IMPACT OF GESTATIONAL TREATMENT OR PRENATAL COCAINE EXPOSURE ON EARLY POSTPARTUM OXYTOCIN SYNTHESIS AND RECEPTOR BINDING

Matthew S. McMurray

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Approved by

Advisor: Josephine Johns, Ph.D.

Reader: Mitch Picker, Ph.D.

Reader: Kathy Light, Ph.D.

ABSTRACT

MATTHEW MCMURRAY: Impact of Gestational or Prenatal Cocaine Exposure on Early Postpartum Oxytocin mRNA Synthesis and Receptor Binding
(Under the Direction of Josephine Johns)

Prior research reported decreased oxytocin levels in specific brain regions and disruptions in maternal care following gestational cocaine treatment in rats. Similarly, prenatal exposure to cocaine impaired maternal behavior in adulthood, but this was not associated with oxytocin level disruptions. To determine if cocaine alters other aspects of the oxytocin system, oxytocin mRNA transcription and receptor binding were examined on postpartum day two in relevant brain regions following gestational treatment with, or prenatal exposure to, either cocaine or saline. Results indicated an increase in oxytocin mRNA levels in the paraventricular nucleus of dams treated with cocaine gestationally with no group differences in brain regions dependent upon prenatal exposure. No significant differences in receptor binding were found in any region examined for either group of dams. These findings suggest that cocaine affects multiple aspects of the oxytocin system in the early postpartum that could be associated with altered maternal behavior.

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ABBREVIATIONS

PPD	Postpartum Day
GD	Gestation Day
FGD	First Generation Dam
CC	Chronic Cocaine
CS	Chronic Saline
mRNA	Messenger Ribonucleic Acid
ER	Estrogen Receptor

INTRODUCTION

Adequate maternal care is unquestionably critical for the normal development of mammalian offspring and the survival of the species as a whole. Rodent models of maternal behavior are particularly useful for the study of this important behavioral set. Rodent offspring are born blind and unable to thermoregulate, defecate, urinate, or protect themselves from attack (Numan, 1994), thus requiring considerable maternal care to survive. Importantly, rodent maternal behavior has been well characterized behaviorally, but has only recently begun to be understood in terms of neurobiological mechanisms.

The postpartum period consists of two distinct phases: the initiation or onset of maternal behavior immediately following birth, and the maintenance of established maternal behavior throughout the remaining postpartum period. The onset of maternal behavior is thought to stem primarily from hormonal changes that occur at parturition (Numan & Insel, 2003; Insel & Harbaugh, 1989; Van Leengoed, Kerker, & Swanson, 1987), while the maintenance of established maternal behavior appears to be less hormone-dependent. The onset of maternal behavior is characterized by a dramatic drop in progesterone levels accompanied by a rise in estrogen, which remains elevated through much of the postpartum (Terkel & Rosenblatt, 1972; Terkel & Rosenblatt, 1968). In addition to estrogen and progesterone, one of the primary hormonal mediators of maternal behavior is oxytocin.

Oxytocin is a nine amino acid peptide synthesized primarily in the paraventricular and supraoptic nucleus of the hypothalamus (Numan et al., 2003). These two regions contain

two oxytocin-producing neuronal subtypes: magnocellular and parvocellular. Magnocellular neurons within both of these regions project to the posterior pituitary for secretion to the peripheral circulatory system. Peripheral oxytocin initiates contractions during labor, facilitates milk letdown during the postpartum period, and modulates the stress response in both humans and rats (Russell & Leng, 1998; Petersson, Alster, Lundeberg, & Uvnas-Moberg, 1996). The parvocellular neurons are found only in the paraventricular nucleus, and project centrally to the bed nucleus of the stria terminalis, medial preoptic area of the hypothalamus, ventral tegmental area, amygdala, hippocampus, olfactory bulbs, and nucleus accumbens, as well as other regions in the brain (Bale, Davis, Auger, Dorsa, & McCarthy, 2001; Ingram & Moos, 1992; Numan & Corodimas, 1985; Pedersen, 1997). The function of oxytocin in these regions appears to play a role in the onset of pup-directed maternal behavior, maternal aggression, social and affiliative behaviors, and stress reactivity.

Interestingly, oxytocin release, receptor number, and mRNA transcription increase several fold throughout the brain at parturition (Lightman & Young, III, 1987; Neumann, Russell, & Landgraf, 1993; Insel, 1990), implying an increase in the functional importance of oxytocin during the early postpartum period. Oxytocin is particularly important to the onset of maternal behavior (proposed as postpartum days one through three in rats), and is essential for the switch from the avoidance of pups to the approach of pups (Fleming, Vaccarino, & Luebke, 1980; Numan et al., 2003). Intracerebroventricular oxytocin antagonist administration (Pedersen, Ascher, Monroe, & Prange, 1982), or lesions of specific oxytocin-containing brain regions, including the paraventricular nucleus (Insel et al., 1989), medial preoptic area (Numan, 1994; Numan, 1986), hippocampus (Kimble, Rogers, & Hendrickson, 1967), and ventral tegmental area (Gaffori & LeMoal, 1979; Numan & Smith, 1984) have

been shown to disrupt maternal behavior during this period. Similarly, oxytocin anti-sense administration into the paraventricular nucleus has been shown to disturb aspects of maternal behavior (Giovenardi, Padoin, Cadore, & Lucion, 1998). Conversely, intracerebroventricular administration of oxytocin in rodents has been shown to dose-dependently increase maternal behavior in usually non-maternal virgin female rats (Pedersen et al., 1982). Despite oxytocin's importance to the onset period of maternal behavior, its role in maintaining maternal behavior through the later postpartum periods is poorly understood.

Gestational cocaine treatment has been shown to disrupt multiple aspects of early postpartum maternal behavior dose dependently (Nelson, Meter, Walker, Ayers, & Johns, 1998; Vernotica, Lisciotto, Rosenblatt, & Morrell, 1996). Cocaine is a potent reuptake inhibitor of serotonin, dopamine, and norepinephrine, all of which play a role in maternal behavior (Numan et al., 2003). Importantly, all of these neurotransmitter systems can also alter aspects of the oxytocin system, including release, reuptake, and binding. Thus, it has been suggested that cocaine's effect on the onset of maternal behavior may stem primarily from its effect on oxytocinergic systems, either directly or indirectly via other neurotransmitter effects (Johns et al., 1998b; Johns, Noonan, Zimmerman, Li, & Pedersen, 1994; Elliott, Lubin, Walker, & Johns, 2001). Given oxytocin's role in the normal induction of maternal behavior, it seems that oxytocin is a likely candidate for mediation of cocaine's effects on this behavioral set.

Chronic cocaine administration to rats throughout gestation has been associated with decreased oxytocin levels in brain structures implicated in maternal behavior and/or maternal aggressive behavior, including the medial preoptic area, ventral tegmental area, and hippocampus (Johns, Lubin, Walker, Meter, & Mason, 1997). When administered directly

into the medial preoptic area and nucleus accumbens, cocaine has been shown to disrupt multiple aspects of maternal behavior (Vernotica, Rosenblatt, & Morrell, 1999). In general, acute cocaine delivered at the time of observation has been shown to alter both the onset of maternal behavior and established maternal behavior, while chronic treatment seems to primarily affect the onset of maternal behavior (Kinsley et al., 1994; Johns et al., 1994; Zimmerberg & Gray, 1992). Importantly, the effects of cocaine on maternal behavior do not result from cocaine withdrawal (Johns, Noonan, Zimmerman, Li, & Pedersen, 1997).

Johns et al. (2005) demonstrated that poor maternal care given by cocaine-treated rat dams rearing even untreated foster pups was subsequently reflected in the maternal behavior of these offspring, who exhibited less pup-directed maternal care when they became mothers. A variety of disruptions in maternal behavior that resulted from rearing condition or prenatal cocaine exposure were demonstrated in offspring, but only rearing by cocaine-treated dams was correlated with oxytocin level changes in the medial preoptic area. This increase in oxytocin levels in the medial preoptic area, seen in cocaine-reared offspring, was unlike the consistent decreases in oxytocin levels seen in the medial preoptic area of cocaine-treated dams. Given the importance of the oxytocin system in maternal care, it is likely that other oxytocin system disruptions play a role in the altered maternal behavior of prenatally exposed offspring.

To date, oxytocin release, binding, synthesis, receptor changes, and clearance have not been systematically examined in the early postpartum period following gestational treatment with cocaine or prenatal exposure to cocaine. The purpose of this project was to examine two aspects of the oxytocin system following gestational treatment with cocaine or prenatal exposure to cocaine: the synthesis of oxytocin as measured by oxytocin mRNA

levels, and oxytocin receptor binding in relevant brain regions. It was hypothesized that gestational treatment with cocaine would result in increased oxytocin mRNA production in the paraventricular and supraoptic nucleus, and would alter oxytocin receptor binding in the medial preoptic, ventral tegmental and hippocampal brain regions. These changes could occur in response other cocaine-induced changes in oxytocin system dynamics such as the decreased levels and increased release previously reported. Additionally, based on previous behavioral and biochemical studies implicating oxytocin system involvement, it was hypothesized that prenatal exposure to cocaine would result in group differences in oxytocin mRNA synthesis and decreased receptor binding in the same brain regions assessed in the original dams.

METHODS

Subjects

Virgin female Sprague–Dawley rats (200–250 grams) were group housed in a temperature and humidity controlled room for a one-week habituation period prior to mating. Females were then singly housed with a sexually mature male until conception was noted by the presence of a sperm plug and, if necessary, confirmation via vaginal smear. On the day of conception, designated as gestation day (GD) zero, the female was removed from the breeding cage, randomly assigned to a treatment group, and individually housed. Pregnant females were maintained on a reverse 12-hour light-dark cycle (lights on at 2100 hours for seven days), then transferred to a room with a regular light cycle (lights on at 0700 hours for the remainder of the experiment); a procedure that results in the majority of dams delivering their litters during daylight hours (Mayer & Rosenblatt, 1998). All procedures were conducted under federal and institutional animal care and use committee guidelines for humane treatment of laboratory animals.

Treatment

Original Dams

On GD zero, pregnant females were randomly assigned to either chronic cocaine (CC) or chronic saline (CS) groups (eight per group). CC- and CS-treated dams received subcutaneous injections twice daily throughout gestation (GD 1-20) on alternating flanks, of 15.0mg/kg cocaine HCL dissolved in 0.9% normal saline (1.0ml/kg total volume), or

1.0ml/kg normal saline respectively, at approximately 0800 and 1600 hours. CC-treated dams had free access to water and food (rat chow). To control for the anorexic effects of cocaine, CS-treated dams were food yoked (pair fed) to match consumption of food by CC-treated dams, as previously described (Johns et al., 1994; Johns et al., 2005).

First Generation Dams

First generation offspring of the dams that were bred and treated as described above were cross-fostered to untreated surrogate mothers. This resulted in offspring prenatally exposed to CC or CS, but reared by drug-naïve dams. Offspring were weaned on postnatal day 21 and bred on postnatal 60. Breeding procedures were as described above, except that no drugs were administered to first generation dams (FGDs) during gestation. All FGDs were maintained on ad libitum food and water. Following delivery, litters were culled to four (two male and two female) and maternal care was monitored until sacrifice.

Maternal Behavior Priming

The procedure for maternal behavior priming has been previously described (Johns et al., 1994). Following delivery of their final pup, original dams and their litters were brought in their home cage to an enclosed behavioral observation room, 400cm x 460cm, where dams were removed from their cage and weighed. Their pups were removed, culled to litters of eight (four male and four female pups), and fostered to an untreated surrogate dam who had delivered within 12 hours of the treated dam. Treated dams were returned to their home cage without pups and the cage placed in a 60cm x 40cm x 50cm dimly lit testing cubicle for a 30-minute habituation period. Simultaneous with the habituation period, the untreated surrogate litters from the surrogate dams that received the treated pups were weighed and culled to four female and four male pups. Surrogate litters were then placed on top of the experimental

dam's testing cubicle in a room temperature plastic cage lined with paper towels. After habituation, nesting material (ten, 2.5cm strips of paper towel) was placed at the back of the dam's cage and the culled surrogate litter was placed in the front of her cage. Dams were allowed access to pups for 30 minutes on postpartum day (PPD) one, and then returned to the animal colony until they were killed 24 hours later.

Brain Collection and Slice Preparation

Twenty-four hours after maternal behavior priming in original dams, subjects were decapitated, the whole brain extracted and frozen immediately on dry ice and stored at -80ºC. The time of sacrifice for FGDs was matched to the original dams, but no behavioral priming occurred. Twenty μm coronal sections were collected from regions of interest as illustrated in the rat brain atlas (Paxinos & Watson, 1997). Regions of interest for *in situ* hybridization included the paraventricular nucleus and supraoptic nucleus of the hypothalamus, while receptor autoradiography focused primarily on the medial preoptic area, ventral tegmental area, and ventral CA1 region of the hippocampus. Slices were thaw mounted onto slides and returned to storage at -80^{o}C until time of assay.

Oxytocin In Situ Hybridization

Sections were fixed in paraformaldehyde and rinsed in PBS. After treatment with triethanolamine and acetic anhydride, they were then defatted in chloroform and dehydrated in a series of graded ethanol concentrations. Each slide was incubated at 37°C overnight with 200μl of hybridization solution containing about 1x10^{6}cpm of ^{35}S-labeled oxytocin oligonucleotide probe. After the incubation, the slides were washed, dehydrated, dried, and placed on Kodak Biomax MR film for one hour and developed.

Oxytocin Receptor Autoradiography

Oxytocin receptor autoradiogaphy was performed using ^{125}I-OTA [d$(CH_2)_5$, O-Me-Tyr^2, Thr^4, Tyr^9, Orn^8]-vasotocin as described previously (Francis, Young, Meaney, & Insel, 2002). Sections were allowed to thaw at room temperature, immersed in a fixative at room temperature for two minutes, and then rinsed twice for 10 minutes in Tris Buffer. For tracer binding, 30mL of tracer solution containing 1,800cpm / 10µl probe was applied to the sections in vertical slide holders at room temperature for 60 minutes. After tracer binding, the slides underwent three five-minute washes with Tris/MgCl buffer at room temperature, followed by one 30-minute wash in Tris/MgCl buffer, and then a final two-second wash in distilled water. Slides were then rapidly dried and, along with a series of ^{125}I-microscale standards, were exposed on film for four days to obtain images for quantification.

Photomicrograph Production and Image Analysis

Binding was quantified from the digitized films using the NIH Image program for the Macintosh®. For the receptor binding studies, optical densities were converted to disintegrations per minute, per milligram tissue equivalents using the ^{125}I-autoradiograph standards developed with the slide images. This conversion was not used on the mRNA film, since standards are not commercially available. Comparisons between treatment groups were based on optical density measurements. Binding in each region of interest was measured bilaterally from six to 11 adjacent sections per region, per animal.

Statistical Analysis

For both *in situ* and autoradiography data, a mean value for each animal for each brain region was calculated after outliers were removed. Due to the nature of our hypotheses, we were able to use one-tailed T-tests with pooled variance to compare the mean

mRNA levels of each group in the original dams; however, two-tailed T-tests were used to examine mRNA levels in the FGDs, and receptor binding in both generations. Statistical significance was set at the $p \leq 0.05$ level. Data are presented as group mean optical density for *in situ* and DPM/mg for autoradiography, with standard error.

RESULTS

Original Dams

Gestational

As shown in Table 1, CC-treated dams gained less weight during pregnancy than CS-treated dams [$F(1,16)=7.134$, $p \leq 0.05$]. Cocaine-treated animals gained an average of 119.6±5.4g during the gestational period, while saline-treated animals gained an average of 139.1±4.9g. There were no between-group differences in gestation length, culled litter weight, or male/female ratio.

Oxytocin *In Situ* Hybridization

As shown in Figure 1, CC-treated dams exhibited significantly higher mean optical densities of binding in the paraventricular nucleus compared to CS-treated animals (CC: 60.59±2.41; CS: 53.93±2.19; $p=0.03$). There was no significant difference between groups in the supraoptic nucleus, although there was a strong trend towards increased transcription in this region in cocaine-treated dams compared to controls (CC: 73.75±2.30; CS: 68.17±2.45; $p=0.06$).

Receptor Autoradiography

As shown in Figures 2, 3, and 4, T-tests revealed no between group differences in autoradiographic oxytocin binding (DPM/mg) in the medial preoptic area (CC: 5484.0±428.0; CS: 5146.1±608.8; $p=0.33$), ventral tegmental area (CC: 12090.0±1480.9; CS:

11939.0±1143.5; p=0.47), or ventral CA1 region of the hippocampus (CC: 61064±3318.8; CS: 64028±4030.9; p=0.29) respectively.

First Generation Dams

Gestational Data

There were no differences between CC-exposed and CS-exposed FGDs on any gestational measure examined. Gestational data for FGDs is presented in Table 2.

Oxytocin *In Situ* Hybridization

As shown in Figure 5, CC-exposed FGDs exhibited no significant differences in mean optical densities of binding in the paraventricular nucleus compared to CS-exposed animals (CC: 73.13±1.99; CS: 74.51±0.86; p=0.25). Although there was no significant difference in the supraoptic nucleus, CC-exposed pups exhibited a strong trend towards increased oxytocin mRNA transcription levels in this region (CC: 81.78±1.49; CS: 76.60±2.06; p=0.06).

Receptor Autoradiography

As shown in Figures 6, 7, and 8, T-tests revealed no between group differences in autoradiographic oxytocin binding (DPM/mg) in the medial preoptic area (CC: 18174±609.91; CS: 18220±1081.9; p=0.97), ventral tegmental area (CC: 11991±1204.9; CS: 11548±608.43; p=0.73), or ventral CA1 region of the hippocampus (CC: 61776±5653.8; CS: 61969±2328.1; p=0.97) of FGDs.

DISCUSSION

The purpose of this study was to determine cocaine's effect on postpartum oxytocin mRNA production and receptor binding in relevant brain regions following gestational treatment with or prenatal exposure to cocaine. As predicted, we found an increase in oxytocin mRNA synthesis in gestationally cocaine-treated dams; most notably, lower levels were reported in the medial preoptic area, hippocampus, and ventral tegmental area on PPDs one and two (Johns et al., 1997). This is potentially in response to other reported changes in oxytocin system dynamics following chronic cocaine impact, or may reflect oxytocin mRNA production increases following overnight exposure and interaction with pups. We did not find any group differences in oxytocin receptor binding in the medial preoptic area, ventral tegmental area, or hippocampus. Although we have reported receptor binding increases following lower oxytocin levels in previous studies on PPD six in the amygdala (Johns et al., 2004), it is clear from this and other studies (Meddle, Bishop, Gkoumassi, van Leeuwen, & Douglas, 2007) that the oxytocin receptor system is complex and varies according to brain region and time of assessment.

The paraventricular nucleus is especially relevant not only for its magnocellular oxytocin producing neurons, but also for its parvocellular oxytocin neurons with central projections to distinct brain regions associated with maternal/social behavior. Unfortunately, we could not clearly visualize a differentiation between the magnocellular and parvocellular neurons, which may have masked any parvocellular-specific effects. Were this effect

specific to magnocellular neurons we would expect that magnocellular neurons within the supraoptic nuclei might also have been significantly altered. However, this was not the case, although there were strong trends toward increased mRNA production in this nucleus. Had our sample size been larger we may have found significant increases in both regions, lending credence to the idea that magnocellular increases may be disguising parvocellular alterations.

With respect to oxytocin mRNA synthesis, regulation is extraordinarily complex, with multiple excitatory and inhibitory mechanisms that cocaine could potentially influence. Very few studies have examined cocaine with respect to oxytocin synthesis, thus non-drug models are useful to examine possible target pathways for cocaine's effects in the present study. As illustrated in Figure 9, the primary mechanism of oxytocin transcription regulation in a non-drug environment occurs through the steroid hormones estrogen and progesterone. Evidence exists supporting the differential modulatory roles of both estrogen and progesterone in cocaine's behavioral effects, including its rewarding and locomotor effects (Niyomchai et al., 2005; Russo et al., 2003), although these effects were not examined in lactating dams. Progesterone administration has also been shown to modulate estrogen's facilitating effects on the self-administration of cocaine, but again this was in non-lactating models (Jackson, Robinson, & Becker, 2006). Additionally, only a handful of studies have examined cocaine's direct effect on these steroids. To our knowledge only one publication has examined cocaine's impact on estrogen (Mello, Mendelson, Kelly, & Bowen, 2000), reporting only minor increases in estrogen levels following acute cocaine administration. Increased progesterone levels, receptor binding, and increased DNA binding formation levels in striatal cell nuclei have all been reported following acute cocaine injections (Wu, Fabian, Jenab, & Quinones-Jenab, 2006). As demonstrated by these studies, acute cocaine can

directly influence progesterone levels, and potentially estrogen levels, which may in turn alter oxytocin mRNA production, but little is known about the effects of chronic gestational cocaine in lactating animals on these systems.

A third system of oxytocin mRNA regulation is mediated through the prolactin system (Ghosh & Sladek, 1995b; Ghosh & Sladek, 1995a; Bakowska & Morrell, 1997; Bakowska et al., 1997). Decreased prolactin release has been shown to reduce oxytocin mRNA production (Grattan, 2001). During lactation, dopamine signaling, which is known to be altered by cocaine, has a direct inhibitory influence on prolactin levels (Byrnes & Bridges, 2007; Mello et al., 1994; Voogt, Lee, Yang, & Arbogast, 2001). Interestingly, acute dopamine D2 receptor activation can also reduce prolactin release, in turn reducing oxytocin mRNA production (Popeski, Amir, Diorio, & Woodside, 2003; Byrnes et al., 2007). Unlike acute cocaine, chronic cocaine administration has been associated with an overall decrease in the number of D2 receptors (Nader et al., 2006), which could then result in an overall increase in prolactin levels and thus contribute to the increase we see in oxytocin mRNA. However, this has not been studied in a lactational model.

Oxytocin receptor activation may also further increase oxytocin release and possibly synthesis (Neumann, Douglas, Pittman, Russell, & Landgraf, 1996), either directly through oxytocin receptor activation, or indirectly by increasing prolactin levels (Samson, Lumpkin, & McCann, 1986) to stimulate oxytocin mRNA synthesis. Chronic cocaine administration has been associated with decreased oxytocin levels in the medial preoptic area on PPD one and in the ventral tegmental area and hippocampus on PPD two (Johns et al., 1997). It is possible that the effects on oxytocin mRNA synthesis seen in the present study reflect higher levels immediately prior to this reported decrease. Given the chronic nature of the

gestational cocaine treatment, it is also possible that the original dams had a depressed level of oxytocin mRNA production, which then increased in response to the overnight pup exposure, resulting in the comparatively higher levels reported here.

Cocaine is a potent reuptake inhibitor of dopaminergic, serotonergic, and less so of noradrenergic systems. In addition to the effects on dopamine receptors previously mentioned, the serotonin and norepinephrine systems have also been shown to increase oxytocin mRNA transcription in both the supraoptic and paraventricular nucleus, though the mechanism of these interactions has not been fully elucidated (Vacher, Fretier, Creminon, Calas, & Hardin-Pouzet, 2002; Jorgensen, Kjaer, Knigge, Moller, & Warberg, 2003). Chronic cocaine administration through its multiple effects on these systems could result in alterations in neurotransmitter receptor levels and an overall change in the sensitivity of these systems.

One highly probable mechanism for cocaine's effects on mRNA is through dopamine reuptake inhibition and the resulting neurotransmitter system changes. In addition to blocking dopamine reuptake, cocaine alters dopamine release and receptors (White, 1990). Manipulations of the dopamine system have been strongly associated with alterations in various aspects of maternal behavior and affect aspects of oxytocin. D2 receptor agonists particularly promote the release of peripheral oxytocin (Amico, Pomerantz, Layden, & Cameron, 1992; Amico, Layden, Pomerantz, & Cameron, 1993; Crowley, Parker, Armstrong, Spinolo, & Grosvenor, 1992; Parker & Crowley, 1992), while administration of dopamine antagonists results in a significant disruption in pup retrieval, nest building, and motor activity in general (Byrnes, Rigero, & Bridges, 2002; Giordano, Johnson, & Rosenblatt, 1990; Keer & Stern, 1999; Silva, Bernardi, & Felicio, 2001; Silva, Bernardi,

Cruz-Casallas, & Felicio, 2003; Stern & Keer, 1999). Additionally, when given chronically, the relatively selective dopamine reuptake inhibitor amfonelic acid increases oxytocin levels and enhances maternal behavior (Johns et al., 1998a; Johns et al., 1996). These findings, in conjunction with those discussed above regarding dopamine and prolactin interactions, suggest that dopamine signaling may play an important role in oxytocin mRNA synthesis, and that cocaine may alter synthesis through this system.

Serotonin, though more strongly associated with aggression, also affects maternal behavior, and is therefore another candidate for oxytocin mRNA changes following cocaine treatment. Similar to cocaine treatment, serotonin reuptake inhibition has been associated with alterations in oxytocin receptor dynamics (Johns et al., 2004), and serotonin agonists alter peripheral oxytocin release, which is important for lactation (Bagdy & Kalogeras, 1993; Bagdy, Kalogeras, & Szemeredi, 1992; Saydoff, Rittenhouse, Van De Kar, & Brownfield, 1991; Uvnas-Moberg, Hillegaart, Alster, & Ahlenius, 1996). Although serotonin's role in oxytocin mRNA production is unclear, Barofsky et al. found impairments in lactation and pup retrieval, and higher incidences of pup cannibalism following specific serotonergic neurotoxin lesions of the median raphe, a major production site for serotonin (Barofsky, Taylor, Tizabi, Kumar, & Jones-Quartey, 1983). The multitude of serotonergic projections from the raphe nuclei to magnocellular neurons in the paraventricular and supraoptic nuclei (Sawchenko, Swanson, Steinbusch, & Verhofstad, 1983) may point to a potential role of these projections in altering oxytocin mRNA production during the stress response, which could indirectly influence maternal behavior and could also explain the trend towards increases in the supraoptic nucleus.

We have previously reported alterations in oxytocin receptor binding on PPD six following chronic cocaine administration and a maternal aggression task (Johns et al., 2004), yet in this study we found no significant differences in receptor binding following priming exposure to pups. This finding, in conjunction with preliminary reports of no alteration in oxytocin receptors in the medial preoptic area, ventral tegmental area, hippocampus, and amygdala of cocaine treated dams without any history of pup exposure indicates that gestational cocaine does not alter receptor binding in these regions with or without pup stimuli present. Receptor changes do not always occur in conjunction with level changes, and the results of studies such as this one are highly dependent on the time of sampling. Level changes are apparent in the medial preoptic area, ventral tegmental area, and hippocampus after pup presentation, suggesting a differential effect on oxytocin levels than receptors at our testing period. Receptor binding dynamics can change rapidly or slowly, and without a thorough investigation of the time course of these changes, cocaine's effect on the oxytocin receptor system are limited to the time points already studied. A time course study using saturation-binding techniques could be beneficial to elucidate any effects of cocaine on this system. Given reported alterations in receptor affinity in the later postpartum period following gestational cocaine treatment (Johns et al., 2004), it is also possible that receptor affinity changes occur earlier in the postpartum period, but the methods used in present study did not allow this determination.

Given previous data regarding effects on maternal behavior and oxytocin in offspring following prenatal cocaine exposure (Johns et al., 2005), another goal of this project was to assess oxytocin system changes other than levels in cocaine-exposed FGDs. Although Johns and colleagues (2005) reported altered maternal behavior in dams following prenatal

exposure to cocaine, there were no significant alterations in oxytocin levels associated with the behavioral alterations, as were shown to occur in cocaine-treated dams. With respect to prenatal cocaine exposure, little has been reported regarding the effects of cocaine on the oxytocin system other than slight level increases following pup stimulation (Johns et al., 2007). Given the previously reported behavioral alterations, some of which are very similar to treated dams, we expected that some aspects of the oxytocin system would be disrupted in prenatally exposed FGDs. Given the functional importance of the oxytocin system, oxytocin mRNA synthesis and oxytocin receptor binding were chosen for assessment in offspring. We found no significant alterations in oxytocin mRNA in the cocaine-exposed FGDs, though there was a strong trend towards increased production in the supraoptic nucleus, but not in the paraventricular nucleus, as was seen in their cocaine-treated biological mothers. Again as in the case of the original treatment dams, the addition of a few more animals to the FGD groups may have led to statistical significance.

The supraoptic and paraventricular nucleus differ in terms of cell composition and function. While the paraventricular nucleus contains both parvocellular and magnocellular neurons, the supraoptic nucleus contains only magnocellular neurons, which project solely to the posterior pituitary for the secretion of oxytocin into the peripheral nervous system. This fact may be particularly important in elucidating possible mechanisms involved in the disrupting effect of prenatal cocaine exposure on maternal and social behaviors.

There is evidence of a parasympathetically driven "anti-stress" system, primarily mediated by oxytocin signaling (Uvnas-Moberg, 1997). If indeed peripheral oxytocin plays a role in stress responding, this interesting trend towards elevated mRNA production in the supraoptic nucleus of cocaine-exposed offspring may reflect subtle dysfunctions in the stress

response of these animals (Huber, Darling, Park, & Soliman, 2001; Molina, Wagner, & Spear, 1994; Wood, Molina, Wagner, & Spear, 1995). Importantly, since all animals were fostered to untreated surrogate mothers, it is unlikely that cocaine-exposed FGDs received abnormal maternal care, though fostering in itself has been shown to affect the stress response (Champagne & Meaney, 2001; Fish et al., 2004).

Prenatal cocaine exposure disrupts many social/aggressive behaviors over the lifetime, and effects seem to manifest during stressful conditions (Johns, Means, Means, & McMillen, 1992; Johns et al., 1992; Johns & Noonan, 1995; Overstreet et al., 2000). As in their mothers, if the stress response were a trigger for oxytocin mRNA changes, it would be extremely important to find a mechanism underlying abnormal stress responses in offspring prenatally exposed to cocaine. Within this framework, the role of oxytocin becomes very exciting.

Both serotonin and norepinephrine have been implicated in the stress response, and if the stress response is indeed altered in these offspring, perhaps cocaine's effect on oxytocin mRNA synthesis may be indirectly related to these two neurotransmitters. As discussed above, both have been implicated in altering oxytocin mRNA levels in these regions (paraventricular and supraoptic nucleus); however, serotonin seems especially pertinent considering serotonin-rich projections from the raphe nucleus to magnocellular neurons that are known to affect oxytocin release (Barofsky & Harney, 1978; Saphier, 1991). Since prenatal cocaine exposure can alter multiple aspects of serotonin signaling, including serotonin receptors, affinity, release, etc. (Akbari, Kramer, Whitaker-Azmitia, Spear, & Azmitia, 1992; Cabrera et al., 1993; Henderson & McMillen, 1993; Johns, Lubin, Lieberman, & Lauder, 2002; McReynolds & Meyer, 1998; Yan, 2002), it seems likely that

any effects cocaine may be having on oxytocin transcription may be related to these serotonergic disruptions.

Additionally, allopregnanolone, which is known to modulate oxytocin mRNA synthesis through the alteration of $GABA_A$ receptor tone (Blyth, Hauger, Purdy, & Amico, 2000), has also been strongly implicated in the stress response (Barbaccia, Serra, Purdy, & Biggio, 2001). Prenatal cocaine's impact on allopregnanolone levels is unknown at this time, but presents an interesting possibility. The involvement of allopregnanolone and GABA is especially interesting given the complex reorganization of the brain that occurs at parturition (Theodosis & Poulain, 2001), bringing more GABA-rich neurons into close proximity to the paraventricular and supraoptic nuclei. More research is needed to determine mechanisms underlying the effects of prenatal cocaine exposure and perhaps of cocaine treatment in general, on these potential oxytocin mRNA changes and the possible stress connection.

As with the mothers of these dams, there was no effect of cocaine exposure on oxytocin receptor binding. Since oxytocin level changes at this time point in cocaine-exposed dams were not apparent, receptor-binding changes may also not occur following prenatal cocaine exposure. This is by no means a definitive finding until more evidence is collected. Further receptor and affinity quantification with more precise methods may indicate differences not obtainable through autoradiography. A complete time course using a variety of methods will prove beneficial in answering these questions.

The results presented here demonstrate that gestational cocaine treatment increases oxytocin mRNA production, which could influence oxytocin signaling throughout the postpartum period. Our findings also indicated that cocaine had minimal impact on oxytocin receptors at the time points tested in the very early postpartum period in both mothers and

their offspring. More studies are clearly needed, including a time course of receptor changes and an examination of other aspects of receptor function such as affinity. Though no behavioral measures were assessed in these studies, these findings add important data to the emerging picture on how cocaine influences oxytocin and perhaps maternal and social behavior through multiple generations. The potential role of stress response mechanisms highlights the exciting possibilities and questions to be explored with respect to these systems.

Table 1.

Original Dam Maternal Gestation and Litter Data

Dam Rearing Conditon	Number of Dams	Gestational Weight Gain (g)	Culled Litter Weight (g)	Number of Pups
CC	8	119.6±5.4 *	37.5±1.0	14.1±0.8
CS	10	139.1±4.9	37.3±0.9	13.9±0.7

Note. CC indicates chronic cocaine, while CS indicates chronic saline. Asterisk indicates significant between groups difference at the $p \leq 0.05$ level.

Table 2.

First Generation Dam Maternal Gestation and Litter Data

Dam Prenatal Exposure	Number of Dams	Gestational Weight Gain (g)	Culled Litter Weight (g)	Number of Pups
CC	8	121.7±16.0	23.3±0.9	13.9±0.9
CS	9	137.4±14.9	24.4±0.8	12.2±0.9

Note. CC indicates prenatal exposure to chronic cocaine, while CS indicates prenatal exposure to chronic saline.

Figure 1.

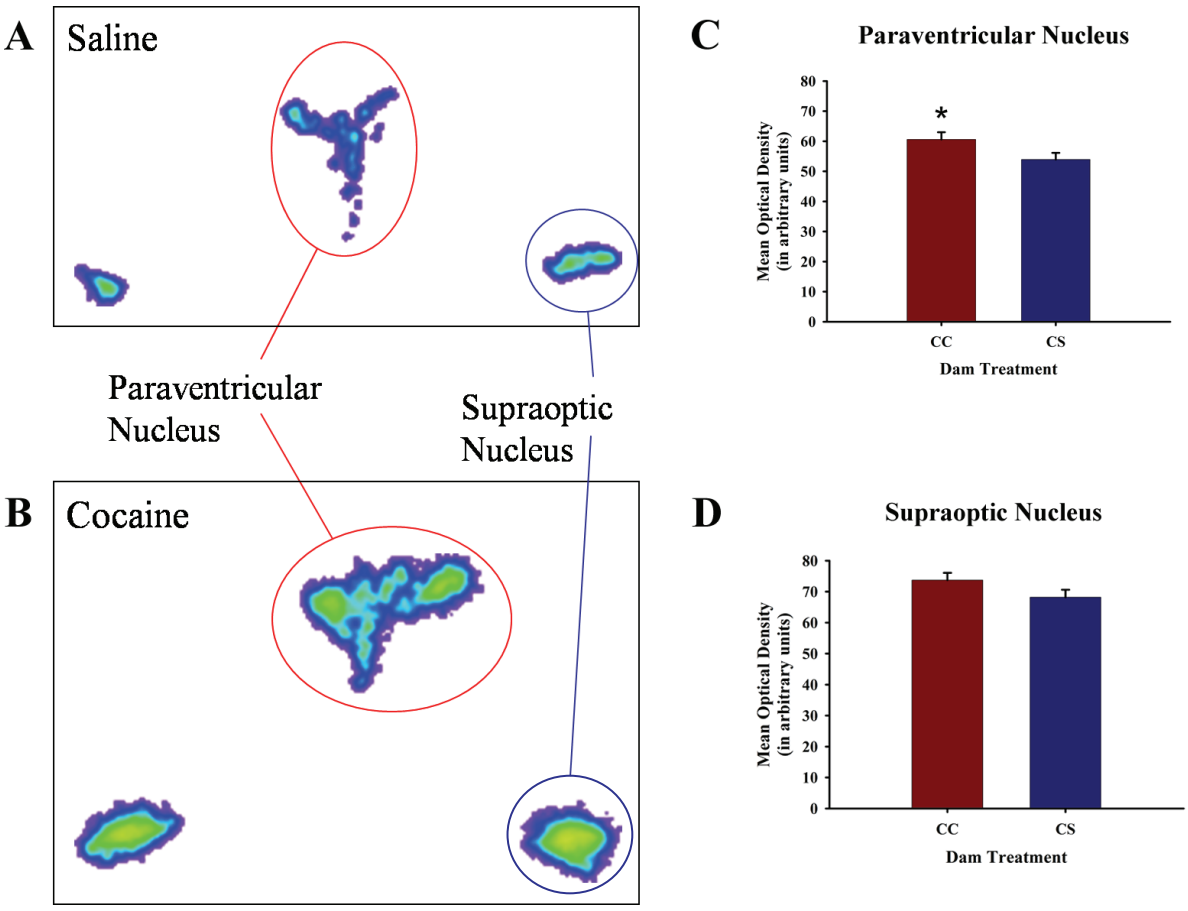

Figure 1: Oxytocin mRNA *in situ* hybridization in the paraventricular nucleus and supraoptic nucleus of Original Dams. Representative radiographs are coronal brain slices taken from saline-treated (A) and cocaine-treated (B) rat dams. Quantification of binding in the paraventricular nucleus (C) and the supraoptic nucleus (D) was performed using NIH Image software, and values are represented as mean optical density area $\pm$ SEM. * $p \leq 0.05$

Figure 2.

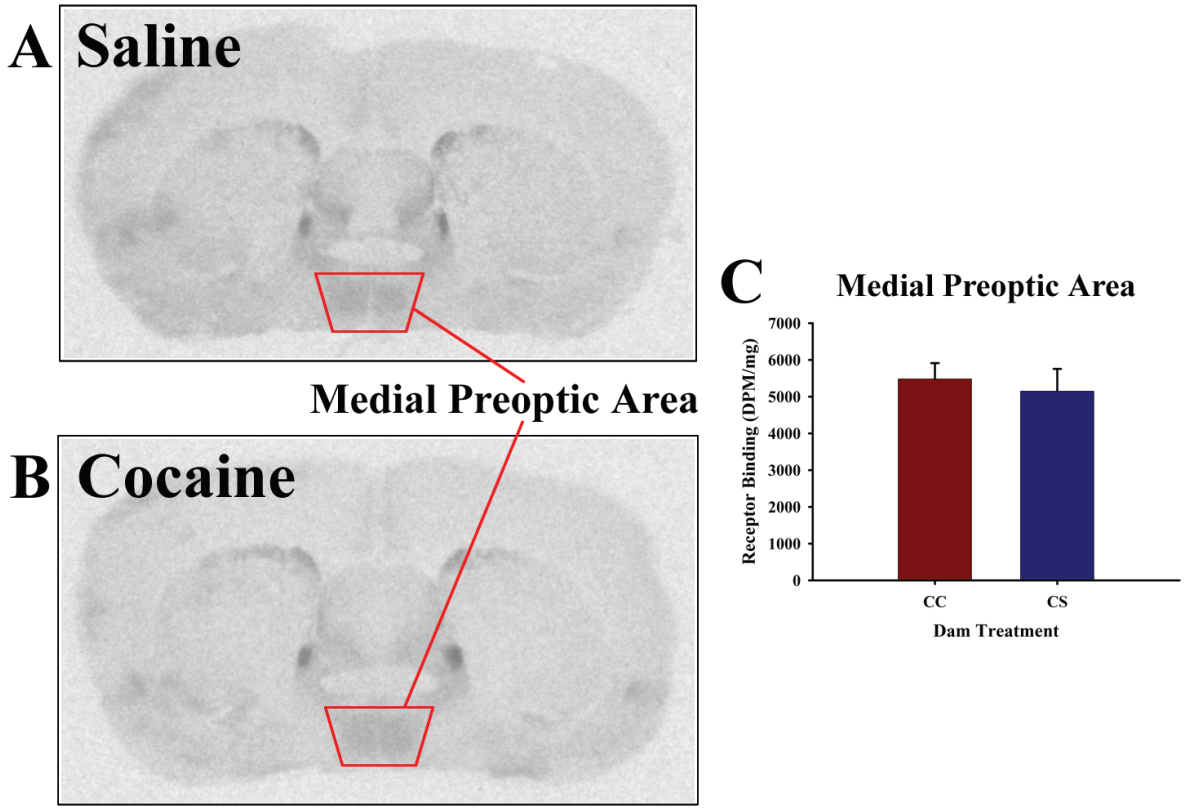

Figure 2: Oxytocin receptor autoradiography in the medial preoptic area of Original Dams. Representative radiographs are coronal brain slices taken from saline-treated (A) and cocaine-treated (B) rat dams. Quantification of binding in the medial preoptic area (C) was performed using NIH Image software, and values are represented as mean DPM/mg $\pm$ SEM.

Figure 3.

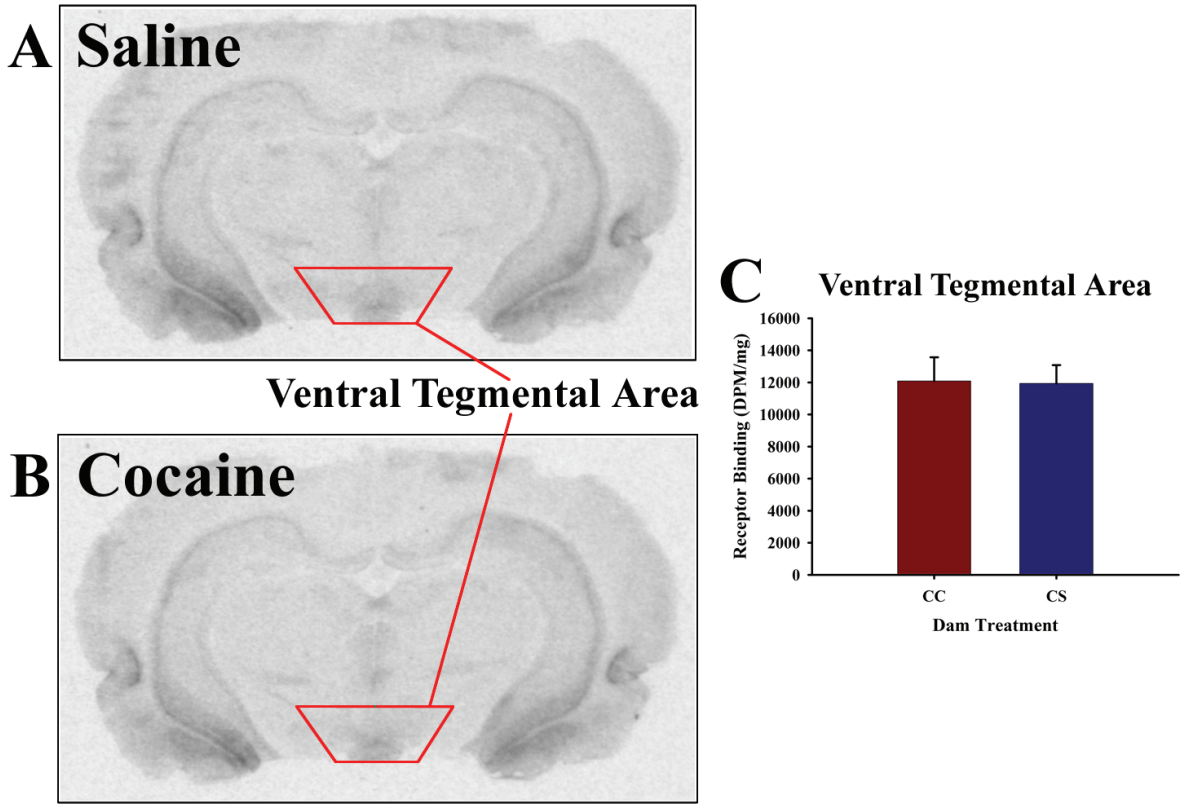

Figure 3: Oxytocin receptor autoradiography in the ventral tegmental area of Original Dams. Representative radiographs are coronal brain slices taken from saline-treated (A) and cocaine-treated (B) rat dams. Quantification of binding in the ventral tegmental area (C) was performed using NIH Image software, and values are represented as mean DPM/mg $\pm$ SEM.

Figure 4.

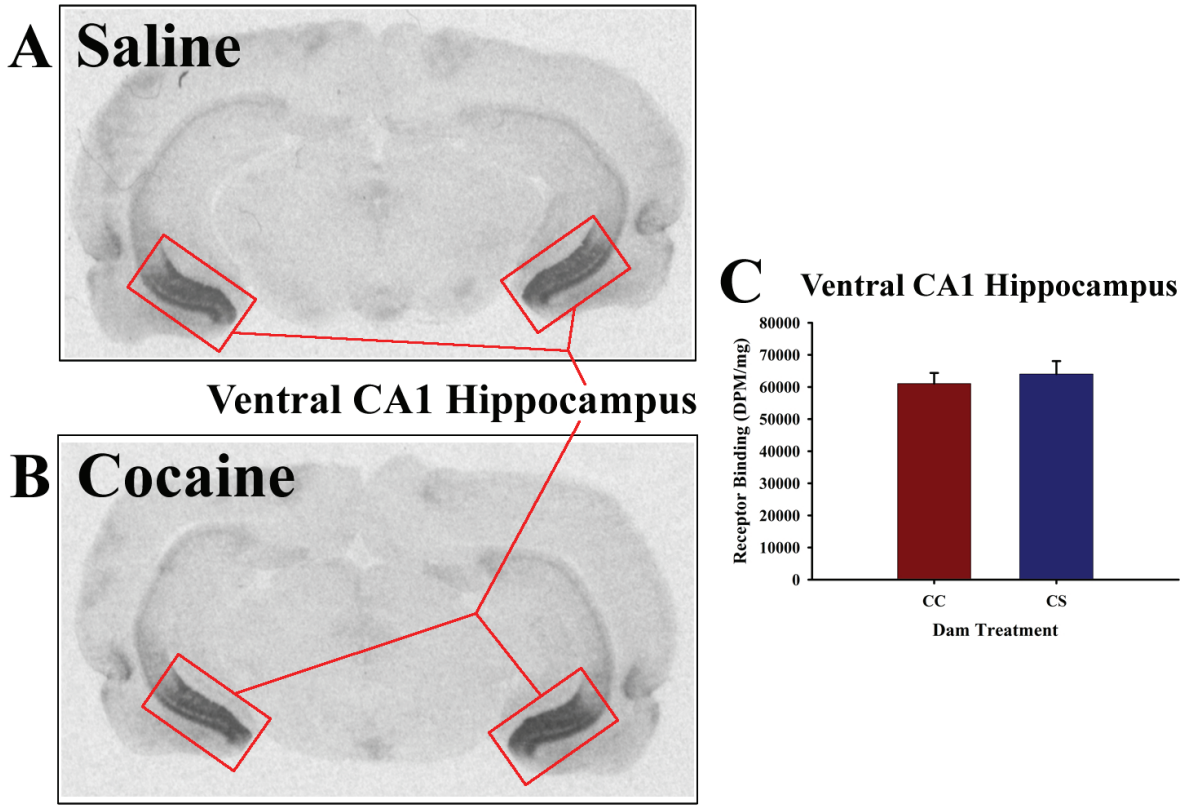

Figure 4: Oxytocin receptor autoradiography in the ventral CA1 region of the hippocampus of Original Dams. Representative radiographs are coronal brain slices taken from saline-treated (A) and cocaine-treated (B) rat dams. Quantification of binding in the hippocampus (C) was performed using NIH Image software, and values are represented as mean DPM/mg ± SEM.

Figure 5.

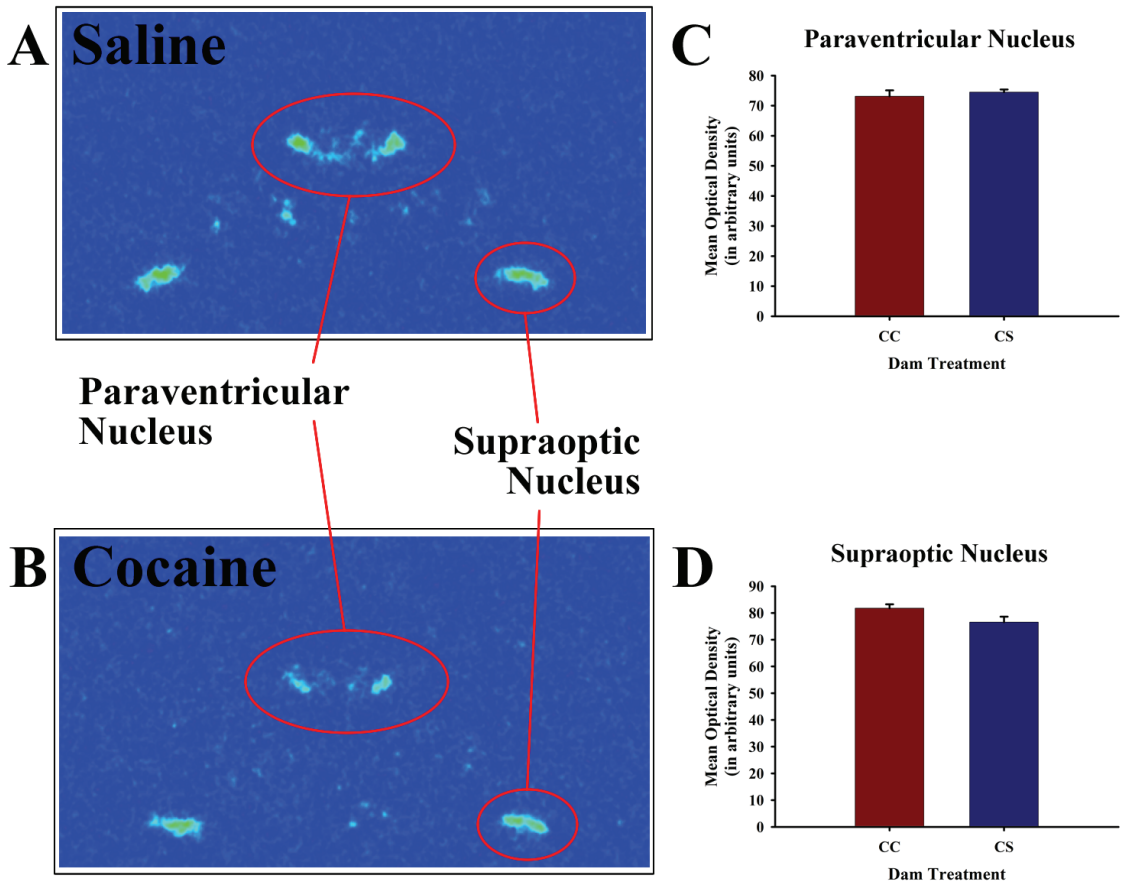

Figure 5: Oxytocin mRNA *in situ* hybridization in the paraventricular nucleus and supraoptic nucleus of First Generation Dams. Representative radiographs are coronal brain slices taken from saline-exposed (A) and cocaine-exposed (B) rat dams. Quantification of binding in the paraventricular nucleus (C) and the supraoptic nucleus (D) was performed using NIH Image software, and values are represented as mean optical density area ± SEM.

Figure 6.

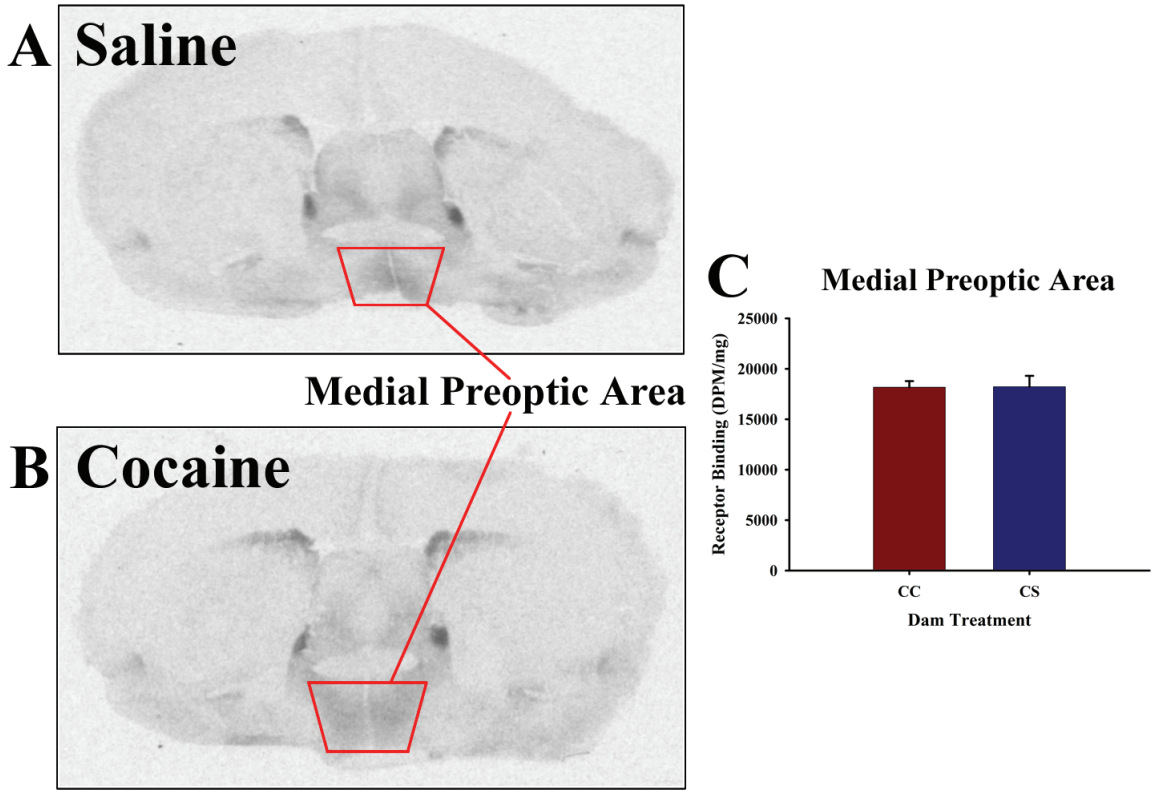

Figure 6: Oxytocin receptor autoradiography in the medial preoptic area of First Generation Dams. Representative radiographs are coronal brain slices taken from saline-exposed (A) and cocaine-exposed (B) rat dams. Quantification of binding in the medial preoptic area (C) was performed using NIH Image software, and values are represented as mean DPM/mg $\pm$ SEM.

Figure 7.

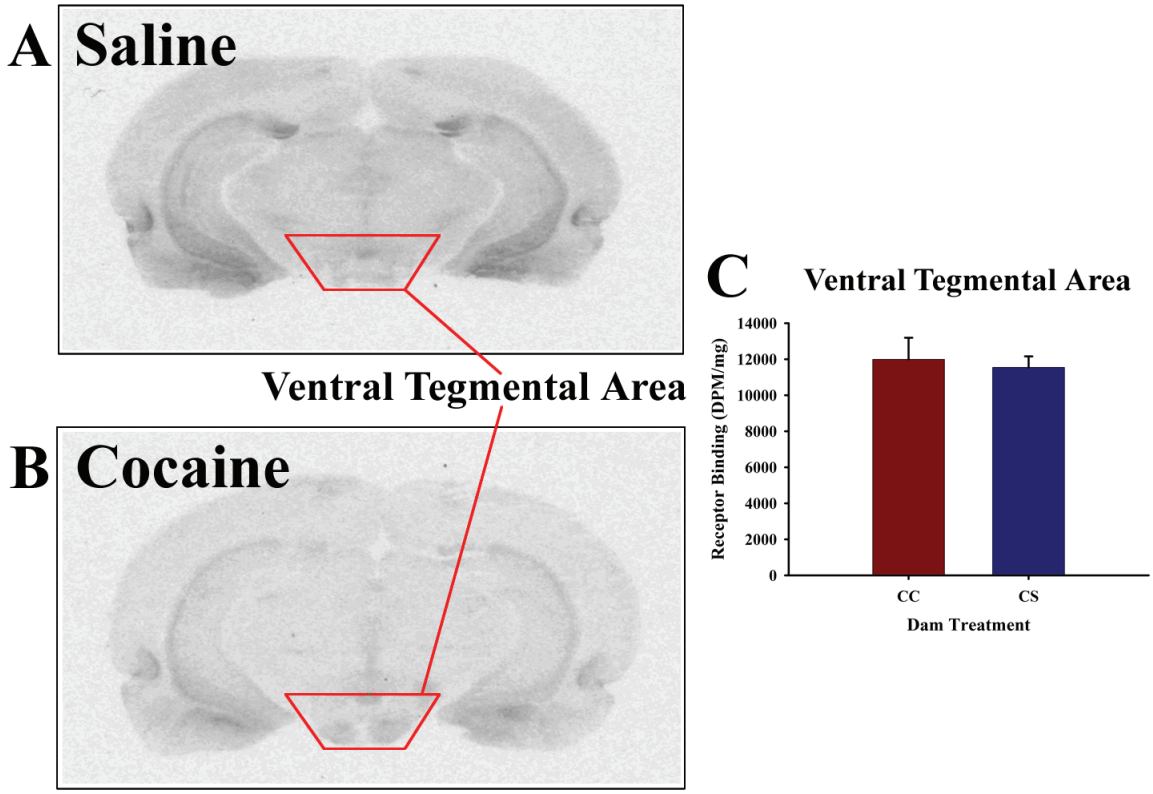

Figure 7: Oxytocin receptor autoradiography in the ventral tegmental area of First Generation Dams. Representative radiographs are coronal brain slices taken from saline-exposed (A) and cocaine-exposed (B) rat dams. Quantification of binding in the ventral tegmental area (C) was performed using NIH Image software, and values are represented as mean DPM/mg $\pm$ SEM.

Figure 8.

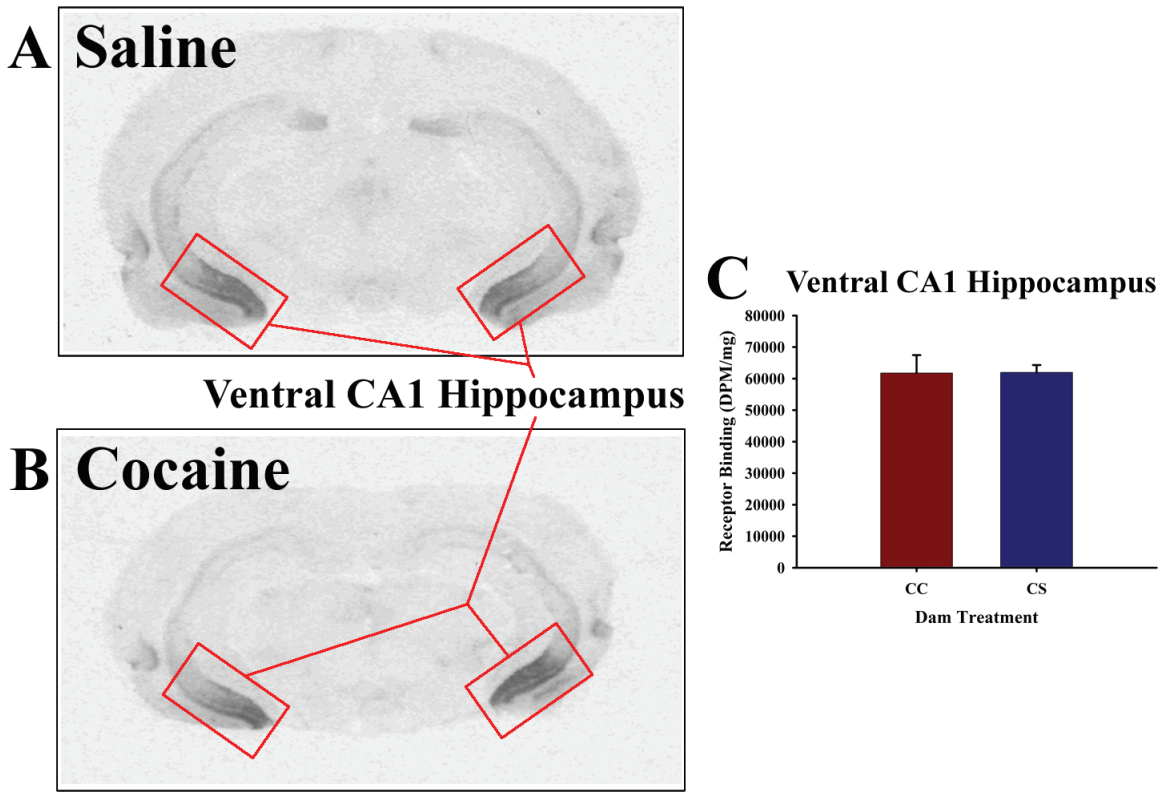

Figure 8: Oxytocin receptor autoradiography in the ventral CA1 region of the hippocampus of First Generation Dams. Representative radiographs are coronal brain slices taken from saline-exposed (A) and cocaine-exposed (B) rat dams. Quantification of binding in the hippocampus (C) was performed using NIH Image software, and values are represented as mean DPM/mg $\pm$ SEM.

Figure 9.

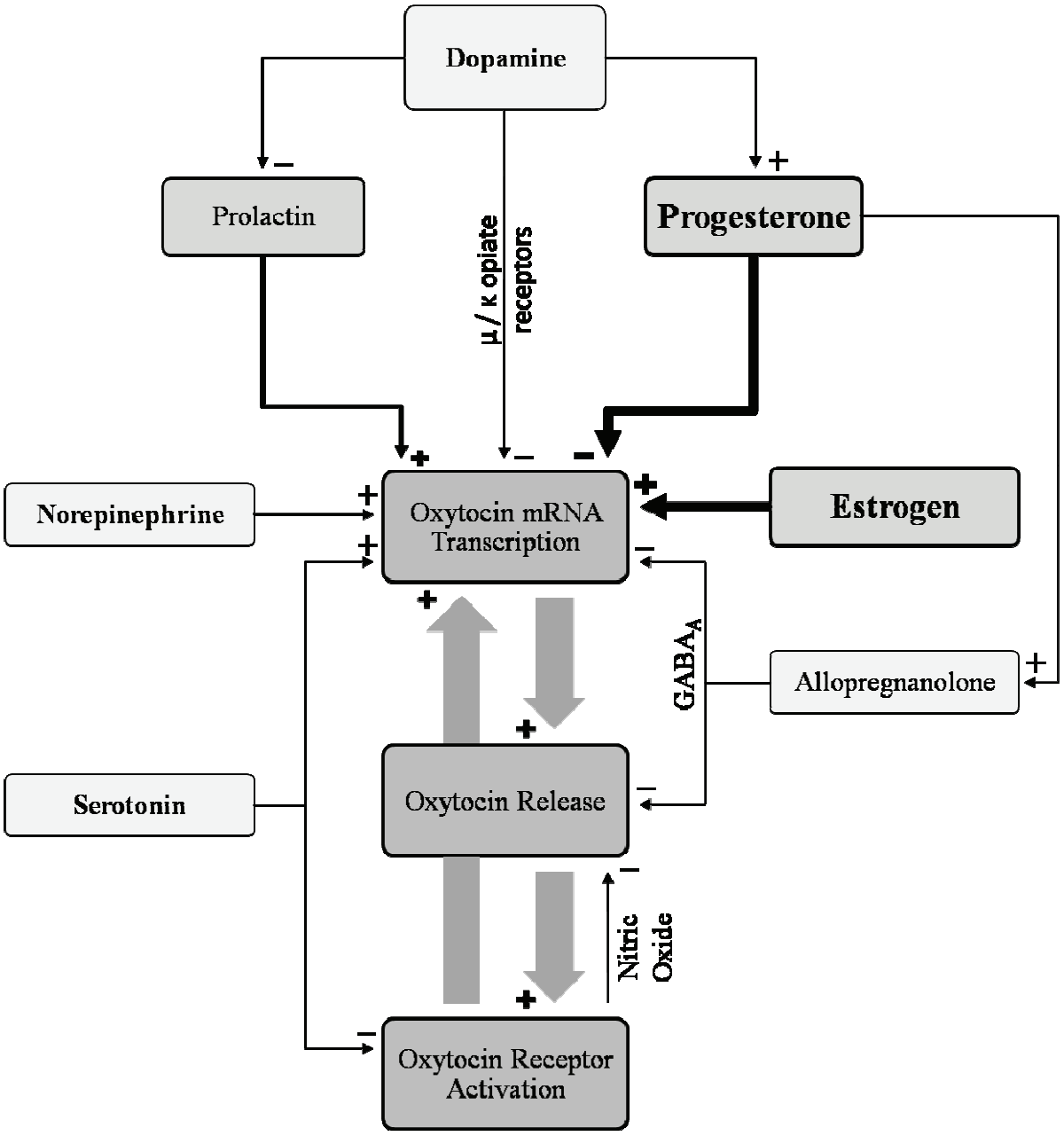

Figure 9: Theoretical diagram of the major regulatory mechanisms of oxytocin synthesis within the paraventricular and supraoptic nuclei. Plus and minus signs indicate direction of modulation of the respective system, while size of sign and arrow indicates relative strength of modulation. Primary modulation occurs through estrogen and progesterone.

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