetuses (Ward and Weisz 1984; Barbazanges et al., 1996), which may itself alter phasic dopamine signaling (Lemos et al., 2012). Another common control is to pair-feed a group of mothers the amount of food that cocaine rats eat, which is less than a free-feeding pregnant rat and can lead to hoarding and even less weight gain than observed in cocaine-exposed rats. Thus, we recognize that several factors contribute to this model of maternal neglect – repeated injections, changes in maternal diet and the various effects of cocaine (physiological, psychological and pharmacological). Another approach for future studies will be to train rats to self-administer cocaine, which has face validity for drug-using human mothers but has the disadvantage of unequal drug exposure. Self-administration models are appropriate for the study of addiction, but as our major focus was the behavioral and physiological effects of cocaine on the mother and offspring, we chose a method that tightly controls the exposure. The subcutaneous route we used is analogous to “snorting” cocaine by humans in terms of absorption rates (Spear et al., 1989), and 15mg/kg in the rat is approximately equivalent to 1 gram of cocaine in a 150 lb. woman. While future experiments will be required to determine which factors associated specifically with the cocaine regimen contribute most heavily to the effects observed here, the data herein provide evidence that the method of FSCV can be used to measure phasic dopamine at baseline and during MB, both in typical rats and in a proven model of blunted maternal response.

We predict that pup-produced stimuli, such as odors and USV cries, can evoke dopamine transients in rat mothers due to their salience and effectiveness to induce MB (Stern, 1997), consistent with theoretical roles of dopamine in reward prediction and approach (Ikemoto and Panksepp, 1999; Redgrave et al., 2008; Schultz 2007). While the effectiveness of pup-produced stimuli to evoke dopamine transients was not directly tested in the present study, we observed that dopamine transients were more frequent in control than in cocaine-exposed rat mothers when they interacted with their litters. The fewer dopamine transients observed in cocaine-exposed mothers could be due to the altered dopamine dynamics we observed in anesthetized rats, perhaps indicating reduced capacity for fast fluctuations in dopamine concentration. Indeed, cocaine-exposed rat mothers exhibit differential maternal response to pup-produced cues (Lippard et al., 2015), which may be due, in part, to deficient dopamine signaling. However, another potential contributor could be differences in the stimuli produced by cocaine-exposed versus unexposed litters. For example, pup urine is a strong stimulus that elicits MB (Londei et al., 1989), and in this study we demonstrate significant differences in the urine composition between cocaine-naïve and exposed pups. Also, ultrasonic vocalizations are altered by prenatal cocaine in rats (Cox et al., 2012, Lippard et al., 2015, McMurray et al., 2013) in ways that are qualitatively similar to exposed human infants (Zeskind et al., 2014). Therefore, it is feasible that stimuli produced by cocaine-exposed pups are less effective to evoke dopamine release in rat mothers. Future studies can test the relative contributions of infant stimuli and maternal dopamine response by measuring dopamine transients with FSCV at the presentation of specific pup-produced stimuli, instead of the entire litter, making this technique valuable for mechanistic approaches to these questions.

In summary, the present results provide evidence that dopamine dynamics are altered postpartum in ways that may modify MB. While the precise circuitry by which mesolimbic dopamine is upregulated at parturition is unclear, one possibility is that hypothalamic

oxytocin regulates dopamine neurons via projections to the VTA. Oxytocin is critical for optimal initiation of MB in several mammalian species, including the rat (e.g., Pedersen et al., 1994), and regulation of the oxytocin system is disrupted by cocaine in brain regions that regulate MB circuitry (Strathearn and Mayes, 2010; Johns et al., 1997b). More work is necessary to tease apart the mechanisms by which MB is supported and can be disrupted in the perinatal period. The FSCV technique could prove very effective for critical preclinical studies as it may point the way toward therapeutic intervention not only in women at risk for maternal neglect due to drug addiction (Strathearn and Mayes 2010), but also in understanding basic maternal brain circuitry response during the early postpartum period.

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Figure 1.

Electrically-evoked dopamine release is influenced by postpartum status and drug history. Panel A: Examples of evoked dopamine release from a virgin rat, an untreated (UN) postpartum rat, and a cocaine-exposed (CC) postpartum rat. The line traces show current at the oxidation potential of dopamine converted to concentration via *in vitro* electrode calibration. The cyclic voltammograms on the left indicate the full current versus applied potential for the signals and reveal the oxidation and reduction peaks characteristic for catecholamines. Panels B & C: The maximum concentration of dopamine (B) and the dopamine released per electrical impulse (C) are higher in UN postpartum than virgin rats, with dopamine in CC postpartum rats intermediate. Panel D: The efficiency of clearance via the dopamine transporter is faster in UN postpartum rats than either virgin or CC postpartum rats. Data are mean $\pm$ SEM, * $p < 0.05$.

Pre-separation

Post-separation

Figure 2.

Duration of specific behaviors expressed by rat mothers during pre-separation (15 min) and post-separation (30-min) phases of the experiment. Individual pup-directed behaviors exhibited by CC and UN rats are shown during the pre-separation (panel A) and post-separation (panel B) periods. Collapsed across specific behaviors, durations of self-directed and pup-directed behaviors were compared between groups during pre-separation (panel C) and post-separation (panel D). CC rats exhibited less pup-directed behavior than UN rats post-separation. Data are mean $\pm$ SEM; + main effect of behavior type, $p < 0.001$; * post hoc comparison versus UN rats, $p < 0.05$; # post-hoc comparison versus UN pup-directed.

Figure 3.

Example of individual dopamine transients measured in the NAc core of UN rat during crouching/nursing her pups on postpartum day 1 during the post-separation period. In the color plot, oxidation and reduction current is expressed in color and plotted by applied potential (y-axis) over time (x-axis). Current due to dopamine oxidation at ~0.65V potential is indicated by the dotted line, and extracted to the trace above the color plot. The asterisks (*) indicate dopamine transients, positively identified via their cyclic voltammograms (correlational analysis $r > 0.86$ to template; see Methods). The cyclic voltammogram (top) represents one of the spontaneous dopamine transients indicated by the asterisks.

A: Overall dopamine transients **B: Dopamine during pup-directed behaviors** **C: Dopamine during self-directed behaviors** **Figure 4.**

Frequency of dopamine transients recorded in the NAc core of CC and UN postpartum rats across pre- and post-separation phases. Panel A: Overall dopamine transient frequency (during all behaviors) declined over the experiment in both groups, but CC rats exhibited significantly fewer dopamine release events than UN rats. Inset: transient frequency of individual rats (CC: black lines; UN: gray lines). Panel B: Dopamine transient frequency only during pup-directed behavior followed a similar pattern. Panel C: No significant differences were observed between groups or phases when transient frequency was assessed during self-directed behavior. Data are mean $\pm$ SEM; * post hoc comparison versus UN post $p < 0.05$, versus CC pre $p = 0.051$.

Figure 5.

Prenatal cocaine exposure alters composition of infant urine on postnatal day 1. Panel A: Glucose was only detected in cocaine-exposed (CC) pup urine. Panel B: Leukocytes were more likely to be present in unexposed (UN) than cocaine-exposed pup urine. Panel C: Ketones were more likely to be present in cocaine-exposed than unexposed pup urine. Panel D: The levels of ketones were also higher in cocaine-exposed than unexposed pup urine.

Proportion of rats demonstrating self-directed and pup-directed behaviors during pre-separation and post-separation experimental phases.

Table 1

	Behavior	Pre-separation		Post-separation	
		CC	UN	CC	UN
Self-directed	Rear/sniff	3/5	3/6	4/5	6/6
	Locomotion	2/5	2/6	5/5	4/6
	Self-grooming	1/5	0/6	5/5	3/6
	Rest away	1/5	1/6	4/5	0/6
Pup-directed	Crouching	4/5	4/6	5/5	6/6
	Lay on pups	4/5	1/6	3/5	3/6
	Rest in nest	1/5	3/6	4/5	6/6
	Lick pup	1/5	2/6	3/5	5/6
	Touch/sniff pup	1/5	2/6	5/5	6/6
	Approach pup	0/5	1/6	5/5	6/6