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Oxytocin receptors in the nucleus accumbens shell are involved in the consolidation of maternal memory in postpartum rats

T.M. D'Cunha [a], S.J. King [a], A.S. Fleming [a,*], F. Lévy [b]

[a] Department of Psychology, University of Toronto at Mississauga, Mississauga, Ontario, Canada L5L 1C6
[b] UMR 6175 INRA/CNRS/Université F. Rabelais/Haras Nationaux, Nouzilly, 37380, France

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ABSTRACT

Female rats with maternal experience display a shorter onset of maternal responsiveness compared to those with no prior experience. This phenomenon called 'maternal memory' is critically dependent on the nucleus accumbens (NA) shell. We hypothesized that activation of OT receptors in the NA shell facilitates maternal memory. In Experiment 1, postpartum female rats given 1 hour of maternal experience were infused following the experience with either a high or low dose of an OT antagonist into the NA shell and tested for maternal behavior after a 10-day pup isolation period. Females receiving a high dose of the antagonist showed a significantly longer latency to exhibit full maternal behavior after the pup isolation period compared to females that received vehicle or a high dose of antagonist in a control region. In Experiment 2, postpartum female rats were infused with either a high or low dose of OT into the NA shell after a 15-minute maternal experience and tested for maternal behavior after a 10-day pup isolation period. There were no significant differences between the females infused with OT and females treated with a vehicle infused into the NA shell or with OT infused into the control region. One possible reason for a lack of facilitation is a floor effect, since females in the control groups displayed a rapid maternal response after the pup isolation period. These findings suggest that OT receptors, likely in combination with other neurotransmitters, in the NA shell play a role in the consolidation of maternal memory.

Introduction

Immediately after parturition, female rats are maternally responsive to their pups. The dam builds a nest, retrieves the pups to the nest, and assumes a nursing posture to allow the pups to suckle. The dam will also lick the pups, both general body licking and licking the anogenital region of the pups to promote urination and defecation (Numan et al., 2006). The dam will continue to respond maternally until the pups are weaned at about 3–4 weeks postpartum (Numan, 1994; Rosenblatt and Lehrman, 1963).

Hormonal changes that occur just before parturition play a key role in the induction and maintenance of maternal behavior in the dam (Orpen et al., 1987). However, studies indicate that hormones alone are insufficient in inducing and maintaining maternal behavior and that direct contact with pups is necessary (Bridges, 1975; Fleming et al., 1990; Orpen and Fleming, 1987). Females that were caesarian sectioned and therefore received no direct contact with pups after surgery display similar maternal responsiveness as virgin females when tested 10 days after parturition (Orpen and Fleming, 1987). Orpen and Fleming (1987) found that females that were allowed to interact and care for pups during a 30-minute exposure period following caesarian section exhibited short latencies to maternal behavior 10 days after parturition and that 15 minutes of exposure was insufficient in achieving similar maternal responsiveness. This long-term retention of maternal responsiveness as a result of prior experience with offspring is referred to as maternal memory (Bridges, 1975; Li and Fleming, 2003; Lee et al., 1999; Orpen and Fleming, 1987).

Maternal memory likely occurs in a number of species. This has been demonstrated in rabbits and also in humans (González-Mariscal et al., 1998; Fleming et al., 1988). Mothers who have earlier postpartum interactions, as opposed to late interactions, with their babies are more affectionate to them on day 2 postpartum (Fleming et al., 1988). Maternal experience is also acquired across successive births and lactation. In comparison to multiparous mothers, first-time mother rats, hamsters, voles, and sheep show deficits in some components of maternal behavior and in some cases take longer to accept their young at suckling (Numan et al., 2006). In humans, prior maternal experience is a reliable predictor of positive responsiveness to offspring. In comparison to primiparous mothers, during the initial postpartum period, multiparous mothers are more attracted to the

* Corresponding author.
E-mail addresses: tracey.dcunha@gmail.com (T.M. D'Cunha), sking3@connect.carleton.ca (S.J. King), alison.fleming@utoronto.ca (A.S. Fleming), levy@tours.inra.fr (F. Lévy).

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body odors of newborn infants and recognize their own baby's odors (Schaal et al., 1980; Fleming et al., 1997), they respond more rapidly and sympathetically to the cry of their own infant (Bernal, 1972) and more sympathetically to pain than to hunger cries (Stallings et al., 2001). Clearly, in most mammalian species, including our own species, a memory of maternity develops through interactions with the young and influences the subsequent expression of maternal behavior.

Previous research has found that the nucleus accumbens (NA), particularly the shell subregion, is essential for the consolidation of maternal memory (Li and Fleming, 2003; Lee et al., 1999). If females are briefly exposed to pups on the day after parturition, postnatal day 1 (PND1), isolated from pups for 10 days and then reintroduced to pups, NA-lesioned females exhibit longer latencies to become maternal compared to animals that received a sham operation. Maternal memory is disrupted both when electrolytic lesions of the NA shell are performed before parturition or after the 1 hour of pup exposure. Maternal memory, however, does not seem to be affected by lesions to other areas such as the dorsal hippocampus and the caudate nucleus (Lee et al., 1999). Central infusion of cycloheximide, a protein synthesis inhibitor, into the shell, immediately before or after 1 hour of maternal experience blocks the retention of long-term maternal memory, indicating that consolidation of maternal memory requires protein synthesis like many other consolidation memories (Fleming et al., 1990). Recent research on the neurotransmitters involved in the consolidation process of maternal memory has revealed a prominent role of dopamine (DA) receptor systems within the NA shell. When an antagonist of D_1/D_2 receptor subtypes is administered into the NA shell immediately following a 1-hour pup exposure and a 10-day pup isolation period, female rats display longer latencies to become maternal (Parada et al., 2008).

At the time of parturition, profound hormonal changes occur in the mother and some of them are associated with the onset of maternal behavior (Numan and Insel, 2003). For example, there is an increase in the synthesis of oxytocin (OT), a nonapeptide hormone synthesized primarily in the supraoptic and paraventricular nuclei of the hypothalamus. In addition, there is an increase of OT release and of OT receptor expression in various brain regions (Meddle et al., 2007; Kendrick, 2000; Broad et al., 1999). The central activation of the oxytocinergic system at parturition plays a pivotal role in the onset of maternal behavior. For example, intraventricular or site-specific administration of OT antagonists reduces the onset of maternal behavior in primiparous females (van Leengoed et al., 1987; Pedersen et al., 1994; Yu et al., 1996). Studies have also found that intraventricular or site-specific administration of OT can induce maternal behavior in virigin female rats (Fahrbach et al., 1984; Yu et al., 1996). Key brain regions where OT acts to induce maternal behavior are the ventral tegmental area (VTA), the medial preoptic area (MPOA), and the olfactory bulb (OB). However, OT receptors are also expressed in the NA (Veinante and Freund-Mercier, 1997; Tribollet et al., 1988) and have been implicated in facilitation of maternal responses (Olazabal and Young, 2006) and mating-induced partner preferences in prairie voles (Young et al., 2001).

Given the role of NA OT receptors in the formation of both mother–young bonds and pair bonds, and the contribution of the NA to maternal memory (Lee et al., 1999; Li and Fleming, 2003; Parada et al., 2008), we hypothesized that activation of OT receptors in the NA would facilitate the consolidation of maternal memory and that antagonism of OT receptors in this region would attenuate the consolidation process. The present study consists of two experiments in which the effects of an infusion of an OT receptor antagonist (Experiment 1) and of OT (Experiment 2) into the NA on the consolidation of maternal memory were investigated . We predicted that animals treated with an OT receptor antagonist immediately following a 1-hour maternal experience would exhibit deficits in maternal behavior after a 10-day pup separation, whereas animals infused with OT after a 15-minute exposure to pups would show a shorter latency to perform maternal behavior after a 10-day pup separation, compared to vehicle-treated animals.

General method

Subjects and housing

Subjects were nulliparous female Sprague–Dawley rats (about 70–90 days of age), housed, and mated at the University of Toronto at Mississauga animal vivarium. Animals were derived from a stock obtained from Charles River Laboratories (St. Constant, Quebec, Canada). The rats were individually housed in transparent Plexiglas cages (47 cm × 26 cm × 20 cm) in a temperature- and humidity-controlled environment (22 °C and 45%–55%, respectively) on a standard 12-hour light–dark cycle (lights on at 0800). Purina rat chow and water were available *ad libitum*, and bedding consisted of wood shavings. A group of dams was also maintained to provide donor pups for retention testing.

All methods used complied with the Canadian Council on Animal Care guidelines for care of laboratory animals and were approved by the University of Toronto Animal Care Committee. All efforts were made to minimize the number of animals used in each experiment.

Procedure

Two females were housed with a proven stud male for 1 week to ensure pregnancy. Gestational days were estimated based on dates animals were first placed with the stud male. On gestational days (GD) 10–14, bilateral guide cannulas were stereotaxically implanted into the targeted brain regions. On GD21, females were checked for parturition at 30-minute intervals between 8 a.m. and 8 p.m. If signs of parturition were present, females were checked at 15-minute intervals, and pups were systematically separated from the dam. When parturition was complete, as assessed by 3 consecutive intervals with no pups present, the females were placed into large transparent maternal observation cages. Females were provided with 2 shredded paper towels to use as nesting material and were then placed in a pup-deprived room.

The day of parturition was designated as PND 0. On PND 1, all rats were exposed to six 1- to 3-day-old donor pups (3 males and 3 females) for a 1-hour period (Experiment 1) or for a 15-minute period (Experiment 2). During the first 10 minutes of the pup exposure period, a baseline maternal test was conducted. Immediately following the pup exposure, the rats were randomly assigned to a infusion group. Female rats were then isolated for 10 days. Starting on PND 11 maternal retention tests were conducted for a maximum of 11 days. The criteria to become maternal were defined as two consecutive days of retrieving all pups to the nest site. The first of the two consecutive days in which the rat met the above criterion was assigned as the maternal latency score. Animals that did not become maternal during the 11-day testing phase were given a maternal latency score of 10. Once behavioral testing was complete, animals were intracardially perfused and histological analysis was conducted to verify the cannula placements.

Stereotaxic surgeries

Rats were anesthetized with a mixture of ketamine (90 mg/kg; MTC Pharmaceuticals) and xylazine (10 mg/kg; Rompun, Bayer Inc., Etobicoke, Ontario, Canada) at 1.5 ml/kg intrapertioneally (i.p.). To provide analgesia, rats were also injected subcutaneously (s.c.) with ketoprofen (Anafen, MERIAL Canada Inc., Morgan Baie d'Urfe, Quebec, Canada; 0.5 ml/kg). Twenty-six gauge stainless steel bilateral guide cannulas (Plastics One, Roanoke, VA) were implanted into the NA shell region (coordinates from bregma: AP +1.56 mm; ML ±0.75 mm; DV −6.5 mm from the skull surface). The coordinates for the guide

cannula placement are based on a standard altas of the rat brain (Paxinos and Watson, 1998). The guide cannula was affixed to the skull using surgical screws and dental cement. In Experiment 1, a control group of females were bilaterally cannulated in the dorsal hippocampal region (coordinates: AP −3.8 mm; ML ±1.5 mm; DV −3.8 mm). In Experiment 2, a control group of females was bilaterally cannulated into the caudate putamen (CP; coordinates: AP +1.44 mm; ML ±3.20 mm; DV −5.5 mm). The hippocampus was chosen as a control site initially because previous work by Lee et al. (1999) showed that lesions to the dorsal hippocampus did not affect maternal memory or expression of maternal behavior. However, given that there is considerable data to suggest the importance of hippocampus to memory, we decided to change our control site to a site that had never been implicated either in mothering or memory.

Initial maternal behavior test

On PND1, female rats were given 6 freshly fed pups (3 males and 3 females) between 1 and 3 days old to interact with for 1 hour (Experiment 1) or for 15 minutes (Experiment 2) before the infusion. A baseline initial maternal behavior test was conducted during the first 10 minutes of maternal experience. The pups were placed diagonal to the nest site in the opposite quadrant of the cage as the female. The BEST collection software (University of Toronto at Mississauga, Mississauga, Ontario, Canada) was used to measure the frequency and duration of several behaviors: (a) pup retrieval—the dam lifted a pup in her mouth and carried it across at least one quadrant; the total number of pups retrieved; (b) pup sniffing; (c) pup licking—general body licking of the pup and licking of the anogenital region of the pup were distinguished; (d) nursing postures—the dam positioned over the pups with an arched back to permit suckling by the pups; high and low crouches were differentiated; (e) hovering—when the dam was on top of the pups performing other maternal behaviors; (f) nest building—the dam picked up nest material and carried it back to the nest site or pushes nest material closer to the nest using her forepaws; (g) general non-pup-directed behaviors—this category included behaviors such as eating, grooming, and general air sniffing.

Infusions

Immediately following the maternal experience period, the pups were removed from the cage, and the female was given bilateral micro infusions. Infusions were delivered over a 60-second period at a rate of 0.5 μl/min via two 500-μl Bas gas-tight syringes (MD-0050; Bio Analytical Systems) connected to PE50 tubing with 26-gauge internal cannula needles that extended 1 mm further than the length of the guide cannula. Infusions were automated with a Harvard infusion pump (Harvard Apparatus Inc. 22, Natick, MA). Injection cannulae remained in place for an additional 60 seconds to minimize backflow into the cannula tracks. Rats were handled on a daily basis to acclimate them to handling by the experimenter, to ensure that the infusion procedure could be completed without anesthesia.

Maternal retention tests

Ten days following the initial pup exposure and subsequent pup isolation period, females were tested for the retention of maternal behavior between 10 a.m. and 2 p.m. The maternal retention tests followed the same procedure as the initial maternal behavior tests, where 6 pups were placed diagonal to the dam's nest site. A spot check was completed after retention test 1 where the position of the pups relative to the nest site and the mother were recorded on a 2-dimensional representation of the maternal cage. The following morning another spot check was completed, and donor pups were removed and returned to their mother. Freshly fed pups were then placed into the cage, and another maternal retention test was conducted. Rats were designated as maternal if for two consecutive days she retrieved all pups into the nest during the 10 minute retention test. The latency to become maternal was scored as the first of two consecutive days that the female fulfilled the maternal criterion.

Histological analyses

Once behavioral observations were complete, animals were overdosed with the ketamine and xylazine cocktail mixture (1.5 ml/kg) and perfused intracardially with 0.9% saline followed by a 10% paraformaldehyde solution. Extracted brains were placed into a 30% sucrose–formalin solution for at least 24 hours and then sliced at 40-μm sections on a cryostat (Leica CM 1850, Nussloch, Germany). Slices were then mounted on gel-coated slides and stained with cresyl violet. Microscopic examination was used to determine the infusion site.

Statistical analysis

A nonparametric between-group Kruskal–Wallis test was conducted on the latency data because of the absence of homogeneity of variance in the data. Post hoc analyses were conducted using Mann–Whitney *U* tests where main effects were found. The cumulative percentages of rats that responded maternally across testing days were compared between groups using χ^2 analysis. Analyses of variance (ANOVAs) were conducted on the frequency and duration of both pup-directed maternal behaviors and the pup-directed behaviors to analyze group differences on testing days.

Experiment 1

Effects of OT receptor antagonist infusion into the NA shell after a 1-hour maternal experience on maternal memory

The aim of this experiment was to examine whether an OT receptor antagonist would produce deficits in maternal memory when injected into the NA shell. Dams received either a high or a low dose of an OT receptor antagonist, (d(CH2)5 Tyr (Me)2, Orn 8)-vasotocin (OTA), into the NA shell after 1 hour of maternal experience and then tested for maternal behavior after a 10-day pup isolation period. Infusion of vehicle into the NA shell or infusion of a high dose of OTA into the dorsal hippocampus served as controls.

Method

Thirty-six female rats were used in this study. Immediately following the 1-hour experience, pups were removed from the cage, and rats were randomly assigned to receive bilateral microinfusions (0.5 μl/side) of one of the following: 0.25 μg /μl of OTA (Bachem, Torrance, CA, USA) into the NA shell (Low OTA–NA group); 0.75 μg/μl of OTA into the NA shell (High OTA–NA group); 0.75 μg/μl of OTA into the dorsal hippocampus (High OTA–HIPP group); artificial cerebrospinal fluid (aCSF; Harvard Apparatus, Holliston, MA, USA) into the NA shell (VEH group). Drugs were dissolved in aCSF before being infused. OTA doses were based on previous studies (Pedersen et al., 1994; Melis et al., 2007).

Results

Histological verification

Of the 36 animals that were cannulated, 31 were cannulated accurately into the nucleus accumbens or the dorsal hippocampus and the 5 animals with cannulas external to the target region were excluded from statistical analyses (Fig. 1A). Statistical analysis was

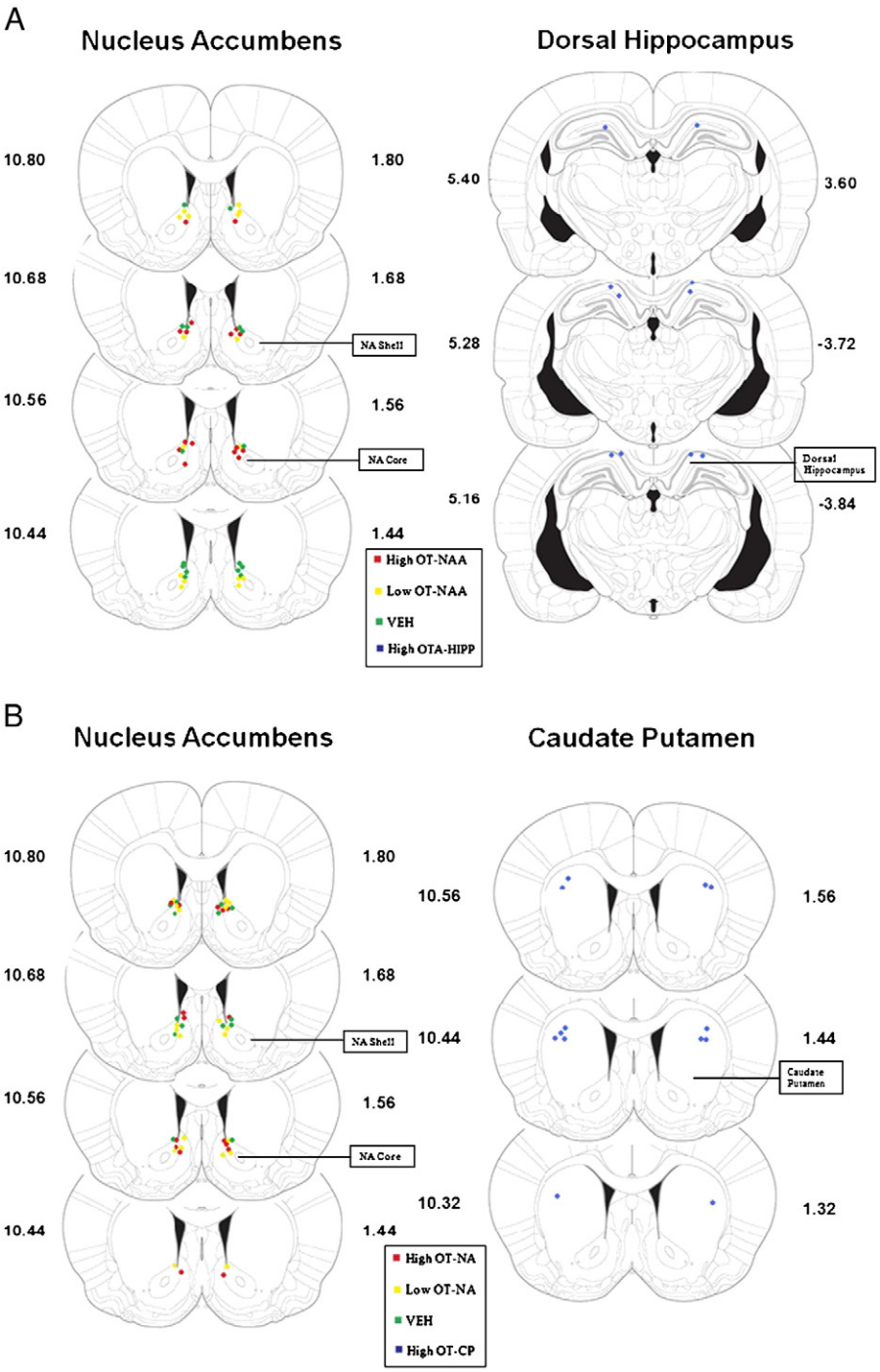

Fig. 1. Placement locations of guide cannulas in Experiment 1 (A) and Experiment 2 (B) into the nucleus accumbens (NA) shell (coordinates: AP +1.56 mm; ML ±0.75 mm; DV −6.5 mm). The control region for Experiment 1 is the dorsal hippocampus (coordinates: AP −3.8 mm; ML ±1.5 mm; DV _3.8 mm); the control region for Experiment 2 is the caudate putamen (coordinates: AP +1.44 mm; ML ±3.20 mm; DV −5.5 mm).
From *The Rat Brain in Stereotaxic Coordinates* (4th ed), by G. Paxinos and C. Watson, 1998, San Diego, CA: Academic Press.

therefore conducted on the following group composition: High OTA–NA, $n=9$; Low OTA–NA, $n=8$; High OTA–HIPP, $n=6$; and VEH, $n=7$.

Maternal behaviors exhibited during pup exposure

All dams gave birth to healthy litters, and during the initial exposure session, all dams exhibited normal maternal behaviors. There were no between-subject differences in displays of pup retrievals, crouching and hovering, licking, or nest-building behaviors during the baseline initial maternal behavior test (data not shown).

Effect of drug treatment on maternal memory

Latency to reach maternal criterion

Following the 10-day isolation period, the 4 groups of rats were assessed for their latency to become maternal using the nonparametric Kruskal–Wallis test, followed by Mann–Whitney *U* tests for post hoc analyses. There was a significant effect of treatment group on latency in days ($\chi^2(3)=7.728$, $n=30$, $p=0.052$; Fig. 2). Post hoc analysis revealed that the High OTA–NA group was significantly different from the VEH group ($\chi^2(3)=7.728$, $n=30$, $p=0.052$). The

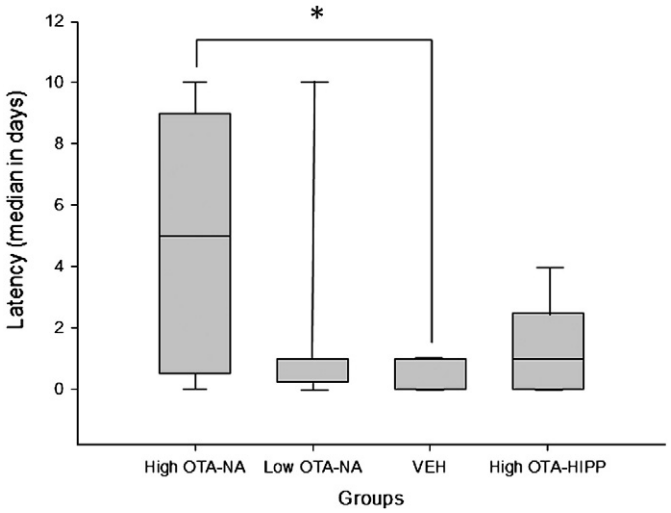

Fig. 2. Median latency and interquartiles (in days) to exhibit full maternal behavior for the treatment groups in Experiment 1 (High OTA–NA, $n=9$; Low OTA–NA, $n=8$; VEH, $n=7$; High OTA–HIPP, $n=6$).

High OTA–NA group took significantly longer to reach maternal criterion over days compared to the VEH group. There were no other significant differences between the other groups.

Cumulative percentage of maternal rats across test days

There was a significant treatment effect found on the percentage of rats to reach maternal criterion within the first 24 hours ($\chi^2(3)=9.960$, $n=30$, $p=0.019$). Post hoc analysis revealed that the High OTA–NA group had a significantly lower percentage of dams reaching the maternal criterion within the first 24 hours (Day 2) of testing compared to the Low OTA–NA group, ($\chi^2(3)=5.130$, $n=17$, $p=0.024$) and compared to the VEH group ($\chi^2(3)=7.467$, $n=16$, $p=0.006$). The percentage of animals reaching the maternal criterion by Day 2 in groups High OTA–NA, Low OTA–NA, VEH, and High OTA–HIPP was 33%, 80%, 70%, and 66%, respectively.

First day of maternal retention testing

A significant difference was found between groups on the duration of time spent nest building on the first day of maternal retention testing ($F(3,29)=7.559$, $p=0.001$). Post hoc analyses revealed that the VEH group spent a significantly longer amount of time nest building compared to the other 3 groups (Fig. 3). There were no other significant differences between treatment groups on any of the other behaviors recorded for the first day of maternal retention testing.

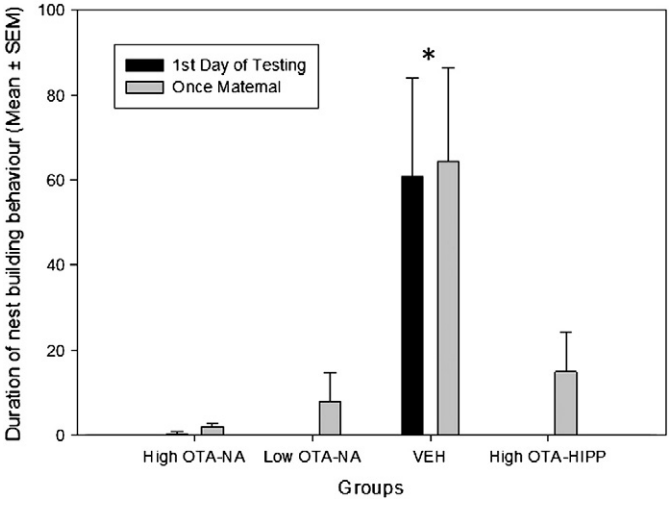

Fig. 3. Mean duration (±SEM) of nest building behavior on the first day of maternal retention testing and once the females reached maternal criterion for all treatment groups in Experiment 1 (High OTA–NA, $n=9$; Low OTA–NA, $n=8$; VEH, $n=7$; High OTA–HIPP, $n=6$).

Effect of drug treatment on the quality of maternal behavior at RT2

Once the dams reached maternal criterion, differences between treatment groups were observed only with regards to nest building. On the first day of maternal criterion, there was a significant difference between groups on time spent nest building ($F(3,26)=5.250$, $p=0.007$). Similar to the pattern found on the first day of retention testing, post hoc analyses revealed that the VEH group spent a longer duration of time nest building compared to the other groups (Fig. 3).

On maternal criterion days for each of the treatment groups, there was a significant difference in the frequency of mouthing behaviors ($F(3,26)=6.311$, $p=0.003$). Post hoc analysis revealed that the High OTA–NA and Low OTA–NA groups had a significantly higher frequency compared to the VEH group (5.86 ± 1.28 and 7.14 ± 1.59 vs 0.79 ± 1.59, $p=0.037$ and $p=0.006$, respectively), and the Low OTA–NA group had a significantly higher frequency compared to the High OTA–HIPP group (7.14 ± 1.59 and 5.86 ± 1.28, $p=0.033$). No significant differences were found between groups on any other maternal behavior examined within the two criterion days.

Experiment 2

Effects of OT infusion in the NA shell after a 15-minute maternal experience on maternal memory

As a follow-up to Experiment 1, Experiment 2 injected OT into the NA shell to examine if it would produce effects opposite to the OT antagonist, that is if it would enhance maternal memory. Dams were given either a high or low dose of OT following a 15-minute maternal experience, and then tested after a 10-day isolation period for their maternal behavior. An experience time of 15 minutes was chosen with a view to provide minimal experience with pups which would result in a high onset latency at test 10 days later and that could be enhanced by an OT agonist. This duration was based on pilot studies in the lab. Control groups consisted of an infusion of the vehicle into the NA shell or an infusion of the high dose of OT into the CP.

Method

Thirty-seven female rats were used in the study. After the 15-minute experience period, pups were immediately removed from the cage, and the females were randomly assigned to receive bilateral microinfusions (0.5 µl/side) of one of the following: 200 ng/µl of OT (Sigma-Aldrich, Missouri, USA) into the NA shell (High OT–NA group); 200 ng/µl of OT into the caudate putamen (High OT–CP group); 100 ng/µl of OT into the NA shell (Low OT–NA group); and aCSF into the NA shell (VEH group). Drugs were dissolved in aCSF before being infused. There is a wide range of effective doses of oxytocin that have been infused directly into different regions in the brain. Effective doses range from 10 to 500 ng to induce or affect various behaviors, such as to decrease maternal aggression (Consiglio et al., 2005), to induce penile erection in male rats (Melis et al., 2007), and to induce grooming (Marroni et al., 2007). Therefore, in the present study, an intermediate dose range of 100 ng and 200 ng was chosen as the low and high doses of oxytocin, respectively.

Results

Histological verification

Histological analyses revealed that 33 of the 37 animals cannulae were acurately located in targeted regions of the brain, either the NA shell or the CP (Fig. 1B). The 4 animals with cannulas outside of the

targeted regions were excluded from statistical analyses. Statistical analysis was therefore conducted on the following group composition: High OT–NA, $n=8$; Low OT–NA, $n=10$; High OT–CP, $n=7$; and VEH, $n=8$.

Maternal behaviors exhibited during pup exposure

All of the dams gave birth to healthy litters. During the baseline test period, 5 dams did not retrieve all of the pups into the nest site and were subsequently removed from the study. There were no between-subject differences in displays of retrieval, crouching licking, hovering, or nest-building behaviors during the baseline initial maternal behavior test (data not shown).

Effect of drug treatment on maternal memory

Latency to reach maternal criterion

Dams were assessed for their latency to become maternal following a 10-day pup isolation period using a nonparametric Kruskal–Wallis test, followed by Mann–Whitney *U* tests for post hoc analysis. There was no significant effect of treatment group on latency in days ($\chi^2(3)=2.598$, $n=33$, $p=0.458$). There were no significant differences between the treatment groups on their latencies to reach maternal criterion (Fig. 4).

Cumulative percentage of maternal rats across testing days

No significant differences were found between groups on the cumulative percentage of rats to reach maternal criterion across testing days.

Effect of drug treatment on the quality of maternal behavior

First day of maternal retention testing

After the 10-day isolation period, dams were tested on their initial responses towards foster pups. Using a one-way ANOVAs followed by Tukey's post hoc analyses, no significant differences were found between groups on pup-directed and non-pup-directed behaviors displayed on the first day of testing.

Effect of drug treatment on the quality of maternal behavior at RT2

Once subjects displayed the full onset of maternal behaviors on two consecutive days (RT2), the frequency and duration of various maternal behaviors were analyzed using one-way ANOVAS followed by Tukey post hoc analysis to assess the effect of oxytocin treatment on the quality of maternal care. There was a significant difference between treatment groups on the frequency of body licking bouts once animals achieved the maternal criterion. As shown in Fig. 5, groups that received OT treatment displayed less frequent body licking bouts compared to the VEH group. Given that we hypothesized a facilitation of the onset of maternal behavior by OT administration, this finding was counterintuitive.

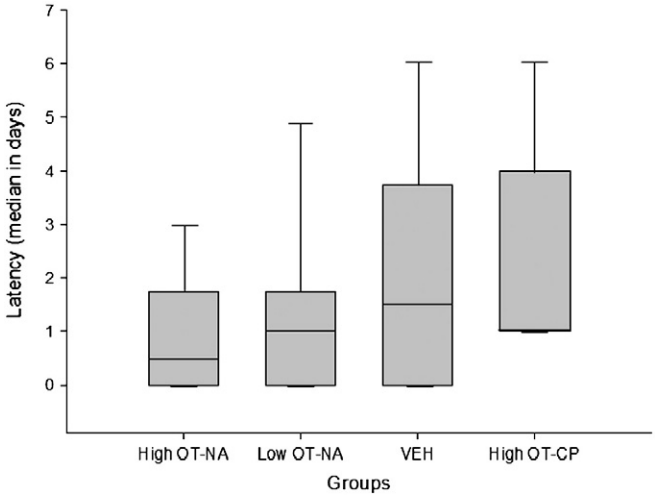

Fig. 4. Median latency and interquartiles (in days) to exhibit full maternal behavior for the treatment groups in Experiment 2 (High OT–NA, $n=8$; Low OT–NA, $n=10$; VEH, $n=8$; High OT–CP, $n=7$).

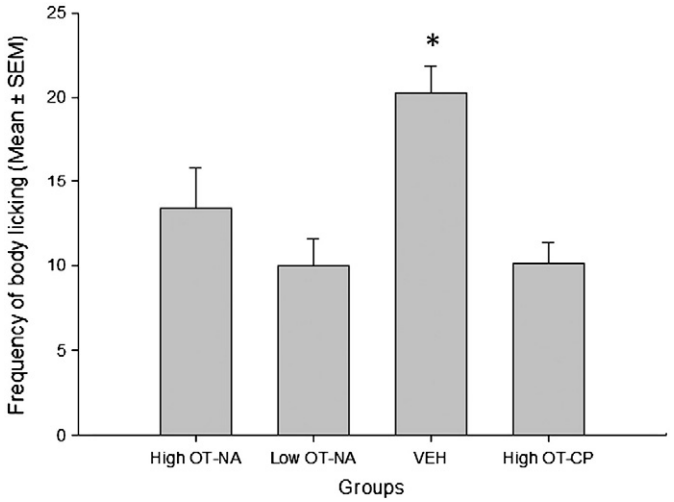

Fig. 5. Mean frequency (±SEM) of body licking behavior once the females reached maternal criterion for all treatment groups in Experiment 2 (High OT–NA, $n=8$; Low OT–NA, $n=10$; VEH, $n=8$; High OT-CP, $n=7$).

No significant differences were found between groups on any other maternal behaviors examined within the two criterion days.

Discussion

The present study indicates that an OT receptor antagonist microinfusion within the NA impairs the consolidation of maternal memory in a dose-dependent manner. We found that microinfusions of the high dose of the OT receptor antagonist (OTA) resulted in a 5-day latency to reach maternal criterion which is significantly longer than the VEH control group, whereas no difference was found between the low-dose OTA group and the VEH control group. In addition, 33% of females receiving the high OTA dose reached maternal criterion by day 1 of retention testing compared to 71% of females in the VEH control group and to 80% of females in the low OTA dose group. Interestingly, mothers infused with similar high doses of OT receptor antagonist in the dorsal hippocampus, which exhibits OT receptor expression (Tribollet et al., 1988), showed no deficit in maternal memory in comparison to the VEH control group. Finally, the effects of OT in the NA shell on maternal memory were likely through action on consolidation of the memory rather than through behavioral expression since the OT antagonist was administered 10 days before maternal retention testing. Overall, these results indicate that consolidation of maternal memory is partly mediated by OT activation in the NA shell. Other regions including the VTA (Numan and Insel, 2003) or other neurotransmitters including dopamine (Parada et al., 2008) or norepinephrine (Moffat et al., 1993) may also contribute to memory consolidation.

Although we used two doses of OT, intra-NA microinfusions of OT did not improve the consolidation of maternal memory. One possible reason for a lack of facilitation is a floor effect, since females in the VEH control groups given 15 minutes of exposure to pups display a rapid maternal response after the 10-day isolation period (50% of the females reached maternal criterion within 24 hours of testing, and the median latency to become maternal was 1.5 days) therefore making it difficult to see an enhancement of maternal memory. Reasons for this high level of responsiveness in the current study and not in the pilot study are unclear, but likely reflect cohort differences, or perhaps differences in

how animals were treated. Pilot animals had not had prior implant surgeries, whereas all of the animals in this study had. However, unless we use caesarean deliveries, which introduce other confounds, we have to manipulate postpartum experience by using a 'pup catching' strategy at the time of parturition. Bridge's 1975 study (Bridges, 1975) reported that immediate removal of pups prevented establishment of maternal memory when subjects were tested 25 days later. An approach using "pup catching" in combination with minimal pup exposure 24 hours later and OT administration could be an effective procedure to determine whether OT itself can stimulate memory when it is tested 25 days later. Another strategy could be to increase the retention interval in order to increase latency to exhibit maternal behavior in control animals. For example, after 24 hours of maternal experience following parturition the latency to reach maternal criterion is less than 1 day after 10 days of pup separation, whereas this latency is increased to 6 days after 30 days of pup separation (Fleming and Sarker, 1990). It is possible that if we had used a 30-day pup separation an effect would have been found. It is also possible that the doses of OT used were too high to be effective. It seems that the general pattern of OT effects in the brain is represented by a *U*-shaped dose–response curve. For instance, OT administration in the amygdala decreases maternal aggressive behavior with 20 ng of peptide, while the dose of 200 ng was ineffective (Consiglio et al., 2005). A similar *U* dose–response curve has been reported for social recognition (Benelli et al., 1995) and sexual behavior in rats (Melis et al., 2007) and in an inhibitory avoidance task in mice (Boccia et al., 1998).

In our study, the OT antagonist was infused 1 day after parturition when females were given 1 hour of exposure to pups and maternal behavior was tested at the end of a 10-day pup isolation. Our findings suggest that OT receptors are activated at the time when mothers are in the presence of pups. While OT receptor activation in the NA during pup exposure has not been demonstrated in rat mothers, a 15-minute exposure to pups in female prairie vole induces an increase in OT receptor binding in the NA for females behaving maternally (Olazabal and Young, 2006). This increased binding of OT receptors in the NA could facilitate mother–young bonding in mothers by reinforcing the association between the rewarding effects of NA activation and the pups.

A similar mechanism has also been found in prairie voles in which OT receptor antagonists applied directly to the NA inhibit mating-induced partner preference formation (Young et al., 2001). However, the present study further indicates for the first time that activation of OT receptors in this brain region is also necessary for long-term social memory. Whether OT acts directly on NA neurons or indirectly to consolidate the maternal memory is not known. Several lines of evidence do suggest, however, that OT interacts with the dopaminergic inputs to the NA. The NA is densely innervated by dopaminergic neurons of various DA receptor subtypes, many of which originate in the VTA (Setlow, 1997) and administration of a dopaminergic D_1/D_2 antagonist in the NA disrupts the consolidation of maternal memory (Parada et al., 2008). In addition, OT–DA interactions in the NA have been shown to regulate partner preference formation in the prairie vole. Blockade of OT receptors prevent partner preferences induced by a D_2 agonist, whereas blockade of D_2 receptors blocked OT-induced partner preferences (Liu and Wang, 2003). In the present study, while the effect of the OT receptor antagonist was evident, it was not long-lasting, and the possibility exists that a stronger effect would be produced if both DA and OT receptor antagonists were administered simultaneously. Similarly, one might predict greater enhancement of maternal memory with combined infusions of DA and OT agonists.

The nature of OT–DA interactions is not known. However, there is some evidence showing a facilitation of catecholamine release by OT. In rodents, OT facilitates noradrenergic release in the olfactory bulb thereby enhancing social recognition through an alpha-adrenergic mediated mechanism (Brennan and Keverne, 1997; Dluzen et al., 2000). In the ewe, OT stimulates the release of noradrenalin in the OB and the MPOA where it facilitates behavior associated with recognition and reduced aggression towards the lamb (Da Costa et al., 1996; Lévy et al, 1995). Infusion of OT directly into the VTA in rats also enhances extracellular DA concentrations in the NA indicating an effect of OT on the activity of dopamine neurons of the VTA (Melis et al., 2007; Shahrokh et al., 2010). Thus, it is possible that OT could participate in the consolidation of maternal memory by facilitating dopamine release within the NA. Recently, Numan and colleagues have proposed neural circuits regulating maternal behavior in rats (Numan and Insel, 2003; Numan et al., 2006; Numan and Stolzenberg, 2009). In particular, connections from the MPOA indirectly through the dopaminergic projections of the VTA to the NA would be essential for maternal behavior. In addition, DA activation of the NA is critically involved in the consolidation of maternal memory (Parada et al., 2008). Thus, in this model, it is tempting to speculate that a co-action of OT and DA at the level of the NA and between the VTA and NA during maternal experience would reinforce and consolidate the memory for the rewarding value of pups.

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