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Oxytocin antagonism alters rat dams' oral grooming and upright posturing over pups

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Abstract

Studies involving intracerebral administration of antiserum or antagonists have demonstrated that central oxytocin (OT) plays a prominent role in initiating but not maintaining postpartum maternal behavior in rats. There has been little investigation, however, of OT's influence on the levels of maternal behavior exhibited during the maintenance phase. We measured rat dam behavior during the 105-min observation periods preceding and beginning 2 h after intracerebroventricular infusion of the selective OT antagonist (OTA) (1 μg), or normal saline (NS) vehicle (5 μl) on postpartum days 2–3 and 6–7. Compared to NS, OTA significantly decreased pup licking as a proportion of dams' total oral grooming, increased self-grooming, decreased the frequency of elevated upright posture over pups and increased the frequency of lying prone on pups. Quiescent, kyphotic nursing was also significantly lower in OTA-treated dams. Other components of maternal behavior were not significantly affected by OTA or NS treatment. These findings suggest that central OT may shift the focus of the dams' oral grooming from self to pups and may also facilitate elevation of dams' upright posture over pups. Acute stress responses, maternal behavior and central OT receptor binding in adult rats have been linked to the amount of maternal licking and arched back, upright nursing received during infancy. OT activity in dams' brains may influence these developmental outcomes in their offspring by selectively regulating their pup licking and crouching posture.

Keywords: Oxytocin; Neuropeptides; Maternal behavior; Grooming; Nursing

1. Introduction

Central administration of oxytocin (OT) antagonists or antiserum or destruction of the hypothalamic paraventricular nucleus, the source of most OT projections within the brain, delayed or prevented the postpartum and ovarian steroid-induced activation of all components of maternal behavior [8,16,31,32,44]. In contrast, intracerebroventricular infusion of OT antagonist (OTA) or paraventricular lesions in rat dams with several days of postpartum mothering experience failed to extinguish any components of established maternal behavior [8,28]. These findings led to the conclusion that OT contributes significantly to initiating but not to sustaining rat maternal behavior [8,27,29]. The behavior measurements in these earlier studies, however, were not sufficiently quantitative to definitively determine whether central OT regulates the amount of maternal behavior exhibited by experienced dams.

Several considerations led us to hypothesize that OT may continue to regulate dams' licking of pups and nursing posture after the postpartum activation of maternal behavior. Oral grooming bouts in rat dams are characterized by oscillations back and forth between pup licking and self-grooming. Central administration of OT robustly stimulates self-grooming in infant, juvenile and adult rats [5,6,17,30,33,43]. In preweaning rats, intracisternal injection of OT also increases licking and forepaw holding of newborn pups [33]. Kyphotic nursing bouts are triggered by pup somatosensory stimulation of the dam's ventral trunk [19–21,36,37,39,40,42]. Central OT release increases during nursing [25] probably in response to nipple stimulation but perhaps other aspects of ventral trunk stimulation as well. Therefore, we tested whether central administration of an OTA in lactating rat dams alters their frequencies of pup licking, self-grooming, upright posturing over pups and upright nursing bouts.

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2. Methods

All experiments were conducted in accordance with NIH Guidelines for the Care and Use of Laboratory Animals and were approved by the University of North Carolina Institutional Animal Care and Use Committee.

2.1. Animals

Primigestational, lateral cerebral ventricle-cannulated Sprague–Dawley rats ($N = 10$) were shipped from Charles River Breeders (Raleigh, NC) to our animal facility on pregnancy day 15. Rats were housed in individual transparent polycarbonate cages ($46 \times 25 \times 20$ cm) from the time they arrived. Each cage was placed in a slot on the end of a cage rack so that events in the cages could easily be observed through the side of the cage. Rat chow (distributed on cage floors) and water from spigots, accessible through a small hole in the back of each cage, were available ad libitum. Lights were turned on at 04:30 h and turned off at 16:30 h. Animals were monitored daily until parturition.

2.2. Stereotaxic surgery

Charles River personnel implanted lateral ventricle cannulae in pregnant dams 1–2 days prior to shipment to our facility. Prior to this procedure, rats were anesthetized with a combination of ketamine (43 mg/kg) and xylazine (8.7 mg/kg) given by intraperitoneal injection. A guide cannula (22 gauge) was stereotaxically implanted in each rat with the end of the cannula positioned just above a lateral cerebral ventricle (coordinates: 0.8 mm posterior and 1.5 mm lateral from bregma and 4.8 mm down from the top of the skull). Each guide cannula was fixed in position by cranioplastic cement anchored to four steel screws implanted in burr holes cut in the rat's skull. Obturators were then inserted to maintain patency of the guide cannulae. Cannulation supplies were obtained from Plastics One (Roanoke, VA).

The accuracy of lateral ventricular cannulae was tested in two ways. First, at least 5 days after final maternal behavior observations and removal of pups from dams' home cages, self-grooming was measured in each animal after intracerebroventricular infusion of 400 ng of OT in 5 μl of buffered normal saline (NS) [5]. Self-grooming was scored in the animal's home cage during 10-s periods at 1-min intervals for 30 successive minutes beginning 15 min after intracerebroventricular treatment. One point was given for each 10-s period during which self-grooming was exhibited (maximum possible score = 30). Rats infused intracerebroventricularly with NS rarely score higher than 6 in this test. Second, after all tests were completed, animals were deeply anesthetized and then infused intracerebroventricularly with 5 μl of concentrated Evans blue dye. Their brains were then removed, frozen and sectioned to examine the cerebral ventricles. Only data from animals that exhibited an OT-stimulated self-grooming score of 12 or greater and a good distribution of dye throughout the lateral and third cerebral ventricles were included in analyses.

2.3. Intracerebroventricular infusions

Obturators were removed and reinserted once on pregnancy day 20 to confirm guide cannulae patency. Otherwise, dams were left undisturbed until they received their intracerebroventricular infusions. These were administered through 26-gauge cannulae that were cut to extend 1 mm beyond the end of the guide cannulae so that infusions could be made directly into the lateral cerebral ventricle. Each infusion was administered slowly over a 1-min period using a 10-μl Hamilton microsyringe connected by a length of PE50 tubing to the injection cannula. Obturators were reinserted after each intracerebroventricular infusion to maintain guide cannulae patency.

2.4. Procedures

Shortly after parturition, litters were culled to 10 pups with as even a sex distribution as possible. Beginning at 1000 h on postpartum day 2 (five dams) or 3 (five dams), events in each dam's home cage were recorded continuously for 105 min on a 120-min VHS videocassette through the transparent 46 cm side wall of the cage by a Samsung camcorder mounted on a tripod. Each camcorder was adjusted so that the cage from which it was recording completely filled the camcorder field of view. A mirror was placed against the long side of each maternity cage opposite to the camcorder so the reflection was recorded along with events in the cage. Each mirror, which was large enough to cover the entire side of the maternity cage, was tilted so that the reflection of events inside the cage was from a somewhat elevated angle allowing a clear view of behavior even when the dam was turned away from the camcorder and adjacent to the back wall of the cage.

Each animal was then removed from its cage and infused intracerebroventricularly with 1 μg of the OTA, $d(CH_2)_5$-[$Tyr(Me)^2$,Thr^4,$Tyr\text{-}NH_2^9$] ornithine vasotocin (OTA, [7]), dissolved in 5 μl of buffered NS or NS vehicle alone and then returned to its home cage. This dose of OTA has been shown to occupy most central OT receptors for 6 h or more after intracerebroventricular infusion of a single 1 μg dose [45]. On each test day, five animals were successively videotaped and infused intracerebroventricularly at 5-min intervals: three animals received one intracerebroventricular treatment and two animals received the other given in random order. Pups were not disturbed during removal or return of dams. Events in each home cage were again videotaped for 105 min beginning 2 h after intracerebroventricular treatments.

These procedures were repeated for the first five dams on postpartum day 6 and for the second five dams on postpartum day 7, except intracerebroventricular treatments were reversed. All procedures were conducted during the light phase.

2.5. Behavioral measurements

During behavior measurements, videocassettes were played on an RCA videocassette recorder (model VR724HF) and displayed on a 25.5-in. (64.8 cm) diagonal Zenith color television (model A25A23W). The 54.5-cm diameter of the image on this television resulted in a slight magnification of events in each 46 cm long maternity cage. A modified frequency coding system [23] using a 15-s interval was employed to score behaviors of interest from the videotapes. The time display on the television screen generated by the VCR during playback was used to ascertain the beginning and end of each 15-s interval. As is described below, brief licking bouts had to be <2 s and nursing posture bouts had to be ≥ 4 s. The duration of each bout in question was measured in real time using a stop watch after careful analysis in slow motion to determine the beginning and end of the bout. We recorded all of the behaviors described below that occurred during each of the 15-s intervals over each 105-min observation period. The score for each of these behavioral categories for each observation period was the number of 15-s intervals during which the animal exhibited that behavior. The maximum possible score during a 105-min period was 420. Behavior was recorded on spreadsheets that were divided into rows, one for each behavior category. Rows were further divided by vertical lines into 40 boxes that represented sequential 15-s intervals. Each behavior that was exhibited during each 15-s interval was coded by placing a check in the appropriate box. Ten minutes of videotape could be coded on each spreadsheet. Therefore, 10.5 sheets were used to record events during each 105-min videotaped observation period.

Animals and videotapes were labeled so behavior measurements could be made blind to treatment. Blindness was compromised, however, by involvement of CAP in intracerebroventricular infusions and coding of tapes (due to limited experience in these areas by other personnel at that time), as well as obvious differences between some animals in the two treatment groups in frequency of self-grooming. Fortunately, blind-to-treatment recoding of 10 videotapes by CAP more than 6 months after the initial coding (see Behavior coding reliability) found a high degree of consistency in behavior measurement.

Definitions of behaviors exhibited by the dams and criteria for scoring each during 15-s intervals were as follows. Pup licking: licking pups for 2 s or longer. Because tongue contact with pups could not always be seen, rhythmic up and down movement of the dam's head with her snout directed toward and adjacent to pups was sufficient to score this behavior. Using these criteria, differentiation from pup sniffing cannot be certain. However, in our observations at closer range, pup sniffing almost always involves lateral, back and forth scanning movements of the head rather than rhythmic up and down movements. Brief licking: licking pups for less than 2 s; bouts must occur two or more seconds apart from pup-licking bouts. Self-grooming: self-directed licking, mouthing or other oral manipulation. Scratching: self-directed hindleg scratching; licking or mouthing of a hindpaw during scratching bouts is not scored as self-grooming. Elevated upright posture over pups: the dam is in an upright, ventroflexed posture with her ventrum elevated off of the floor of the cage. She supports her weight with front and/or hindlegs, her hindlegs remain stationary and she has at least one pup under her. This posture must be held for at least 4 s. The dam may engage in other activities such as pup licking, eating, self-grooming and nest building while in the elevated upright posture. This behavior includes hovering, low crouch, high crouch and partial kyphosis as defined by Stern [20,21,36]. Prone on pups: the dam is in an upright posture over pups but is lying on them and not supporting her weight with front or hindlegs for at least 4 s. Supine nursing: the dam is nursing one or more pups for at least 4 s while lying on her side or back. Retrieving: the dam carries a pup in her mouth or otherwise moves a pup from outside into the nest. Carrying: the dam carries a pup in her mouth from one position to another outside of the nest. Regrouping: the dam carries a pup in her mouth from one position within the nest to another in-nest position. Nest building: moving wood chip bedding toward the nest from outside of the nest or moving wood chips from the center to the walls of the nest. Locomoting: moving all four legs while outside the nest. In addition, eating, drinking and rearing were scored.

During each 15-s interval, it was also recorded whether the dam was in or out of the nest and whether all, some or none of the pups were in the nest. To be scored as out of the nest, the dam's body, other than her tail, or pups had to be outside of the plane extending perpendicularly from the top of the wall of the nest. Dams or pups could be scored as in and out of the nest within a 15-s interval if their location changed during the interval. Nest quality was not measured because only 1.5 cm of wood chip bedding was provided to prevent dams from building high nest walls that would interfere with behavior observations.

We measured the number of 15-s intervals that were entirely embedded within kyphotic nursing bouts as defined by Stern and Keer [40]: periods of two or more minutes during which dams maintained an elevated, upright posture and were continuously suckled by four or more pups. Milk ejection is more likely to occur during nursing bouts that meet these criteria [34,39]. To identify nursing bouts, we first examined behavior coding sheets for periods of eight or more consecutive 15-s intervals (i.e., 2 min or longer) during which elevated upright posture over pups and no other behaviors were coded. Videotape records were then reviewed to discern those periods during which dams remained quiescent (exhibiting only occasional slight leg and head movements) in an elevated upright posture over at least four nursing pups throughout the entire period. The minimum interbout interval was one 15-s interval during which this posture was not maintained.

Stretch responses exhibited by suckling pups were counted during each observation period conducted on post-

partum days 6/7 as an indirect measure of milk ejection frequency. This was not done on postpartum days 2/3 because it is difficult to reliably detect stretch responses in pups this young.

2.6. Behavior coding reliability

All behavior measurements were made from videotapes. To assess intraobserver reliability of measurements over time, 10 videotapes were recoded blind to treatment group more than 6 months after the original coding and the results compared. An overall average of 92% agreement between the initial and second coding of videotapes was obtained for the behavior categories used in this study. The lowest reliability in any individual category was 86% for pup licking.

2.7. Statistical analyses

Behavioral data from the experimental subjects were analyzed with a three-way repeated measures analysis of variance (ANOVA) with order (OTA on postpartum day 2/3 or OTA on postpartum day 6/7) as the between-subjects variable and day (postpartum day 2/3 or postpartum day 6/7) and time (before or after intracerebroventricular treatment) as the within-subjects variables. Effect of treatment was embedded in day because half the animals received OTA on postpartum day 2/3 while the other half received OTA on postpartum day 6/7. Therefore, this ANOVA was used only to test for three-way interaction effects to address the hypothesis that behavior changed from before to after treatment for OTA but not NS. Significant three-way interactions were further investigated with Bonferroni post hoc tests to determine significant effects.

Some behaviors occurred infrequently and were dropped from further analysis. Analyses were conducted on pup licking, brief licking, self-grooming, elevated upright posture over pups, prone posture over pups, supine nursing, nest building, scratching, eating/drinking, locomoting and rearing. In addition, pup licking as a proportion of total oral grooming (pup licking + self-grooming) and elevated upright posture over pups as a proportion of total upright posture (elevated upright posture + prone over pups) were also computed and subjected to ANOVAs. The frequency of oral grooming periods and pup stretch responses was also analyzed. Finally, a three-way repeated measures ANOVA was conducted on the total number of 15-s intervals in the periods of quiescent, upright nursing identified as described above.

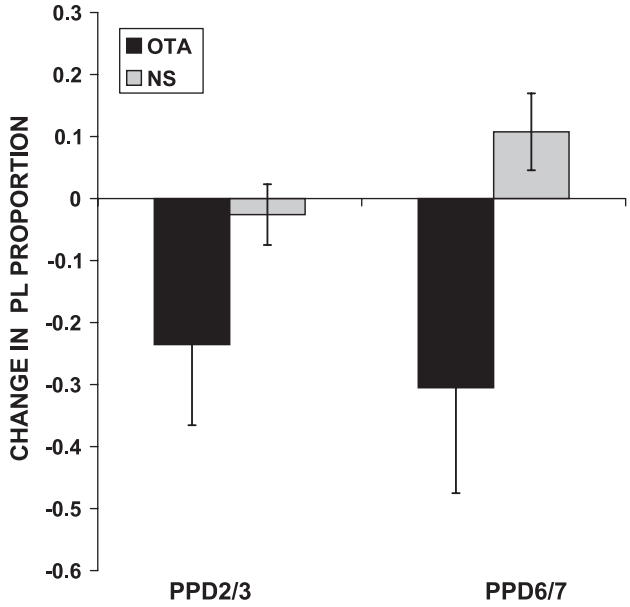

Fig. 1. Mean (± S.E.M.) change in pup licking (PL) as a proportion of total oral grooming in lactating rat dams after intracerebroventricular administration of 1 μg of OTA or buffered NS vehicle on postpartum days 2/3 (PPD2/3) and PPD6/7. Compared to NS, OTA significantly decreased pup-licking proportion.

3. Results

Table 1 contains the mean (± S.E.M.) pre- and post-intracerebroventricular treatment scores for each behavior

Table 1
Mean modified frequencies and proportions (± S.E.M.) of dams' behavior during 105-min observation periods before and after intracerebroventricular administration of 1 μg OTA or NS

Behaviors	Postpartum day 2/3				Postpartum day 6/7			
	OTA		NS		OTA		NS	
	Before	After	Before	After	Before	After	Before	After
PL frequency	86 ± 8	54 ± 5	73 ± 2	77 ± 11	49 ± 7	27 ± 7	78 ± 15	71 ± 16
SG frequency	33 ± 5	67 ± 20	27 ± 7	32 ± 7	14 ± 2	46 ± 23	33 ± 4	19 ± 7
PL proportion	0.73 ± 0.04	0.50 ± 0.08	0.74 ± 0.04	0.72 ± 0.05	0.77 ± 0.04	0.46 ± 0.12	0.68 ± 0.06	0.79 ± 0.04
BL frequency	7 ± 2	26 ± 5	6 ± 2	6 ± 1	6 ± 2	12 ± 4	4 ± 2	7 ± 3
EUP frequency	370 ± 10	344 ± 22	377 ± 11	355 ± 21	355 ± 22	233 ± 14	275 ± 16	301 ± 31
PP frequency	43 ± 10	96 ± 31	40 ± 10	45 ± 14	31 ± 12	157 ± 22	107 ± 26	69 ± 21
EUP proportion	0.90 ± 0.03	0.79 ± 0.07	0.90 ± 0.02	0.89 ± 0.03	0.92 ± 0.03	0.60 ± 0.04	0.73 ± 0.05	0.81 ± 0.06

Frequencies are numbers of 15-s intervals out of 420 in each observation period.
PL = pup licking, SG = self-grooming, BL = brief (< 2 s) pup licking, EUP = elevated upright posture, PP = prone posture, PL proportion = PL/PL + SG, EUP proportion = EUP/EUP + PP.

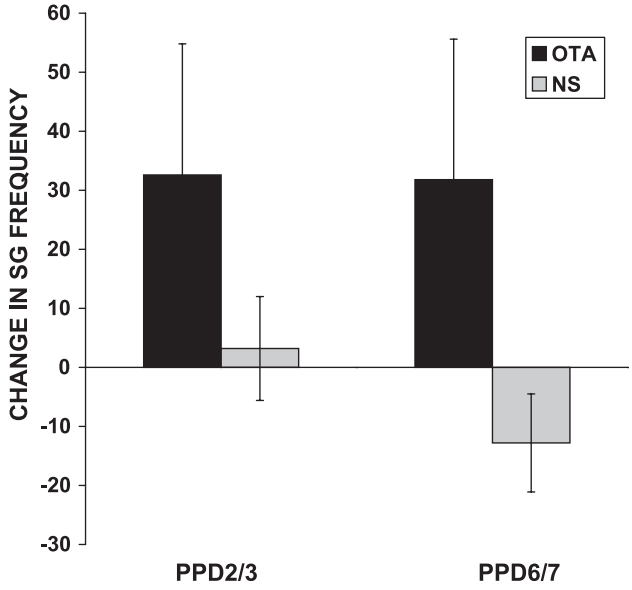

Fig. 2. Mean (± S.E.M.) change in frequency of self-grooming (SG) in lactating rat dams after intracerebroventricular administration of 1 μg of OTA or buffered NS vehicle on postpartum days 2/3 (PPD2/3) or PPD6/7. Compared to NS, OTA significantly increased self-grooming.

category for OTA- and NS-treated rats on postpartum day 2/3 and postpartum day 6/7. Pup licking decreased more after intracerebroventricular infusion of OTA than after intracerebroventricular NS, although the effect of OTA only approached significance [$F(1,8) = 4.168$, $P = .075$; Table 1]. Pup licking as a proportion of total oral grooming (pup licking + self-grooming) dropped significantly after intracerebroventricular OTA but not after intracerebroventricular NS [$F(1,8) = 17.184$, $P = .003$; Fig. 1]. The rise in this variable was, in part, the consequence

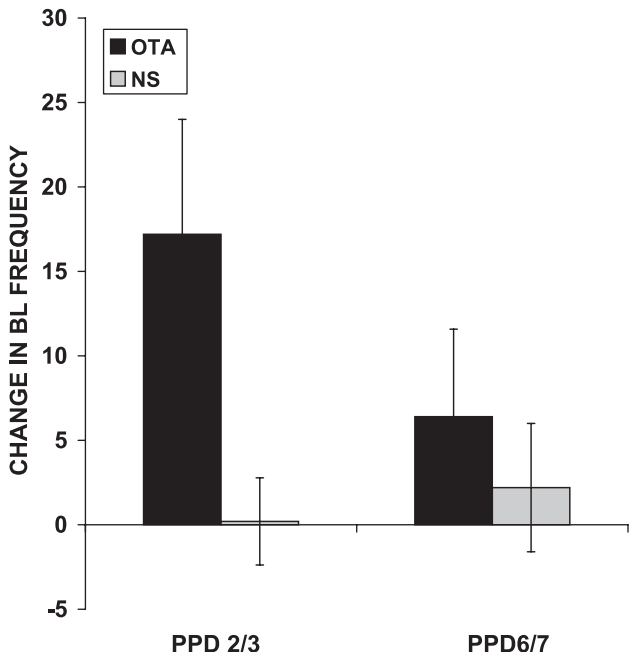

Fig. 3. Mean (± S.E.M.) change in frequency of brief (< 2 s) pup-licking (BL) bouts after intracerebroventricular administration of 1 μg of OTA or buffered NS vehicle (5 μl) on postpartum days 2/3 (PPD2/3) and PPD6/7. Compared to NS, OTA significantly increased the frequency of brief licking bouts directed toward pups.

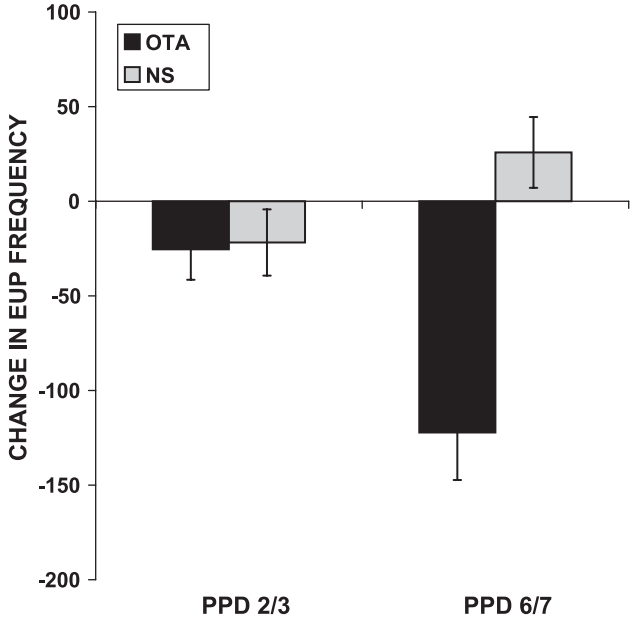

Fig. 4. Mean (± S.E.M.) change in frequency of elevated, upright posture over pups (EUP) after intracerebroventricular administration of 1 μg of OTA or NS vehicle on postpartum days 2/3 (PPD2/3) and PPD6/7. Compared to NS, OTA significantly decreased the frequency of elevated, upright posture over pups.

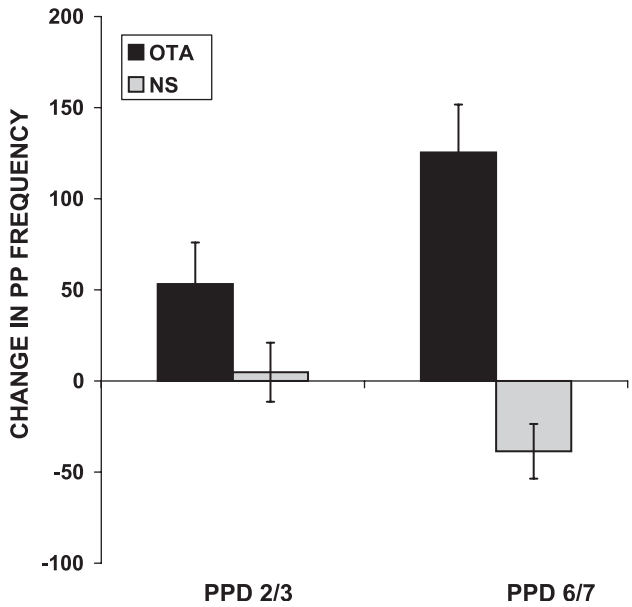

Fig. 5. Mean (± S.E.M.) change in frequency of prone posture over pups (PP) after intracerebroventricular administration of 1 μg of OTA or NS vehicle on postpartum days 2/3 (PPD2/3) and PPD6/7. Compared to NS, OTA significantly increased the frequency of prone posturing.

of a significant increase in self-grooming scores after OTA but not after NS administration [$F(1,8) = 6.593$, $P = .033$; Fig. 2]. Changes in total oral grooming scores were not significantly different between treatment groups [$F(1,8) = 0.316$, $P = .589$]. OTA but not NS treatment also significantly increased brief pup-licking scores [$F(1,8) = 9.435$, $P = .015$; Fig. 3]. When brief licking was scored as pup licking, changes in pup-licking scores as a proportion of total grooming scores after intracerebroventricular infusions were still significantly different between OTA and NS treatments [$F(1,8) = 14.623$, $P = .005$].

Upright posture over pups was significantly altered by central OTA administration. Scores for elevated upright posture over pups decreased significantly [$F(1,8) = 15.965$, $P = .004$; Fig. 4], and scores for prone posture increased significantly [$F(1,8) = 27.604$, $P = .001$; Fig. 5] following OTA but not NS administration on postpartum day 6/7. Consequently, elevated upright posture as a proportion of total upright posture over pups decreased significantly following OTA but not NS [$F(1,8) = 33.242$, $P < .001$; Table 1]. The decline in elevated upright posture scores after OTA administration was not related to the rise in self-grooming scores. When data from intervals in which self-grooming occurred were dropped from the analysis, elevated upright posture over pups scores still declined significantly after OTA but not after NS [$F(1,8) = 18.754$, $P = .003$]. When the analysis was restricted to 15-s intervals embedded within 2 min or longer quiescent nursing bouts, scores also decreased significantly after OTA but not after NS [$F(1,8) = 7.579$, $P = .025$; Table 2].

The frequency of stretch responses by suckling pups did not change significantly after intracerebroventricular infusion of OTA or NS on postpartum days 6/7 [$F(1,4) = 0.104$, $P = .76$: pre-OTA 19.3 ± 3.3, post-OTA 20.7 ± 1.7; pre-NS 15.5 ± 2.5, post-NS 15.5 ± 1.5].

In summary, intracerebroventricular administration of OTA, but not NS, significantly decreased pup licking as a proportion of total oral grooming, increased the scores for self-grooming and brief (< 2 s) pup-licking bouts, decreased the scores for total and within-nursing bout (≥ 2 min) elevated upright posture over pups and increased the score for prone posture over pups. Scores for other behaviors were not significantly affected by OTA treatment.

Table 2
Mean modified frequencies (± S.E.M.) of quiescent upright nursing before and after intracerebroventricular administration of 1 μg OTA or NS

	Postpartum day 2/3		Postpartum day 6/7	
	Before	After	Before	After
OTA	239 ± 10	189 ± 26	277 ± 20	150 ± 20
NS	277 ± 11	227 ± 37	130 ± 21	203 ± 16

Frequencies are the numbers of 15-s intervals out of 420 in each observation period that were embedded within nursing bouts ≥2 min in duration.

4. Discussion

The effects of intracerebroventricularly administered OTA reported above suggest that central OT continues to enhance pup licking and elevated upright posture over pups well after the immediate postpartum period when OT facilitates the onset of all components of maternal behavior. Previous studies unanimously found that central administration of OT antiserum or antagonists, or lesioning the hypothalamic paraventricular nucleus, the origin of almost all OT projections within the brain, delayed or prevented the initiation of all components of maternal behavior in parturient or ovarian steroid-treated rats [8,16,31,32,44]. On postpartum days 4–5, however, intracerebroventricular infusion of OTA or paraventricular nucleus lesioning failed to eliminate any component of maternal behavior [8,28]. These findings led to the conclusion that central OT contributes significantly to the postpartum and ovarian steroid-induced initiation of maternal behavior but plays no role in maintaining established maternal behavior [8,27,29]. Unfortunately, the behavior measurements employed in these studies were only sensitive enough to detect very large changes in maternal behavior. In postpartum day 5 dams, Fahrbach et al. [8] scored maternal and other behaviors every 5 min during two 1-h observation periods following intracerebroventricular administration of OTA or NS. Numan and Corodimas [28] scored the presence or absence of nursing behavior every minute during a single 20-min period each day prior to and after PVN lesioning on postpartum day 4. In contrast, the 420 observations made during each 105-min observation period in the current study provided sufficient sensitivity and statistical power to demonstrate significant 20–40% declines in pup-licking proportion of total oral grooming and elevated upright posture over pups after intracerebroventricular administration of OTA.

OT knock-out mouse mothers have been reported to exhibit all components of maternal behavior [26]. Nulliparous C57BL mice, the strain in which the OT gene knockout was generated, rapidly exhibit vigorous maternal behavior toward young pups. The lack of qualitative postpartum change in maternal responsivity in this mouse strain makes it impossible to draw conclusions about the role of OT in the dramatic shift from infanticidal to nurturing responses to pups that occurs in parturient wild mice [35]. Nishimori et al. [26] found no differences in retrieval, time in nest or pup grooming between homozygous and heterozygous OT knock-out dams, suggesting that OT does not influence frequencies of maternal behavior in this strain of mice. Unfortunately this report includes no description of how maternal behavior was measured so interpretation of the results is difficult.

Rat dams intermittently exhibit sustained periods of oral grooming. When in the nest, the focus of dams' licking and grooming typically oscillates back and forth between pups and themselves. The observation that perioral anesthesia decreases pup licking but increases self-grooming [38,41]

adds to the impression that these oral grooming behaviors are interrelated in rat mothers. In the current study, intracerebroventricularly administered OTA decreased the frequency of longer pup-licking bouts (≥ 2 s) as a proportion of total oral grooming, increased the frequency of short pup-licking bouts (<2 s) and increased the frequency of self-grooming. These results suggest that central OT may shift the balance in dams' oral grooming behavior toward pups and away from themselves and may prolong pup-licking bouts. Our findings appear to conflict with reports that intracerebroventricular administration of OT increased self-grooming in adult male and nulliparous female rats [5,30,43]. Intracisternal injection of OT, however, in pre-weaning juvenile rats, which readily exhibit maternal behavior toward young pups [2,24], increased both their self-grooming and pup licking [33]. Pup stimuli may alter central mechanisms mediating OT effects on grooming behavior, especially in animals in which maternal motivation is high. OT stimulation of postpartum maternal behavior and self-grooming has been localized to the medial preoptic area, ventral tegmental area and nucleus accumbens [6,17,32]. These may also be sites in which OT regulates pup licking and/or the balance between pup licking and self-grooming in lactating dams.

Lactating dams usually nurse pups in an upright posture throughout lactation, although supine nursing increases as pups grow older. For a variable period after initiating an upright posture over pups, dams typically lick the pups or engage in other motorically active behaviors. Stern [36] referred to this as hovering. Somatosensory stimulation of its ventral trunk by suckling pups gradually lulls the dam into quiescence and promotes kyphotic posturing [36,37,39,40,42]. During upright nursing, the degree of kyphosis and elevation of the dam's ventrum shifts up and down. Dams intermittently rise up into an exaggerated kyphosis or high crouch during which their ventral trunk is quite elevated. Abrupt arching into this posture usually precedes milk ejection. Dams then gradually relax into a less pronounced kyphosis or low crouch in which their ventrum is less elevated. Often, dams' upright posture will slump further until they are lying prone on pups and are no longer bearing weight on their legs. Shifts from lower to higher upright postures occur with regularity as well. OT release and milk ejection are more likely to occur in dams that maintain quiescent kyphotic postures over four or more suckling pups for two or more minutes [39]. Shorter bouts of elevated upright posturing over pups are not uncommon though.

In this study, we examined the frequency of two distinct levels of elevated upright posturing over pups. The first included posturing for four or more seconds over one or more pups. The second was restricted to quiescent nursing bouts at least 2 min in length during which dams were suckled by at least four pups. We found that intracerebroventricularly administered OTA decreased frequencies of both levels of elevated upright posturing and increased prone posturing on postpartum days 6/7 but not postpartum days 2/3. These results suggest that central OT may facilitate all postures over pups in which the dam's ventrum is raised, including sustained crouched nursing. This mechanism may play an increasingly significant role in elevated upright posturing as pups grow and the dam must raise her ventrum higher to accommodate nursing. Kyphotic nursing is triggered by pup somatosensory stimulation of the dam's ventrum [36,37,39,40,42]. Central OT release increases during nursing [25], but it is unclear whether this is produced by nipple stimulation alone, as is peripheral OT release, pup somatosensory stimulation of the ventrum in general or other pup stimuli. OT immunostaining fibers as well as OT receptor mRNA (but not OT binding) are located in the rat periaqueductal gray [12], an area that has been implicated in the regulation of kyphotic nursing [19–21,42].

Milk ejection is facilitated by OT release in the hypothalamic paraventricular nucleus as well as the lateral septum and bed nucleus of the stria terminalis [11–14]. Therefore, central administration of OTA has the potential to suppress milk ejection, which could alter pup behavior and thereby maternal behavior [36,40]. A decline in milk ejection frequency probably did not contribute to the decline in pup licking and elevated upright posture over pups that we found after intracerebroventricular infusion of OTA because this treatment did not alter the frequency of pup stretch reflexes, an index of milk ejection rate. We cannot rule out OTA-decreasing milk ejection volume, which we did not monitor because pretreatment removal of pups for weighing could have influenced dams' behavior [1,3,18]. If OTA had decreased milk volume, we would expect an increase in kyphosis rather than the decline we observed because hungrier pups would root more in search of milk and produce greater somatosensory stimulation of the dam's ventrum [40].

Testing only a single dose of OTA is a significant weakness of the current study. Also, while OTA is most effective at blocking OT receptors, it is a relatively potent V1a receptor antagonist [22]. So OTA inhibition of pup licking and elevated upright posturing over pups could be the result of combined OT and V1a receptor antagonism or, less likely, V1a receptor antagonism alone. Previously, we found that centrally administered V1a antagonist significantly disrupted the postpartum onset of maternal behavior although not as effectively as OTA [32].

Our findings are consistent with recent evidence that OT is involved in the intergenerational transmission of similar levels of maternal behavior. The frequency of maternal licking and "arched-back" nursing received during infancy determines how frequently lactating rat dams' direct these maternal behaviors toward their own pups as well as OT receptor concentrations in those areas of their brains implicated in OT activation of maternal behavior [4,9,10,15,32]. In addition, the high frequency of pup licking exhibited by dams that had received high frequencies of maternal licking was significantly lowered by intracerebroventricularly ad-

ministered OTA [4], a result that is similar to our present findings. While arched-back nursing was not clearly described in previous reports, we are told by one of the authors (Plotsky, personal communication) that it is similar to high crouching as defined by Stern [36]. Because the nursing bouts identified in the current study included both low crouching and high crouching, it remains to be determined whether OTA would inhibit one or both of these categories of upright nursing. Nevertheless, our results do suggest that central OT may facilitate pup-licking and arched-back nursing, those components of maternal care that determine maternal behavior and central OT receptor expression during lactation in female offspring.

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