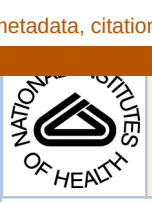

Published in final edited form as:
Behav Neurosci. 2003 April ; 117(2): 195–201.

An Oxytocin Antagonist Infused Into the Central Nucleus of the Amygdala Increases Maternal Aggressive Behavior

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Abstract

Decreased oxytocin levels in the amygdalas of rat dams following chronic gestational cocaine exposure have been correlated with heightened maternal aggressive behavior. In this experiment, drug-naive dams were implanted with bilateral cannulas into the central nucleus of the amygdala (CNA) or control area and infused with 1,000 or 500 ng of an oxytocin antagonist (OTA) or buffer, 4 hr before testing. Behavior was compared among dams infused with OTA into target areas just outside the CNA and cocaine-treated dams (infused with buffer). Dams infused with 1,000 ng OTA attacked intruders significantly more often than buffer-infused dams. OTA did not affect other behaviors, suggesting that disruption of oxytocin activity in the CNA may be sufficient to selectively alter maternal aggressive behavior.

Maternal aggressive behavior is a robust type of offensive aggressive behavior found in lactating female rats (Erskine, Barfield, & Goldman, 1978; Flannelly & Flannelly, 1987; Olivier & Mos, 1992). It has been characterized by a set of postures, threats, and attacks used by mothers to protect their young from intruders that may attack or kill them (Numan, 1994). Although specific components may vary across species, virtually all mammals systematically protect their offspring (Nelson, 1995). Therefore, maternal aggressive behavior is generally considered to be adaptive, helping to ensure that pups survive what would otherwise be the most vulnerable period of their lives.

However, following chronic gestational cocaine administration, rat dams can become highly aggressive toward an intruder (relative to saline-treated dams), leaving their young unprotected and vulnerable or allowing, and in some cases causing, offspring to be injured during the interaction (Johns, Faggin, Noonan, Li, Zimmerman, & Pedersen, 1995; Johns, Noonan, Zimmerman, Li, & Pedersen, 1994, 1997). This heightened level of maternal aggressive behavior is evidenced by a decreased latency to attack intruders and an increased frequency of attacks (Heyser, Molina, & Spear, 1992; Johns et al., 1994, 1995) and is not

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simply a result of cocaine withdrawal (Johns, Noonan, et al., 1997). Chronic cocaine-induced increases in maternal aggressive behavior have been reported to occur during the midlactational period, on post-partum days (PPDs) 6 and 10 (Heyser et al., 1992; Johns et al., 1994, 1995; Johns, Noonan, et al., 1997), but not during the early postpartum period (Lubin, Meter, Walker, & Johns, 2001) when cocaine-induced disruptions of maternal behavior have been reported (Johns et al., 1994). Potentiated aggressive behavior during the midlactational period has been consistently correlated with decreased levels of the neuropeptide oxytocin (OT), specifically in the amygdala (Johns et al., 1995; Johns, Noonan, et al., 1998), a structure implicated in both normal maternal behavior (Fleming, Vaccarino, & Luebke, 1980), and pup cannibalism and viciousness (Grossman, Grossman, & Walsh, 1975).

A growing body of literature reveals that pregnant rats given cocaine daily throughout gestation have decreased amygdala OT levels and increased maternal aggressive behavior on PPD 6 relative to saline-treated controls (Johns et al., 1995). Conversely, females given acute injections of cocaine have increased amygdala OT levels (Elliott, Lubin, Walker, & Johns, 2001) and decreased maternal aggressive behavior compared with saline-treated controls on PPD 6 (Johns, Nelson, et al., 1998). This inverse relationship between OT levels in the amygdala and maternal aggressive behavior suggests that neuropeptide activity in this structure may play an important role in mediating this complicated behavior.

Central OT activity may therefore play a critical role in the regulation of maternal aggressive behavior. Whereas Neumann and colleagues (Neumann, Tosch, Ohl, Torner, & Krömer, 2001) found that an intracerebroventricular infusion of OT antagonist did not increase maternal aggression, Giovenardi, Padoin, Cadore, and Lucion (1997) demonstrated that lesions of the parvocellular division of the paraventricular nucleus (PVN) of the hypothalamus (the main site of central OT synthesis) or local blockade of OT by specific antisense oligonucleotides in the PVN (Giovenardi, Padoin, Cadore, & Lucion, 1998) resulted in increased maternal aggressive behavior in rat dams. However, disruption of OT activity in the parvocellular region of the PVN is nonselective because this region sends OT projections to various targets throughout the central nervous system and therefore subserves a variety of behavioral and physiological outcomes. Accordingly, manipulation of OT activity in discrete brain areas such as the amygdala may provide a more conclusive understanding of the role of this neuropeptide in maternal aggression.

Specific attributes of a discrete division of the amygdala, the central nucleus of the amygdala (CNA), implicate it in this behavior–peptide interaction. Functional OT receptors have been localized to the CNA (Condes-Lara, Veinante, Rabai, & Freund-Mercier, 1994), and although some OT binding sites have also been found throughout the medial nucleus, they are virtually absent in the basolateral and other divisions of this structure (Veinante & Freund-Mercier, 1995, 1997). OT fibers are also largely localized to the central and medial nuclei of the amygdala (Richard, Moos, & Freund-Mercier, 1991). Finally, the CNA has the greatest neuropeptide levels of all nuclear divisions in the amygdala and receives input from most sensory cortices or pathways, as well as other divisions of the amygdala (Simerly, 1989 & Canteras, 1995, as cited in Swanson & Petrovich, 1998).

Therefore, given that cocaine-induced increases in maternal aggression and decreases in OT levels in the whole amygdala were found on PPD 6 (Johns et al., 1995; Johns, Noonan, et al., 1998), the current investigation was conducted to determine whether antagonism of OT activity in the CNA on PPD 6 was sufficient to increase maternal aggressive behavior in cocaine-naive lactating rat dams. The secondary aim of the investigation was to determine whether putative changes in maternal aggressive behavior caused by OT antagonism in untreated dams would be similar in magnitude and character to that elicited by chronic

cocaine administration. It was hypothesized that blockade of OT activity in the CNA would elicit levels of maternal aggressive behavior comparable to those induced by chronic gestational cocaine administration.

Method

Subjects

Virgin female Sprague–Dawley rats (200–225 g) were group housed in a temperature and humidity controlled room for a 1-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 confirmation by a vaginal smear, if necessary. On the day a sperm plug was present (Gestation Day 0), the female was removed from the breeding cage, assigned to a treatment group, and individually housed. Singly housed pregnant females were maintained on a reversed 12-hr light–dark cycle (with lights out at 0900) for 8 days, then transferred to a room with a regular light cycle (lights on at 0700) for the remainder of the experiment, a procedure that generally results in the majority of dams delivering their litters during daylight hours (Mayer & Rosenblatt, 1998). The reverse light cycle was used largely as a matter of practicality, in that staff could immediately weigh, count, sex, and cull litters so that no dam had prolonged exposure to a larger litter than any other dam. This handling procedure has been consistent across investigations in our laboratory.

Treatment

Pregnant females were assigned to one of seven groups and weighed every 5 days to verify maintenance of pregnancy. All females were allowed ad-lib access to food and water. On Gestation Day 15, four groups (10 rats each) had cannulas implanted bilaterally into the CNA (see Table 1). Three groups (10 rats each) had cannulas implanted bilaterally into the ventral tegmental area (VTA). The VTA was selected for comparison because it has been implicated in normal maternal behavior (Gaffori & Le Moal, 1979; Numan & Smith, 1984), and chronic cocaine-induced alterations of OT levels in this site have been correlated only with disruptions in maternal behavior, not maternal aggressive behavior (Johns et al., 1994; Johns, Lubin, Walker, Meter, & Mason, 1997). Therefore, no OT antagonist (OTA)-induced alterations in maternal aggressive behavior were anticipated following infusion into the VTA.

Each group remained untreated throughout gestation except one group of dams (with bilateral amygdala cannulas) that was given cocaine throughout gestation. Cocaine-treated females were weighed daily and given subcutaneous injections of 15 mg/kg of cocaine HCl (dose calculated as the free base; Sigma Chemical Company, St. Louis, MO) dissolved in 0.9% (wt/vol) normal saline, twice daily (on alternating flanks) for a total daily dose of 30 mg/kg, in a total volume of 2 ml/kg. On the day of surgery (and on the morning following surgery), no cocaine injections were administered. As skin lesions are prone to develop following repeated subcutaneous administration of cocaine solution, injection sites were varied. Any skin lesions that developed were cleaned with a Betadine wash and treated with a topical antibacterial ointment (Polymycin–Bacitracin–Neomycin, Burroughs Wellcome, Raleigh, NC) as soon as they were discovered.

Drug

Cocaine was stored in amber bottles and maintained in a standard refrigerator when not in use. New solutions were prepared approximately every 3 days.

Surgery

On Gestation Day 15, each dam was anesthetized with ketamine (55 mg/kg; Abbott Laboratories, Chicago, IL) and xylazine (10 mg/kg; Phoenix Scientific, St. Joseph, MO) and surgically implanted with bilateral 22-gauge stainless steel cannulas into either the CNA or the VTA. The stereotaxic coordinates for the implantation of the cannulas into the amygdala were −2.8 posterior, 4.5 lateral, and −6.9 ventral to bregma according to the atlas of Paxinos and Watson (1997). The coordinates for the implantation of the cannulas into the VTA were −4.8 posterior, 1 lateral, and −8.0 ventral to bregma according to Paxinos and Watson (1997). Three stainless steel screws were anchored in the skull to provide structural support for the skull cap (made of dental acrylic). Finally, stainless steel inserts with plastic caps were screwed into each cannula to prevent any debris from entering the brain and to maintain patency of the hollow metal cylinder. These inserts extended 1 mm beyond the tip of the cannulas, reflecting the ultimate desired depth of the apparatus and the actual depth the infusion needle reached during antagonist or buffer infusion. Following surgery, the females were allowed to recover and were returned to the colony room. They were monitored daily for any signs of distress or infection.

Culled Litters

Pregnant rats typically delivered their litters on Gestation Day 21 (designated PPD 1). Immediately following parturition, pups were removed, counted, sexed, and weighed. Each litter was culled to eight male pups and returned to the respective dam. Cocaine-treated dams received surrogate pups to control for potential differences in maternal behavior resulting from possible alterations in pup-derived cues from offspring that were exposed to cocaine in utero. Each dam and her culled litter were then returned to their home cage in the colony room until aggression testing and monitored daily to ensure that dams were adequately feeding and caring for offspring.

Infusion

On PPD 6, each rat and her litter were retrieved from the colony room. Dams were given bilateral infusions of buffer or 250 or 500 ng OTA(d(CH_2)$_5$, [Tyr(Me)^2-Thr^4-Tyr-NH_2^{9}]–vasotocin; BACHEM Bioscience, King of Prussia, PA) dissolved in neutral buffer solution (plus 10 μl acetic acid to aid drug dissolution) for final concentrations of 0, 250, and 500 ng/0.5 μl. Each infusion was delivered over 30 s. Therefore, dams received a total dose of 0, 500, or 1,000 ng OTA in 1.0 μl of buffer, administered over 1 min (see Table 1). Although these concentrations of OTA are relatively high, they were selected in order to maximize the potential of OTA to bind to OT receptors and have been used in other investigations (Caldwell, Johns, Faggin, Senger, & Pedersen, 1994). Following the targeted infusion, each dam was placed in her home cage with her litter in an observation room and allowed to remain undisturbed for 4 hr prior to the aggression testing session. The 4-hr interval between infusion and testing was chosen for several reasons. Not only does this allow any acute effects of the treatment to subside, but central OT receptors are also almost completely blocked after 6 hr of intracerebroventricular infusion of OTA and continue to be blocked almost 18 hr postadministration (Witt & Insel, 1991). Therefore, there is good evidence that receptor blockade would be maximal at 4 hr after infusion. Furthermore, behavioral manifestations of OT receptor blockade may take many hours to become manifest. Caldwell and colleagues (1994) found that OTA must be administered centrally 4 hr prior to testing for blockade of sexual behavior in females.

Following histological analysis at the conclusion of the experiment, dams that had inappropriate cannula placements (just outside the CNA) and were infused with either dose of OTA were combined into an eighth group. The behavior of this group was compared with

all other treatment groups and allowed for analysis of the specificity of the CNA in mediating the behavioral effects of OT activity (see Table 1).

Maternal Aggression Testing

On PPD 6, each mother was infused with either OTA or buffer and then replaced in her home cage with her litter in an observation room. A smaller (relative to the rat dam; 175–200 g) intruder male was introduced into the home cage 4 hr following the dam's infusion (between 12 and 2 p.m.). Each intruder was used in only one aggression session. Videotaping with a VHS recorder with low-light sensitivity began as soon as the intruder was placed in the cage and continued for 10 min. The following 11 behaviors, which have been described previously (Johns et al., 1994; Lubin et al., 2001), were assessed by two independent observers who were unaware of the rats' treatment conditions. They include *push/box/kick* (dam pushes or kicks the intruder male), *maternal behavior* (dam licks, retrieves, or crouches over pups), *rough groom* (dam grooms intruder male roughly), *self-groom* (dam grooms herself), *lateral/front threat* (dam threatens intruder male while approaching laterally or face to face), *fight attack* (dam lunges quickly at intruder male, usually followed by rolling, biting, and fur pulling directed toward the neck and back regions of the intruder), *rear/sniff* (dam rears on hind legs and sniffs the top or sides or rear of cage), *nip/bite* (dam nips or bites intruder male; distinct from fight attack); *chase male* (dam chases intruder); *aggressive posture* (dam forces intruder into a full submissive posture by pushing with extended front paws), and *other* (any behavior other than those included in the categories above).

Test sessions were immediately discontinued if the intruder either attacked the pups or was seriously wounded by the female, and data from such a session were not included in the statistical analysis. Two observers independently scored behavior for frequency, duration and latency, using a computer program specifically designed for that purpose. Interrater agreement was within at least 90% for frequency and latency and within at least 80% for duration of each behavior. Following the testing procedures, the rats' brains were processed for verification of cannula placement.

Histology

Following aggression testing, each dam was given an overdose of sodium pentobarbital (Abbott Laboratories), and 1 μl of dye (blue ink) was infused into the cannulas. The female was then decapitated. Her brain was quickly removed, immediately placed on a tissue chuck, and frozen. The brains were later sliced on a cryostat to verify appropriate cannula placement according to the atlas of Paxinos and Watson (1997). Figure 1 provides an illustration of relevant brain slices with outlines defining the areas within the amygdala (CNA) and midbrain (VTA) where cannula placement would be considered a "hit."

Data Analysis

Frequency, duration, and latency of each behavior, as well as gestational variables, were independently analyzed for between-group differences by means of a one-way analysis of variance. Tukey's honestly significant difference was used for post hoc comparisons of significant main effects. A probability level of $p \leq .05$ was established as the significance level for rejection of the null hypothesis.

Results

Gestational Variables

There were no significant treatment-related differences in the length of gestation, maternal weight gain, number of live versus dead pups, number of male versus female pups, or litter weight among groups (*p*s > .05; data not shown).

Behavior

This experiment was designed to investigate whether antagonism of OT activity, specifically in the CNA, was sufficient to increase levels of maternal aggressive behavior on PPD 6 and whether this heightened aggressive activity would be similar to that seen in dams given cocaine throughout gestation (without direct OT antagonism). As illustrated in Figure 2, the group of dams given a 1,000-ng OTA infusion into the CNA fought with or attacked an intruder male significantly more often, $F(7, 61) = 4.72$, $p < .01$, than each of the other groups except cocaine-treated dams that were given a buffer infusion and dams infused with 500 ng OTA into the CNA. Untreated females given 1,000 ng OTA also attacked intruders for a longer duration, $F(7, 61) = 3.56$, $p < .01$, than untreated dams given a buffer infusion into the CNA ($p < .02$) and 1,000 ng infused into VTA ($p < .05$); they also had a faster onset of attacking behavior, $F(7, 61) = 4.76$, $p < .01$, compared with untreated dams infused with buffer into the CNA ($p < .04$; data not shown).

Figure 3 demonstrates that although there is considerable variability within treatment groups, dams with the high dose of OTA infused into the CNA and those given cocaine throughout gestation (and a buffer infusion) restrained intruder males using an aggressive posture more often, $F(7, 61) = 3.03$, $p < .01$, than dams given an infusion of buffer into the CNA, dams with cannula placement just outside the CNA, and dams infused with OTA or buffer into the VTA. However, post hoc comparisons reveal that the number of aggressive postures performed by dams given cocaine throughout gestation or those untreated dams infused with 1,000 ng OTA into the CNA was only statistically significantly higher than the number performed by untreated dams given 1,000 ng OTA into the VTA.

Although the group of dams that received a 1,000-ng infusion of OTA into the CNA were significantly more aggressive than other groups (except those given cocaine throughout gestation), there were no statistically significant treatment-related differences in maternal behavior, self-groom, rear/sniff, rough groom, nip/bite, chase male, or threats. In fact, it is striking to note the absence of effects of OT antagonism in the CNA on various mildly aggressive and nurturing behaviors in contrast to its more robust effects on the most aggressive behaviors. For example, there were no treatment-dependent differences in maternal behavior, $F(7, 61) = 0.21$, $p > .98$, as evidenced by the data presented in Figure 4.

Discussion

Previous investigations have established that chronic cocaine administration throughout gestation is correlated with both decreased OT levels in the amygdala and increased maternal aggressive behavior on PPD 6 (Johns et al., 1995; Lubin et al., 2001). The data collected from the present experiment suggest that blockade of OT activity directly in the CNA (with 1,000 ng OTA) is sufficient to increase maternal aggressive behavior. Although the comparison of the cocaine-treated (buffer-infused) cohort and untreated dams given OTA is intuitively interesting, experimental differences (daily injection stress and fostering of pups in the cocaine-treated group) preclude definitive interpretation of the mechanism driving the congruous behavioral outcomes observed in cocaine-treated and untreated dams infused with 1,000 ng OTA into the CNA.

Whereas dams infused with 1,000 ng OTA into the CNA were significantly more aggressive than those infused with buffer, cocaine-treated dams were not statistically significantly different than buffer-infused dams, given intergroup variability. Therefore, the difference in aggressive behavior between cocaine-treated dams and control-treated dams was not as robust as reported in previous investigations (Johns et al., 1994; Johns, Noonan, et al., 1997), perhaps due in part to the unique surgical procedures used in this experiment or differences between cocaine-exposed and untreated groups in terms of injection stress and fostering of pups.

Still, it is important to note that there was specificity in the effects of direct OT antagonism and chronic cocaine administration. That is, most of the observed behaviors, including threats and maternal behaviors (licking, grouping, or crouching over pups), were unaffected by any of the treatments on PPD 6. These findings lend indirect support to the hypothesis that cocaine-induced decreases in amygdala OT activity play an important role in selectively augmenting maternal aggressive behavior in rats. They also bolster previous investigations indicating that OT activity in the VTA is critical to the onset of maternal behavior, but not to its maintenance (Pedersen, Caldwell, Walker, Ayers, & Mason, 1994). Taken together, these data provide evidence for unique mechanisms driving maternal behavior and maternal aggressive behavior.

In general, the mechanism (or mechanisms) by which altered OT activity in the amygdala affects maternal aggression is unknown. Ferguson, Aldag, Insel, and Young (2001) demonstrated that, in mice, OT in the medial amygdala is necessary for the initial processing of olfactory information and subsequent social recognition of conspecifics. It is possible that OT activity in the rat amygdala is also critical for processing information regarding social interactions, particularly in threatening situations. Therefore, it is plausible that decreased OT activity in various amygdalar nuclei enhances maternal aggression, in part, by interfering with perception of the threat represented by the intruder male. The results of the current investigation cannot address this possibility, although they clearly suggest that antagonism of OT activity in the CNA potentiates maternal aggressive behavior in rats, whereas OT antagonism just outside of the amygdala has virtually no effect on maternal aggression. Nevertheless, it is possible that given the volume and concentration of the OTA infusion in the current experiment, some drug may have diffused into neighboring nuclei of the amygdala, thereby contributing to the potentiated behavioral response. Also, given the high concentration of OTA, it is theoretically possible that there may have been some paradoxical agonist activity affecting behavioral outcomes. Future investigations using direct OT agonists will explore this possibility.

Alternatively, reduced OT activity in the CNA may affect aggressive behavior by influencing various systems responsible for fight-or-flight responding, including the sympathetic and parasympathetic nervous systems. Projections from the CNA synapse on relevant brainstem and spinal cord nuclei that regulate autonomic activity (Richard et al., 1991). Uvnas-Moberg (1997) suggests that there is an oxytocin-mediated, parasympathetically driven “anti-stress” system. It is possible that if such a system were dysregulated by decreased OT activity, aggressive responding would be increased.

Given that cocaine-induced increases in aggressive behavior have been correlated with decreased levels of OT in the amygdala (Johns et al., 1995) and that blockade of OT activity in the CNA of untreated animals increased maternal aggressive behavior, pilot data are being collected to determine if cocaine-induced maternal aggression can be ameliorated by an infusion of OT into the CNA. Additional investigations will be critical in characterizing the ability of OT activity in the amygdala to alter maternal aggressive behavior. These would include an investigation of the effects of OT antagonism in the amygdala during the

early lactational period, when even cocaine-induced increases in aggressive behavior are meager (Lubin et al., 2001), or toward the end of the lactational period, when aggressive behavior begins to wane (Erskine et al., 1978; Flannelly & Flannelly, 1987; Mayer, Reisbick, Siegel, & Rosenblatt, 1987). Also, further investigations examining whether OT agonists could decrease cocaine-induced increases in maternal aggression would be informative. In addition, a microdialysis study evaluating OT levels during or just after an aggression session in untreated, cocaine-treated, and saline-treated dams may provide a better understanding of treatment-related differences in OT activity directly at the site and time of interest. Finally, investigations of the effects of chronic cocaine (relative to saline-treated controls) on OT receptor number or affinity would help to more fully characterize the potential effects of cocaine on the OT system in relevant brain areas. Potential cocaine-induced alterations of OT receptor dynamics are currently under investigation in our laboratory (Lubin, Johns, & Walker, 2002).

In conclusion, this experiment demonstrates that blockade of OT activity specifically in the CNA is sufficient to increase maternal aggressive behavior on PPD 6. This finding provides additional evidence supporting the hypothesis that maternal aggressive behavior is potentiated, at least in part, by decreasing OT activity in the amygdala. We are currently investigating whether an infusion of OT can ameliorate cocaine-induced increases in maternal aggressive behavior.

Acknowledgments

This work was supported by National Institutes of Health Grants DA13362–01 and DA13283–01A1.

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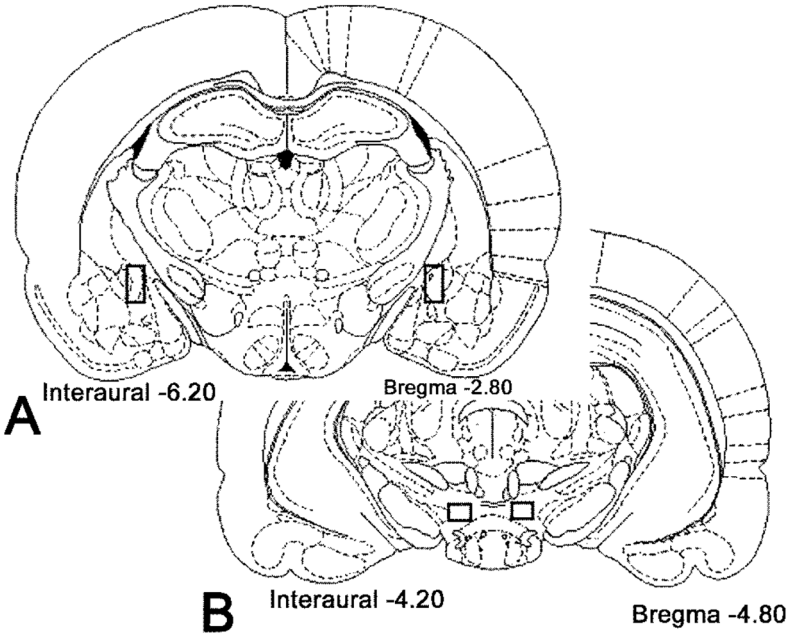

Figure 1.

A: Rectangles indicate the area within which infusions were considered "hits" within the central nucleus of the amygdala. B: Rectangles indicate the area within which infusions were considered hits within the ventral tegmental area. Reprinted from *The Rat Brain in Stereotaxic Coordinates*, 3rd ed., G. Paxinos and C. Watson, Figures 29 and 37, Copyright (1997), with permission from Elsevier Science.

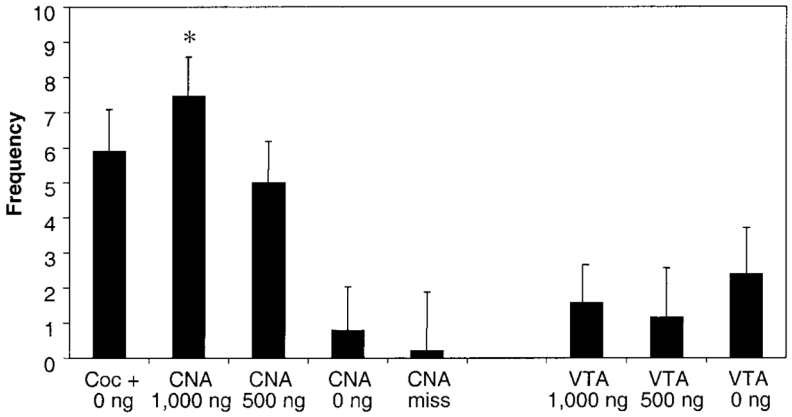

Figure 2.

Least squares means (±*SEM*) fighting and attacks (in number per 10-min test session) exhibited by dams infused with 1,000 or 500 ng oxytocin antagonist (OTA) or buffer. Asterisk indicates that mothers given a 1,000-ng OTA infusion into the central nucleus of the amygdala (CNA) fought more often than those infused with buffer into the CNA ($p < .01$), those infused with OTA just outside the CNA (CNA miss; $p < .01$), those infused with either 1,000 or 500 ng OTA into the ventral tegmental area (VTA; $p < .02$), and almost significantly more often than dams given an infusion of buffer into the VTA ($p < .08$). Coc = cocaine.

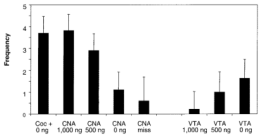

Figure 3.

Least squares means (±*SEM*) frequency (in number per 10-min test session) of aggressive postures assumed by dams infused with 1,000 or 500 ng oxytocin antagonist (OTA) or buffer. Mothers given either a 1,000-ng OTA infusion into the central nucleus of the amygdala (CNA) or cocaine (Coc) throughout gestation performed more aggressive postures during the 10-min test session than those infused with 1,000 ng OTA into the ventral tegmental area (VTA; $p < .05$ and $p < .04$, respectively). CNA miss indicates dams infused with OTA just outside the CNA.

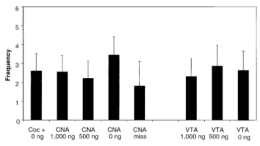

Figure 4.

Least squares means (±*SEM*) maternal behaviors (in number per 10-min test session) exhibited by dams infused with oxytocin antagonist (OTA) or buffer. There were no treatment-related differences observed during the test session. CNA miss indicates dams infused with OTA just outside the central nucleus of the amygdala. Coc = cocaine; VTA = ventral tegmental area.

Table 1

Experimental Design and Group Sizes

		Infusion		
Treatment	**Cannula placement**	**500 ng OTA**	**1,000 ng OTA**	**Buffer**
None	CNA	10	11	9
None	Just outside of the CNA	5	5	
30 mg/kg sc cocaine on GD 1–20	CNA			10
None	VTA	7	9	8

Note. OTA = oxytocin antagonist; CNA = central nucleus of the amygdala; GD = gestational day; VTA = ventral tegmental area.