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OXYTOCIN RECEPTORS IN THE NUCLEUS ACCUMBENS FACILITATE "SPONTANEOUS" MATERNAL BEHAVIOR IN ADULT FEMALE PRAIRIE VOLES

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Abstract—Oxytocin and the nucleus accumbens have been extensively implicated in the regulation of maternal behavior, and the processing of pup-related stimuli relevant for this behavior. Oxytocin receptor density in the nucleus accumbens is highly variable in virgin female prairie voles, as is their behavioral response to pups, ranging from neglecting and infanticidal to full maternal behavior. We hypothesized that oxytocin receptor in the nucleus accumbens facilitates the expression of "spontaneous" maternal behavior in prairie voles. Forty sexually-naive adult females were exposed to pups for the first time and tested for maternal behavior. Oxytocin receptor binding in the nucleus accumbens and other brain regions was later determined using autoradiography. Females that showed maternal behavior (lick and groom the pups and hover over them for at least 30 s, $n=24$) had higher oxytocin receptor density in the nucleus accumbens (shell subregion) ($P<0.05$) than females that did not show maternal behavior or attacked the pups ($n=16$). No differences were found in other brain regions (medial preoptic area, septum, prelimbic cortex).

In a second experiment, we tested whether infusions of the oxytocin receptor antagonist ($d(CH_2)_5^1$,Tyr(Me)^2,Orn^8)-AVT into the nucleus accumbens would block "spontaneous" maternal behavior. As a control region, oxytocin receptor antagonist was also infused into the caudate putamen. Ten females were infused bilaterally into the nucleus accumbens or caudate putamen with either 2 ng/0.5 μl of oxytocin receptor antagonist or CSF (vehicle). While five of 10 nucleus accumbens CSF-infused animals showed maternal behavior, none of the nucleus accumbens oxytocin receptor antagonist-infused subjects did (0/10; χ^2, $P<0.01$). Nucleus accumbens oxytocin receptor antagonist-infused females recovered the next day and were not different from controls. Animals infused with CSF or oxytocin receptor antagonist into the caudate putamen did not differ (four/10, four/10). This is the first study to show that the nucleus accumbens is involved in the regulation of "spontaneous" maternal behavior and that oxytocin receptor in this brain region facilitates maternal responses.

Key words: aversion, crouching, pups, *Microtus ochrogaster*.

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Abbreviations: CP, caudate putamen; LS, lateral septum; MPOA, medial preoptic area; NA, nucleus accumbens; OTA, oxytocin antagonist; OTR, oxytocin receptor; PLC, prelimbic cortex.

Prairie voles (*Microtus ochrogaster*) display biparental and high levels of affiliative behavior (Thomas and Birney, 1979; Getz et al., 1981; McGuire and Novak, 1984; Salo et al., 1993; DeVries et al., 1997; Olazábal and Young, 2005). Interestingly, approximately half (~55%) of naïve adult female prairie voles show "spontaneous" maternal behavior when first exposed to pups (Roberts et al., 1998; Olazábal and Young, 2005). These females lick, groom and hover over the pups immediately after the first exposure. However, ~45% of adult female prairie voles either ignore/neglect the pups or display infanticidal behavior (Roberts et al., 1998; Lonstein and DeVries, 2000, 2001; Olazábal and Young, 2005).

Several studies show that oxytocin facilitates positive social interactions, including maternal behavior, in several species (Pedersen and Prange, 1979, 1985; Insel, 1992; Keverne and Kendrick, 1994; Uvnas-Moberg, 1998; Cho et al., 1999; Carter, 2003). In fact, oxytocin has been proposed to facilitate the process of bringing conspecifics into close proximity for the formation of a social bond (Insel, 1992; Carter, 2003). If this is the case, oxytocin may also be critical in the initiation of contact between naïve female prairie voles and pups, resulting in the expression of maternal behavior.

Although the role of the nucleus accumbens (NA) in regulating maternal behavior is still unclear, as is the case for the rest of the behaviors it modulates (Groenewegen et al., 1996; Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999; Robbins and Everitt, 2002; Salamone and Correa, 2002; Berridge, 2003; Kelley, 2004; Numan et al., 2005), there is evidence that the NA is involved in promoting maternal responses and the processing of pup-related stimuli (Hansen et al., 1993; Lonstein et al., 1998; Keer and Stern, 1999; Li and Fleming, 2003ab; Numan and Insel, 2003; Champagne et al., 2004; Olazábal and Morrell, 2005).

Previous studies suggest that differences in oxytocin receptor (OTR) distribution may be related to the species-typical pattern of social behavior, in particular affiliative behavior (Insel and Shapiro, 1992). We have recently found that OTR distribution may also be related to the presence or absence of juvenile maternal behavior (also called alloparental behavior) across species (Olazábal and Young, 2006). Juvenile female meadow voles and mice do not show positive responses to pups at the age ~20 days while juvenile female rats display maternal responses only after 2–3 days of pup exposure. These three species have very low levels of OTR in the NA. In contrast, juvenile prairie voles show spontaneous alloparental behavior and

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have significantly higher density of OTR in the NA. In addition, prairie voles show significant individual variability in OTR binding in the NA (Young, 1999), and this binding is positively correlated with the quality of alloparental behavior displayed by juvenile females (Olazábal and Young, 2006).

Given this relationship between OTR in the NA and juvenile maternal behavior across species and within juvenile prairie voles, we hypothesized that OTR in the NA plays a role in regulating adult female "spontaneous" maternal behavior. In the present study we investigated whether OTR binding in the NA of adult, naïve female prairie voles is associated with the response to pups during a 15 min interaction test. Other brain regions with high density in OTR or implicated in maternal or social behavior, such as lateral septum (LS, Numan and Insel, 2003), medial preoptic area (MPOA, Numan et al., 2005), and prelimbic cortex (PLC, Young et al., 2001), were also investigated. In a second experiment we directly tested the role of NA OTR in the regulation of spontaneous maternal behavior by infusing OTR antagonist into the NA and a control site (caudate putamen, CP).

EXPERIMENTAL PROCEDURES

Subjects

All subjects were naïve female prairie voles from our colony maintained at the Yerkes Laboratory Animal Facility at Emory University. This facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Prairie voles are regularly weaned in our animal facility at age 19–21 days, and maintained in same-sex groups of two to three in cages 28×17×13 cm with transparent Plexiglas walls under a 12-h dark/light cycle and a stable environmental temperature of 22 °C with access to food (LabDiet® rabbit, Purina, Richmond, IN, USA) and water *ad libitum*. Bed-ócobs® Laboratory Animal Bedding (OH, USA) was used as bedding material.

All procedures used in this study have been conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and approved by the Institutional Animal Care and Use Committee of Emory University (IACUC). Every effort was made to minimize the number of animals used and their suffering. Additional adult lactating females, not included in the experiment, served as donors of pups for the maternal behavior test.

Maternal behavior test

Subjects were individually housed in a clean cage and allowed to habituate for 45–90 min before the maternal behavior test began. Maternal behavior test has been described elsewhere (Olazábal and Young, 2005) and will be briefly summarized here. Two pups (2–5 days old) were placed into the cage opposite to where the subject was located. The behaviors scored for 15 min included: number of animals that attacked pups, time spent licking and grooming, time hovering immobile over at least one pup (quiescence crouching) or doing other activities (active crouching). Our criteria for considering an animal maternal were that it licked the pups >5 s, spent >30 s adopting crouching postures over the pups, and never attacked the pups. Most of the maternal animals spend more than 8 min adopting crouching posture. However, those animals that licked and crouched at least 30 s over the pups did not ignore or avoid the pups, so they were also considered maternal. Animals that neither reached the criteria for maternal behavior nor attacked the pups during the 15 min test period were categorized as females that "ignored" the pups. Pups were removed from the cage at the end of the test, or immediately after being attacked by the subject, in order to avoid injury. Pups that received serious injuries, despite our precautions, were killed. Subjects that performed the attack were categorized as females that "attack" pups.

Experiment I

Brain tissue collection and radioligand receptor autoradiography. Subjects were 40 adults (60–90 days of age) exposed to pups as described above. Four days later, all animals were deeply anesthetized with Isofluorane (Novaplus™; Abbott Laboratories, IL, USA) and decapitated. Brains were removed from the skulls, frozen immediately on dry ice and stored at −80 °C until sectioned. A cryostat was used to obtain five serial sets of 20-μm-thick frozen sections of the brains. Sections collected extended from the olfactory nuclei to the caudal region of the basolateral amygdala and were mounted in separate Superfrost plus slides (Fisher, Pittsburgh, PA, USA) and stored at −80 °C until used for autoradiographic binding.

Slides were processed for receptor autoradiography using ^{125}I labeled OTR antagonist, [^{125}I]-ornithine vasotocin analog (NEN/PerkinElmer, ^{125}IOVTA, 2200 Ci/mmol) using standard procedures in our laboratory (Lim et al., 2004). On the day of receptor autoradiography, the sections were removed from the freezer and allowed to dry and equilibrate to room temperature for 1 h before the binding started. Sections were immersed in 0.1% paraformaldehyde in phosphate-buffered saline (pH 7.4) for 2 min at room temperature. Slides were then rinsed twice in Tris–HCl buffer (pH 7.4) and later incubated for 60 min in 50 pM ^{125}I-OVTA in Tris with 10 mM $MgCl_2$, 0.1% bovine serum albumin (RIA grade, fraction V, Sigma) and 0.05% bacitracin. Unbound ligand was removed by four washes in 50 mM Tris pH 7.4, 10 mM $MgCl_2$. The slides were finally quickly dipped in dH_2O and rapidly dried and exposed to BioMax MR film (Kodak, Rochester, NY, USA) along with ^{125}I autoradiographic microscale standards (Amersham Biosciences) for 48 h. To control for variability all slides were processed at the same time and sufficient amount of the solutions were prepared to use the same solutions for all slides. All further details are described elsewhere (Lim et al., 2004).

Quantitative analysis. The analysis of the autoradiography was performed by applying our standard previously published methods (Phelps and Young, 2003). Optical density readings were measured and converted to decompositions per minute (d.p.m./milligram tissue equivalent) based on I^{125} autoradiographic standards (Amersham Biosciences) by using our automated computer-based image analysis system and AIS™ software version 6.0 (Imaging Research Inc.).

OTR binding for the CP, LS, MPOA, NA (shell and core subregion), and PLC was measured using ~four (CP, LS, NA, and PLC) or two (MPOA) brain sections for each brain region and the average reading recorded. Background reading, taken from an adjacent area with no OTR binding, from two sections of each brain was also recorded and averaged. Specific binding was calculated subtracting the average background reading for each brain from the readings for all brain regions taken from that specific animal. All further details of our quantitative methods were carried out as previously specified in Phelps and Young (2003).

Experiment II

Oxytocin antagonist infusions into the NA and CP. Subjects were 40 adults (60–90 days of age). Animals were anesthetized with isofluorane (3%), placed in a Kopf stereotaxic apparatus and implanted with a 26-gauge double guide cannula (C235G, Plastics One Inc.) aimed at the NA (nosebar at −2 mm; 1.6 mm rostral, 0.85 mm lateral, and −4.5 mm ventral to the bregma) or CP

(1.4 mm rostral, 1.5 mm lateral, and −.3 mm ventral to the bregma). These coordinates were chosen based on pilot studies. The axis was moved down and the guide cannula inserted into two holes drilled in the skull following the NA or CP coordinates. The guide cannula was fixed using a drop of instant adhesive (Loctite^{R} 454 Prism^{R}) attached to part of the skull and the lateral part of the guide cannula. After the instant adhesive dried, the lower part of the cannula was covered by cranioplastic powder (Plastics One Inc.) mixed with fast curing acrylic liquid (Ortho-Jet Liquid; Lang Dental Manufacturing Co. Inc., IL, USA). The dental cement was allowed to dry for 10 min and then the guide cannula was separated from the holder. Immediately after surgery, females were injected with buprenorphine (0.01 μg/30 g; Reckitt Benckiser Pharmaceuticals Inc., Richmond, VA, USA) as an analgesic. Animals were allowed to recover for one week and then anesthetized again using isofluorane (3%) to place the 33-gauge needle (C235I, Plastics One Inc.) into the guide cannula. The needle extended 0.5 mm beyond the guide cannula into the brain and was connected to Hamilton syringes with PE-50 tubes (Plastics One Inc.). Ten animals were injected bilaterally either with the OTR antagonist d(CH_2)$_5$,[Tyr(Me)^2,Thr^4,Orn^8,Tyr^9-NH_2]-vasotocin (OTA) 2 ng/0.5 μl in CSF (ALZET^{R} artificial CSF) or vehicle (CSF). Two hours later these animals were exposed to pups for 15 min and tested for maternal behavior as described above. A previous study (Young, 1999) examining the role of the NA OTR in pair bonding found that a similar dose (1 ng/side) was effective when injected into the NA, but not the adjacent caudate region. This OTA has very high affinity for OTR and occupies the receptor for more than 10 h (Witt and Insel, 1991). After the behavioral test, animals were maintained in the same cages until next day when were tested again for maternal responses to pup exposure. Finally, all subjects were deeply anesthetized with isofluorane, killed, and the brains removed and sectioned to verify proper cannula placement. Verification of cannula placement was done by visualizing the location of the track left by the cannula using high magnification of fresh mounted sections. The most ventral portion of the track left by the cannula was drawn on photocopies of frontal plane diagrams of the brain.

Statistical analysis

For experiment I, all animals were categorized as being maternal or non-maternal (ignore or attack). OTR binding in the CP, LS, MPOA, NA (shell and core), and PLC was analyzed by *t*-test. Spearman rank correlation was applied to investigate whether a relationship existed between OTR binding and time spent in quiescence crouching posture in maternal females. Data from experiment II were analyzed by chi-square. Statistical significance was $P<0.05$. Data are expressed as means±S.E.

RESULTS

Experiment I

Relationship between OTR density in the brain and maternal response. Twenty-four of the 40 females reached our criteria for maternal behavior while the rest (16) did not show any maternal response. There was significant variability in the OTR binding mainly in the NA, and LS (Fig. 1). When maternal and non-maternal animals were compared, maternal females showed higher OTR density in the NA (shell subregion) than animals that did not show any maternal response ($P<0.05$) (Figs. 2, 3). OTR density in the core subregion of the NA did not reach statistical significance ($P=0.08$) but was higher in maternal than non-maternal females (Fig. 2). OTR density in the CP, MPOA, LS, and PLC was not different in maternal (1207±167; 222±30; 586±54; 1986±79 respectively) or non-maternal females (1057±141; 219±25; 551±59; 1927±73 respectively). The time spent adopting crouching posture also varied among the maternal females (Fig. 4). OTR binding in the shell and core subregions of the NA was positively correlated to time spent adopting quiescence crouching posture in maternal prairie voles (rho=.53, $P<0.01$; and rho=.60, $P<0.005$ respectively). There was a positive correlation between OTR binding in the CP and time adopting crouching posture in maternal prairie voles (rho=.56, $P<0.01$). There was no correlation between NA OTR density and licking and grooming (shell rho=−.029 $P>0.89$; core rho=.01 $P>0.96$) or time spent in active crouching (shell rho=−.25 $P>0.20$; core rho=−.18 $P>0.38$). No significant correlation was found between OTR binding in the MPOA, LS or PLC and quiescence crouching posture in maternal animals (rho=−.01, $P=0.95$; rho=−.05, $P=0.80$; rho=.3; $P=0.12$ respectively).

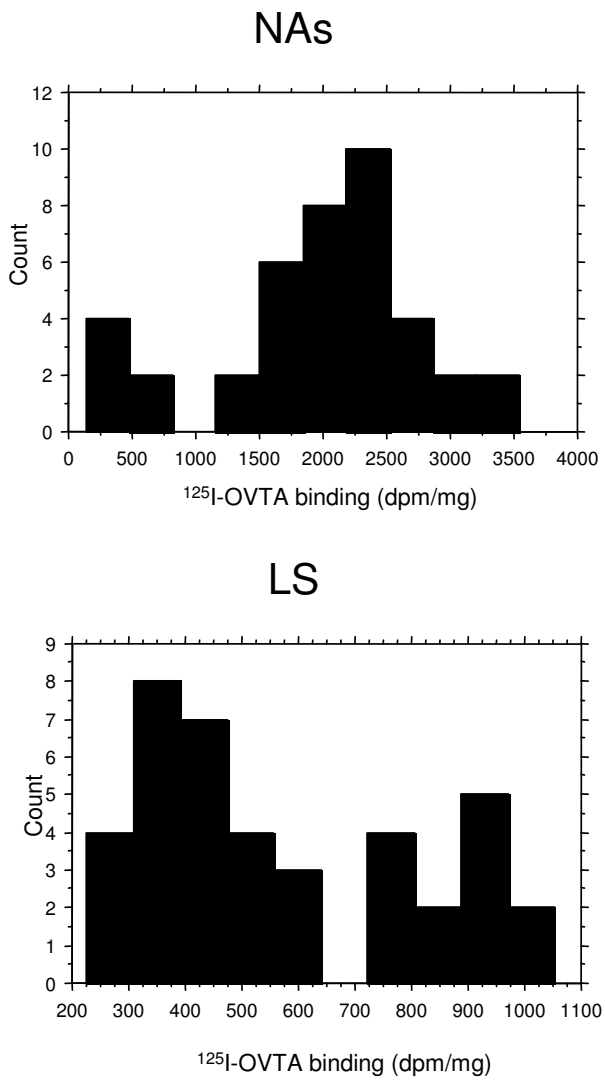

Fig. 1. Frequency distribution of OTR binding in adult female prairie voles. Histograms show the density of OTR binding in the shell subdivision of the nucleus accumbens (NAs) and the LS of the animals used in experiment I.

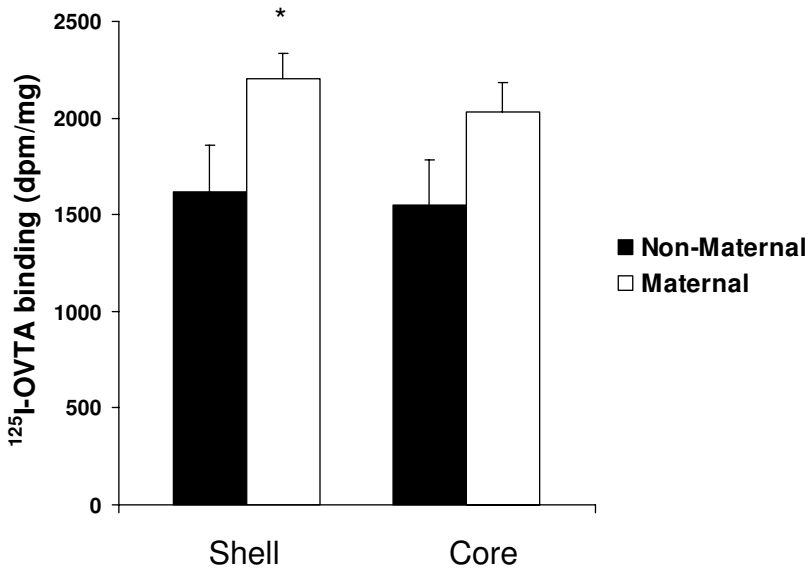

Fig. 2. OTR binding in maternal and non-maternal animals. OTR binding in the shell and core subdivisions of the NA was higher in maternal (white bars) than in non-maternal females (black bars). Data are expressed as means±S.E. (* $P<0.05$).

Experiment II

Oxytocin antagonist infusion into the NA, but not the CP, blocks spontaneous maternal behavior. Cannula placement analysis showed that all animals had cannula properly placed in the NA area as shown in Fig. 5. Cannula placement extended along the rostral and caudal NA. Females infused with OTA into the NA showed no maternal response while control CSF-infused females showed the previously described variability in maternal response; five maternal and five non-maternal (χ^2, $P<0.05$; Fig. 6). There was no difference between the groups in the latency to approach pups, number of approaches to pups, and time

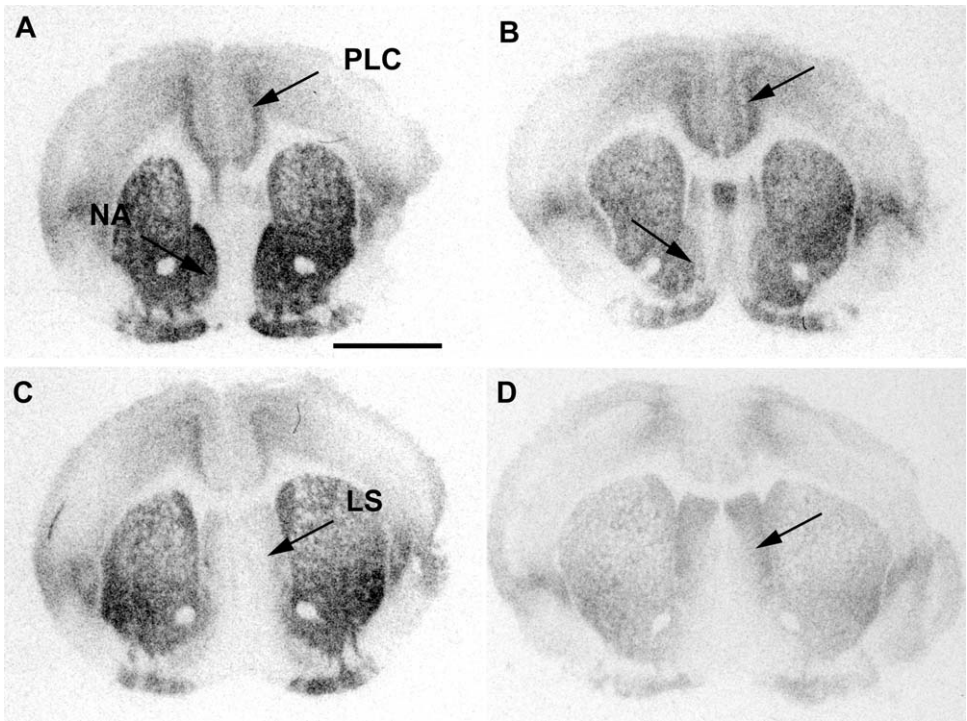

Fig. 3. Pictures of sample brain sections showing the autoradiographic signal for I^{125} OTA for two animals representative of the group of maternal (A, C) and non-maternal females (B, D). Note that while OTR binding is clearly higher in the NA of the maternal animal, no differences are seen in the PLC between maternal and non-maternal females. Note also that in these sections, the LS binding is higher in non-maternal females, demonstrating that the difference is not due to overall decrease in OTR binding or technical artifacts (C, D). Scale bar=2 mm.

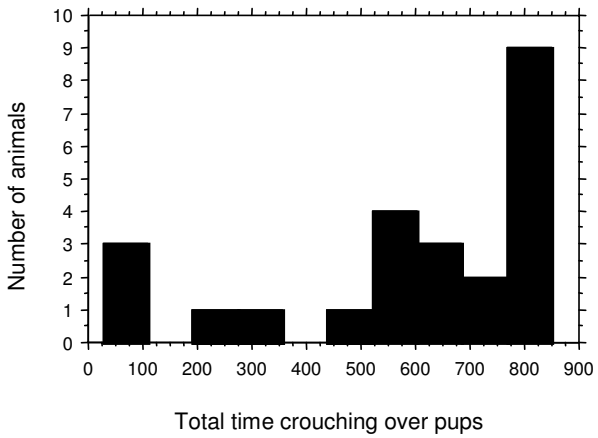

Fig. 4. Frequency distribution of time spent crouching over the pups. Histogram shows the time that maternal females from exp 1 spent crouching over the pups.

sniffing pups (Table 1). When the animals were tested on the next day, no difference was found between control and OTA infused groups, four in nine OTA-infused females licked and groomed the pups and adopted crouching posture for more than 5 min. One animal was not retested because its cannula was removed overnight. Five additional naïve pre-screened maternal females were also infused with a lower dose of OTA (0.5 ng/.5 μl) and all of them showed all the components of maternal behavior, licking and grooming the pups and crouching over them more than 5 min.

Appropriate cannula placement in the CP was also confirmed (Fig. 5). Two CP- and CSF-infused animals were excluded because cannula placement was too rostral and out of the striatum. The rest of the cannulae were distributed in the medial and lateral CP. Females infused with OTA or CSF into the CP did not differ in their response to pups, except for their latency to approach to them (Table 1). Both groups showed the normal variability in maternal behavior (four/10 maternal in CSF and four/10 maternal in OTA; Fig. 6). Maternal animals infused with CSF and OTA only differed in their latency to approach to pups (10 ± 6 and 83 ± 43 respectively, $P<0.05$). No significant differences were found in the latency to retrieve all pups (464 ± 134; 420 ± 104); time licking and grooming (113 ± 61; 104 ± 86) or total time adopting crouching postures (123 ± 59; 85 ± 58). When OTA-infused animals were tested again on the next day, only one more OTA-infused animal showed maternal care (five/10).

DISCUSSION

In the present study we found for the first time that the NA is critical for the expression of “spontaneous” maternal responses and that OT in this brain region, extensively associated with the reward pathway, and the processing of

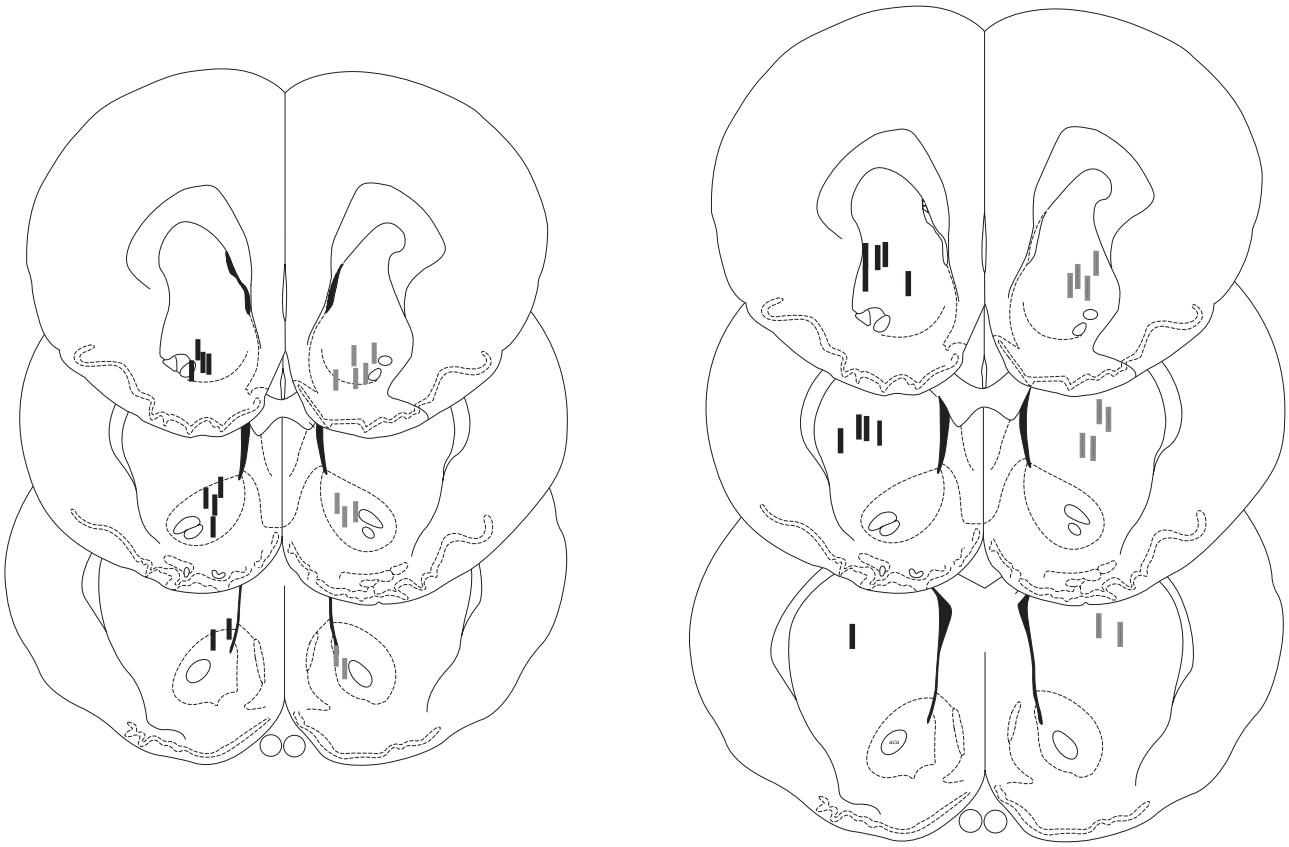

Fig. 5. Cannula placement. location of cannulae across the rostral and caudal NA and CP for each control CSF- (left) and OTA- (right) infused animal is represented by black and gray bars respectively.

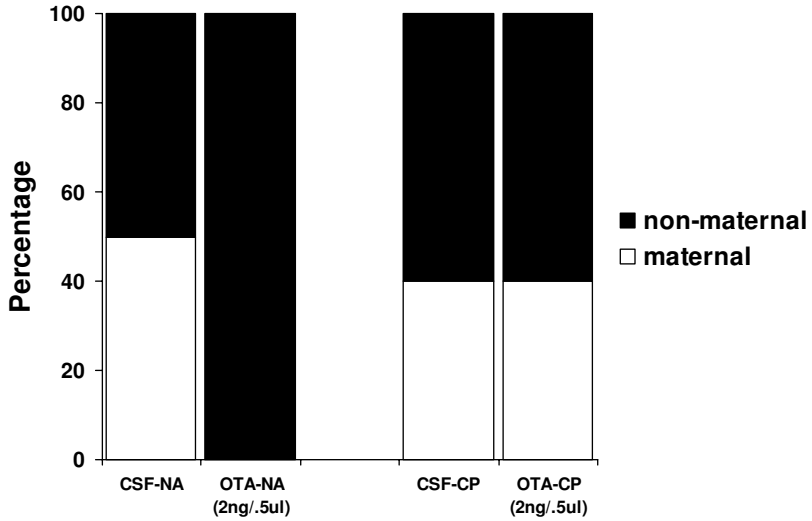

Fig. 6. OTA into the NA, but not the CP, blocks maternal response. Bars show percentage of maternal (white) and non-maternal (black) animals. A higher number of CSF-NA-infused controls showed maternal responses than OTA-NA-infused animals ($P<0.05$). There was no difference between OTA-CP- and CSF-CP-infused animals.

novelty and salient stimuli, facilitates maternal responses. This and previous studies (Olazábal and Young, 2006) from our laboratory suggest that OTR in the NA play a critical role across species and within species in facilitating the initial interaction of naïve subjects with pups. We have shown recently that the binding of OTR in the NA is correlated to the quality of maternal behavior displayed by juvenile prairie voles (Olazábal and Young; 2006). In the present study we also found that naïve adult female prairie voles that behave maternally when exposed to pups had higher OTR binding in the NA than females that attacked or ignored them. In addition we confirmed the role of these receptors in facilitating maternal behavior by transiently disrupting maternal responses with oxytocin antagonist infusions into the NA.

In these experiments, pups were a novel stimulus for our female subjects. Maternal responses are known to be affected by novelty (Mayer and Rosenblatt, 1975, 1979; Fleming and Anderson, 1987; Olazábal and Morrell, 2005) and previous selection of maternal females for this experiment would remove this important aspect of female–pup interaction. In experiment 2, the normal response to pups shown by control animals demonstrates that there was no effect of cannula implant or the brief period of anesthesia on the maternal response. Previous studies show that a minor stress does not affect maternal response in females (Bales et al., 2006). All animals were healthy and OTA infusion effects were transient as shown by the recovery of the maternal response on the next day. The lack of effect of similar dose of OTA infused into the CP (brain region with high OTR density and near the NA), suggests that the disruption of maternal behavior after NA OTA infusions is specific, and not consequence of the diffusion of OTA to other brain regions.

It is important to point out that prairie voles are very unique in their interaction with pups. They are highly responsive to pups both as juveniles and adults, and they spend most of the 15 min maternal test licking/grooming and hovering over the pups. Neither rats, meadow voles nor mice show this highly intense spontaneous maternal behavior. Interestingly, in contrast to other species, adult female prairie voles have a very high density of OTR in the NA (shell and core). This increased binding of OTR in the NA suggests several interesting possibilities on oxytocin and dopamine interactions in this brain region, as suggested previously by other authors (Kovacs et al., 1990; Liu and Wang, 2003). For example, oxytocin may interact with the dopaminergic inputs to the NA to mediate the attractive value of pup related stimuli or facilitating maternal response by other mechanisms. Oxytocin may also act in the NA to disinhibit the approach to the novel stimuli (pups), to reduce the reactivity to pup-related stimuli, increase the reinforcement properties of pups, and/or to reduce locomotor activity facilitating the contact with pups and pup stimulation. The time females spent interacting positively with pups, and specifically adopting crouching postures may then be increased by all these mechanisms.

Maternal responses of naïve females from several species are known to be affected by high reactivity to pups as novel stimuli, generally called neophobic response (Mayer

Table 1. Initial approach to pups displayed by OTA and CSF infused female prairie voles

Treatment	Latency to approach pups (s)	Number of approaches/ withdrawals	Time sniffing pups (s)
CSF-NA, $n=10$	122±87	3±1	38±16
OTA-NA, $n=10$	205±85	3±0.8	46±14
t-Test	$P>0.50$	$P>0.95$	$P>0.70$
CSF-CP, $n=10$	18±6	1.4±0.3	11.5±4.4
OTA-CP, $n=10$	164±65	1.0±0.3	11.3±4.5
t-Test	$P<0.05$	$P>0.26$	$P>0.98$

Data are expressed as mean±S.E.M.

and Rosenblatt, 1975; Fleming and Anderson, 1987). Then, it is possible that oxytocin in prairie voles may be implicated in this process. However, maternal behavior may also be facilitated by the reduction of the reactivity to the new testing environment. Previous studies in rats show that oxytocin facilitation of maternal behavior may be dependent on the exposure to a novel environment (Fahrbach et al., 1986; McCarthy et al., 1992). Oxytocin has also been implicated in the mediation of stress and corticosterone responses (Amico et al., 2004; Mantella et al., 2005; Neumann et al., 2000; Uvnas-Moberg et al., 2000; Petersson et al., 2005; Windle et al., 2004). If as proposed by those studies, oxytocin reduces corticosterone and the response to stress, then in experiment I, the differences in the response to pups found in animals with high or low OTR density in the NA might be partially due to differences in the response to the mild stressful or challenging condition of the maternal test. In experiment II, our NA OTA-infused animals may have also shown a transient deficit in their capability to cope with the novelty of pup's related stimuli or the challenging conditions of the maternal test.

Pedersen et al. (1994) suggested that oxytocin may also be necessary to activate circuits in the mesolimbic system that motivates rat dams to direct maternal behavior toward pups. Previous studies found that centrally infused OT facilitated maternal behavior (Pedersen and Prange, 1979, 1985) and i.c.v. administration of OT antagonist or antiserum (Fahrbach et al., 1985; Pedersen et al., 1985; van Leengoed et al., 1987) disrupted or delayed maternal behavior in rats. The site of action of oxytocin to facilitate maternal behavior in rats has been unclear. However, one of the places where oxytocin infusions have facilitated maternal behavior in rats is the ventral tegmental area. Pedersen et al. (1994) found that antagonists in the ventral tegmental area blocked retrieval and nursing postures, while other studies found some facilitation after oxytocin infusions into this brain region (Fahrbach et al., 1986; McCarthy et al., 1992). The ventral tegmental area sends dense dopaminergic terminals to the ventral striatum and, in prairie voles, may interact with oxytocin in the NA to increase the attractive value of pups.

A third alternative explanation may be that oxytocin interacts with dopamine in the NA to reduce locomotor activity during female–pup interaction. The NA has been previously implicated in the mediation of crouching posture in rats (Stern and Taylor, 1991; Keer and Stern, 1999). Blockage of the dopaminergic activity in this brain region facilitates passive component of maternal behavior increasing the time dams spend nursing over pups. In particular, Keer and Stern (1999) found that that the D1/D2 antagonist cis-flupenthixol infused into the NA enhanced kyphotic nursing and low dosages of haloperidol resulted in more rapid onset and longer duration of nursing (Stern and Keer, 1999). In addition, another study showed that this facilitation of nursing postures occurred both in female and male prairie voles (Lonstein, 2002). In the present study, we found that OTR binding in the NA was positively correlated to the time maternal females spent adopting quiescence crouching posture. Stern and Lonstein (2001) propose that ventral stimulation and suckling-induced inhibition of dopamine may be required for kyphosis and quiescent nursing. The NA (shell subregion) send projections to the ventrolateral part of the periaqueductal gray region (Groenewegen et al., 1996), that has been implicated in the mediation of quiescence crouching posture in rats. Whether a similar mechanism can be triggered, for example, through ventral stimulation of naïve female prairie voles by the very active prairie vole pups needs to be further investigated. Other studies also suggest that oxytocin interacts with dopamine action in the NA and prevents the ability of cocaine to induce an increase in spontaneous locomotor activity (Kovacs et al., 1990).

OTR density in the CP was also positively correlated with time spent adopting quiescence crouching posture. However, note that we have previously reported that OTR density in the NA and CP is highly correlated (R=.89; Olazábal and Young, 2006). In contrast to OTA infusions into the NA, OTA infusions into the CP delayed the approach to pups. However, that delayed response did not block the emergence of full maternal behavior in OTA-infused animals. This finding cannot be explained as a consequence of diffusion of OTA to the NA because OTA-NA- and CSF-NA-infused animals did not differ in their latency to approach to pups. In addition, the latency to approach to pups in OTA-CP-infused females was different to controls but in the range of the latency to approach to pups shown by animals with CSF and OTA infusions into the NA.

OTR binding variability in the brain has previously been associated with individual differences in maternal responses (Francis et al., 2000, 2002; Champagne et al., 2001). For example, increased OTR binding in the MPOA, the LS, the central nucleus of the amygdala, paraventricular nuclei of the hypothalamus, and the bed nucleus of the stria terminalis were associated with increased levels of licking and grooming in lactating females (Champagne et al., 2001). In the present study we found no relationship between spontaneous maternal behavior and OTR density in the MPOA, LS and PLC. Previous studies in prairie voles also failed to find a positive relationship between OTR binding in the LS and maternal behavior (Insel and Shapiro, 1992; Wang et al., 2000; Olazábal and Young, 2006). Comparisons across species and within juvenile prairie voles also showed that OTR binding in the MPOA does not contribute to our understanding of variability in pup-induced maternal behavior (Olazábal and Young, 2006). Although both Champagne et al. (2001) and Francis et al. (2000) found changes in OTR density in the MPOA associated with lactating maternal behavior, note that in non-lactating females (similar to our present paradigm) Francis et al. (2000) did not find any difference in OTR density in the MPOA. However, the difference between previous findings in rats (Champagne et al., 2001) and ours in prairie voles may be also due to the species-specific function of oxytocin in accordance with the species behavioral and reproductive strategy (Insel and Shapiro, 1992).

Our finding on spontaneous maternal behavior in prairie voles agrees with Pedersen and Prange's (1985) sug-

gestion that oxytocin participates in the mediation of "non-hormonal" or pup-induced maternal behavior. There is extensive evidence showing that the presence or not of the ovaries does not affect pup-induced maternal behavior in virgin rats (Rosenblatt, 1967; Mayer and Rosenblatt, 1979; Stern, 1987). On the other hand, there is no evidence that fluctuations in the gonadal hormones of virgin animals can influence maternal responses or OTR density. OTR density in the NA of prairie voles reaches mature levels around 20 days of age and there is no further developmental change after that (D. E. Olazábal and L. J. Young, unpublished observations). In addition, no sexual dimorphism has been reported for OTR in the NA. However, chronic absence of gonadal hormones or drastic changes in prenatal or early postnatal exposure to gonadal hormones might influence spontaneous maternal behavior. In adult prairie voles, OTR density is extremely resistant to hormonal or other environmental changes. However, whether a brief interaction with pups can modify NA OTR binding in maternal animals has never been investigated before.

Oxytocin has multiple functions as described in the introduction and OTR distribution and expression may affect other behaviors or physiological functions. We must point out that the OTR receptor in the NA also facilitates pair bonding in prairie voles and perhaps also same-sex interactions. Central infusions of OT have been shown to enhance social interactions in male and female prairie voles (Witt et al., 1990; Cho et al., 1999), while oxytocin antagonists (i.c.v. or in the NA) reduced the time a female prairie voles spent with her male partner (Insel et al., 1995; Young, 1999). Then, it is possible that oxytocin in prairie voles increases positive social interactions, including interaction with pups, either disinhibiting the animal to approach to conspecifics, reducing social avoidance or/and increasing the attractive value of social stimuli. Whether OT infusions into the NA also influence male spontaneous parental responses, same-sex social interaction, or affect the response to novelty or exploratory behavior needs to be further investigated.

It must be clearly stated that oxytocin may facilitate maternal responses in brain regions that do not show any difference in OTR binding across species or through development, as is apparently the case for the MPOA (Pedersen et al., 1994). However, we have found that comparative and developmental studies can be very useful to understand individual differences in behavior. Based on the evidence that OTR binding in the NA of rats declines through development as maternal response to pups does (Mayer and Rosenblatt, 1979; Shapiro and Insel, 1989) and OTR binding in the NA is higher in prairie voles, that show very intense spontaneous maternal responses, compared with other three rodent species (Olazábal and Young, 2006), we hypothesized and found evidence that OTR binding variability in the NA of juvenile (Olazábal and Young, 2006) and adult prairie voles was associated with the quality of their maternal responses. We finally further tested this relationship with oxytocin antagonist infusions in the NA and confirmed OT facilitation of "spontaneous" maternal responses. Future studies will investigate the mechanisms underlying NA OTR facilitation of "spontaneous" maternal responses and the effect of OTR overexpression in the NA in species that show no or minimal "spontaneous" positive interaction with pups.

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