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Oxytocin Has Dose-Dependent Developmental Effects on Pair-bonding and Alloparental Care in Female Prairie Voles

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Abstract

The present study examines the developmental consequences of neonatal exposure to oxytocin on adult social behaviors in female prairie voles (*Microtus ochrogaster*). Female neonates were injected within 24 hours of birth with isotonic saline or one of four dosages of oxytocin (OT). As adults, females were tested in an elevated plus-maze paradigm (a measure of anxiety and exploratory behavior), and for alloparental behavior and partner preferences. At 2 mg/kg OT, females took longer to approach pups, but were the only group to form a statistically significant within-group partner preference. At 4 mg/kg OT, females retrieved pups significantly more frequently but no longer displayed a partner preference; while females treated developmentally with 8 mg/kg spent significantly more time in side-to-side contact with a male stranger than any other treatment group. OT may have broad developmental consequences, but these effects are not linear and may both increase and decrease the propensity to display behaviors such as pair-bonding.

The neuropeptide oxytocin (OT) has been implicated in social behavior in both male and female mammals. OT's peripheral effects include the induction of parturition and milk let-down, while central OT has been associated with the onset of maternal behavior (Pedersen and Prange Jr., 1979;Pedersen et al., 1982;Pedersen and Boccia, 2002), and other positive social behaviors such as pair-bonding (Insel and Hulihan, 1995;Carter and Altemus, 1997;Cho et al., 1999) and male parental care (Bales et al., 2004b). It is also becoming evident that manipulations of OT and a related peptide, arginine vasopressin (AVP) during the perinatal period of life can have life-long implications for social behavior and neuroendocrine function (Stribley and Carter, 1999;Diaz-Cabiale et al., 2000;Carter, 2003;Lipschitz et al., 2003). These exposures can be either endogenous, through responses to touch, warmth (Uvnas-Moberg, 1998) or perhaps through OT in breast milk (Leake et al., 1981); they could also be pharmacological, for example through use of pitocin or an oxytocin antagonist to manipulate labor (Husslein, 2002).

In this context, we have begun to examine the consequences of OT manipulations in early life for subsequent social behaviors and neuroendocrine variables. Because we were particularly interested in the developmental control of social behaviors such as pair-bond formation and biparental behaviors, we chose to study the socially monogamous prairie vole (*Microtus ochrogaster*). In this species both males and females display parental care and form selective partner preferences indicative of pair-bonding, although the mechanisms for these behaviors

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may differ between the sexes (DeVries et al., 1996;DeVries and Villalba, 1997). OT may be particularly related to pair-bond formation and parenting behavior in female voles (Liu and Wang, 2003;Olazabal and Young, 2006), and is also associated with mating (Carter, 1992).

Initial studies involved injecting newborn prairie voles with OT at a dosage used in previous studies of rats [1 mg/kg; (Sohlstrom et al., 2000)]. In voles treated with this dosage, the effects of OT manipulations were especially apparent in males. For example, in male prairie voles neonatal OT facilitated pair-bond formation (Bales and Carter, 2003b); neonatal OT was also associated with a later, sexually dimorphic increase in AVP (V1a) receptor binding in the ventral pallidum and cingulate cortex (Bales et al., 2007b). Males of this species treated with an OTA (at 0.1 mg/kg) exhibited decreased levels of parental care (Bales et al., 2004c), a tendency to be less aggressive (Bales and Carter, 2003a), and as adults showed lower levels of V1a binding in the bed nucleus of the stria terminalis, the lateral septum, and the medial preoptic area (Bales et al., 2007b).

In females neonatal treatment with OT (1 mg/kg) was associated in adulthood with an increase in female-female aggression after exposure to a male (“mate-guarding”); however, other behaviors including partner preferences and parental care were not measurably affected by this treatment (Bales and Carter, 2003a;Bales et al., 2004c). Thus, in this series of studies of the developmental effects of OT the most marked behavioral changes were observed in males (Carter, 2003). These studies left open the possibility that female prairie voles were not responsive to the developmental effects of OT or that the dose level of OT previously used in females was insufficient to affect behavior. The present study examines the hypothesis that female prairie voles will show a dose-dependent response to developmental exposure to OT. In this study a single exposure intraperitoneal (i.p.) injection of one of four dosages of OT (1, 2, 4, or 8 mg/kg) or the isotonic saline vehicle was administered to prairie vole pups within 24 hours of birth. We hypothesized that behaviors such as female parental care and pair-bonding that rely on OT would be facilitated by neonatal exposure to OT, and that anxiety, which in adult voles may be reduced by OT (Carter, 1998), would decrease following neonatal exposure to additional OT. The wide range of doses used here was selected because, although the behavioral effects of peptides are often dose-dependent, it is common for the effects of comparatively low versus high doses to differ.

Methods

Subjects and Neonatal Treatments

All procedures adhered to the standards set forth in the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committees at the University of Illinois at Chicago and the University of California, Davis. Subjects were laboratory-bred female prairie voles, descendants of a wild stock originally caught near Champaign, Illinois. Stock was systematically outbred. Prairie voles were maintained on a 14:10 light:dark cycle and allowed food (Purina high-fiber rabbit chow) and water *ad libitum*. Breeding pairs were maintained in large polycarbonate cages (44 cm long × 22 cm wide × 16 cm high) and provided with cotton for nesting material. At 21 days of age, females were weaned and housed in same-sex sibling pairs in smaller (27 cm long × 16 cm wide × 13 cm high) cages. The same-sex sibling pairs then were housed in a single-sex colony room.

Within 24 hrs of birth, experimental subjects were briefly removed from the cage, sexed, weighed, and toe-clipped for identification. Female voles were randomly assigned to treatment groups, receiving either an intraperitoneal injection of isotonic saline (SAL), 1 mg/kg OT, 2 mg/kg OT, 4 mg/kg OT, or 8 mg/kg OT. Injections were given based on an average weight of a newborn vole pup of 3 grams. All injections were 25.0 μl in volume and administered in a 250 μl gas-tight Hamilton syringe. Other studies have shown that neonatal OT, as administered

here, can at 1 mg/kg produce a rapid change in cFos expression (Cushing et al., 2003). Only one female in each litter was given a particular neonatal treatment. An initial set of subjects was studied at the University of Illinois at Chicago; following the move of the first author, a second set of subjects (including appropriate controls) were added to the study at the University of California, Davis. Methods and husbandry at both sites were administered by the first author and were as identical as possible.

Behavioral Testing

During behavioral testing, females were fully adult and sexually mature, although prairie voles are induced ovulators (Carter et al., 1987) and estrus stage was therefore not a concern. At day 55, females were tested in an elevated plus-maze (EPM). The EPM consisted of two open and two closed arms, each 67 cm long and 5.5 cm in width (Insel et al., 1995). Each vole was placed in the neutral area in the center of the EPM, and behavior was recorded for 5 min using Behavior Tracker 1.0 (www.behaviortracker.com). Any vole that jumped or fell off the EPM was immediately replaced and the test finished (this happened 6 times). Statistical analysis was performed both with and without the voles that fell, and their inclusion or exclusion made no difference to the significance of the results. The time spent in the open arm divided by the total time spent in the open and closed arms was analyzed. Following the EPM test, the voles were returned to their home cage. In rats, more time spent in the open arm of the EPM is considered indicative of higher levels of exploratory behavior and lower levels of anxiety. In voles, behavior in the EPM is responsive to changes in state. For instance, mating experience (Insel et al., 1995), treatment with AVP (Dharmadhikari et al., 1997) and neonatal handling (Bales et al., 2007a) are associated with increased time spent in the open arm of the EPM in prairie voles. Auto-grooming, which may also be indicative of anxiety, also was quantified during the EPM test.

On day 62 (1 week later), voles were tested for alloparental behavior in response to a pup. Procedures for testing parental behavior were modeled on previous work done in this lab (Roberts et al., 1998;Bales et al., 2004b;Bales et al., 2004c;Bales et al., 2006). Females were first given 45 min to adjust to an empty testing arena. The test apparatus consisted of two small cages (sizes the same as previously mentioned) connected by a short, clear tube. At the end of the acclimation period, the subject was removed from the testing arena and an unrelated 1−3 day old pup was placed in the front cage. The subject then was placed into the tube, and behavior was recorded for 10 min on videotape. If the test subject displayed aggression towards the pup, the test was stopped immediately, and the pup was removed and returned to its parents. In most cases, the pup was not injured, and this protocol resulted in a full recovery for the pup. Tapes were scored by an experimentally blind observer using Behavior Tracker 1.0 software. Following the test, females were returned to their home cages. Behaviors scored included latency to approach within one inch of the infant, sniffing the infant, retrievals, licking the infant, or non-huddling contact, which indicated that the test animal was touching the infant without engaging in any of the other behaviors.

On day 69 each female was tested for the development of a partner preference following a protocol that has been used extensively to study pair bonding in voles (Williams et al., 1992). The experimental female was exposed to a previously unfamiliar, randomly selected male for a 30-min period. This short cohabitation period was necessary, as a 1-hr cohabitation period was sufficient for the development of female partner preference under our present colony conditions. Cohabitation began between 9 and 10 am near the onset of the light cycle (which was at 6 am). Randomly assigned male and female partners were placed in a neutral cage during this period. Immediately following the cohabitation period, females were placed in a testing apparatus where they were given a choice over a 3-hr test period of spending time with the now familiar partner; with an unfamiliar opposite-sex animal (otherwise comparable to the

partner; here called the stranger); or in an empty, neutral cage. Testing was conducted using an apparatus consisting of three identical polycarbonate cages (mouse cages as described above), attached by Plexiglas tubes (7.5 × 16.0 cm). The experimental vole was free to move about the apparatus while the two stimulus voles were loosely tethered within their own separate chambers. It was physically possible for copulation to occur; however, because prairie voles are induced ovulators (Carter et al., 1980;Carter et al., 1987), it was unlikely. In addition, cohabitation periods and testing were monitored to insure that no copulation took place. Tests were recorded in time-lapse video and scored later by an experimentally blind observer on Behavior Tracker 1.0, with the relevant variables being the time spent in physical side-to-side contact with the partner vs. the stranger. Social preferences also were examined as the difference in time spent in side-to-side contact with the partner and time spent in side-to-side contact with the stranger. Following the test, females were returned to their home cage.

Data analysis

Data analysis for continuous variables was carried out by analysis of variance, a.k.a. ANOVA (Littell et al., 1996) in SAS 8.2. Residuals were checked for normality and other conditions and, if non-normal, data were transformed using the log or square root transformations, or analyzed using a non-parametric Kruskal-Wallis test when transformation was unsuccessful (Sokal and Rohlf, 1981). Following significant ANOVA, post-hoc tests were carried out via least-squared means. All tests were two-tailed and significance was set at $p < 0.05$.

Results

Plus-maze behavior:

Treatment did not affect the ratio of time that females spent in the open arm [divided by time spent in the open arm + time spent in the closed arm; $F(4) = 1.07$, $p = 0.375$]. Means ± standard errors for this ratio were as follows (lower ratios reflect more time spent in the closed arm): SAL: 0.32 ± 0.07; 1 mg/kg OT: 0.36 ± 0.08; 2 mg/kg OT: 0.44 ± 0.09; 4 mg/kg: 0.48 ± 0.08; and 8 mg/kg: 0.31 ± 0.06.

Early OT treatment also did not affect time spent in the open arm [$F(4) = 1.45$, $p = 0.226$], the closed arm [$\chi^2(4) = 2.43$, $p = 0.658$], the center of the maze [$\chi^2(4) = 2.27$, $p = 0.687$], or time spent autogrooming [$\chi^2(4) = 3.08$, $p = 0.544$].

Alloparental care:

Proportion of females attacking pups did not differ between treatment groups (Fisher's exact test, $p = 0.609$). SAL females attacked in 41% of tests, 1 mg/kg OT females 21%, 2 mg/kg OT females 20%, 4 mg/kg OT females 31%, and 8 mg/kg OT females attacked in 27% of tests. Further analyses of individual behaviors were carried out only on females that did not attack pups.

Significant treatment differences were found in the frequency of retrievals [overall ANOVA: $F(4) = 3.39$, $p = 0.015$; Figure 1] and in the latency to approach the pup within one inch [overall ANOVA: $F(4) = 2.81$, $p = 0.034$; Figure 2]. Females treated with 4 mg/kg OT retrieved significantly more frequently than SAL females (post-hoc test; $t = 3.43$, $p < 0.01$), while females treated with 1 mg/kg OT showed a non-significant trend to retrieve pups more frequently (post-hoc test; $t = 1.74$, $p = 0.089$). Latency to approach the pup was significantly higher in females treated with 2 mg/kg OT than in SAL females (post-hoc test; $t = 2.15$, $p = 0.036$). All other variables did not differ significantly by treatment, and their means and standard errors are presented in Table 1.

Partner preference behavior:

Early treatment with OT significantly affected the amount of time that females spent in side-to-side contact with a stranger [Figure 3; Kruskal-Wallis test, $\chi^2(4) = 10.76$, $p = 0.029$], but not time spent in side-to-side contact with the partner [Kruskal-Wallis test, $\chi^2(4) = 2.39$, $p = 0.665$] or total time spent in side-to-side contact [Kruskal-Wallis test, $\chi^2(4) = 3.44$, $p = 0.487$].

In post-hoc tests on time spent in side-to-side contact with the stranger, females treated with 8 mg/kg OT spent significantly more time in this behavior than any other treatment group except for the 4 mg/kg OT group (difference from SAL, Mann-Whitney U test, $U = 306.00$, $p = 0.021$; from 1 mg/kg OT, $U = 211.0$, $p = 0.01$; from 2 mg/kg OT, $U = 218.0$, $p = 0.004$; from 4 mg/kg OT, $U = 189.0$, $p = 0.313$). In contrast, females treated with 2 mg/kg OT were the only group to exhibit a statistically significant preference for their partner (Figure 3, indicated by an asterisk; $t = 2.42$, $p = 0.031$).

We also examined a more synthetic measure of preference (difference between time spent in side-to-side contact with the partner and time spent in side-to-side contact with the stranger). The effect of early OT treatment on this variable was also statistically significant (Kruskal-Wallis test, $\chi^2(4) = 10.05$, $p = 0.04$). Using this measure, females in the 2 mg/kg OT group differed significantly in their pattern of preferences from the 8 mg/kg OT group (Mann-Whitney U test, $U = 105.5$, $p = 0.004$), and tended to differ from the SAL group (Mann-Whitney U test, $U = 348.5$, $p = 0.081$).

Early treatment with OT also affected time that females spent totally apart from social contact in the empty cage [Kruskal-Wallis test, $\chi^2(4) = 11.89$, $p = 0.018$]. Means and standard errors were as follows: SAL females, 17.4 ± 1.6% time in empty cage; 1 mg/kg OT: 21.9 ± 3.4%; 2 mg/kg OT 20.5 ± 1.9%; 4 mg/kg OT: 19.3 ± 2.5%; 8 mg/kg OT: 11.6 ± 2.2%. The 8 mg/kg OT females spent significantly less time in the empty cage than all other dosages in post-hoc tests (difference from SAL, Mann-Whitney U test, $U = 149.0$, $p = 0.008$; from 1 mg/kg OT, $U = 121.0$, $p = 0.037$; from 2 mg/kg, $U = 99.0$, $p = 0.001$, from 4 mg/kg, $U = 121.0$, $p = 0.032$).

Discussion

Research in socially monogamous prairie voles has revealed that both OT and AVP play major roles in the expression of social behaviors, including pair bonding (Winslow et al., 1993;Williams et al., 1994;Cho et al., 1999) and parental behaviors (Wang et al., 1994;Wang et al., 1998;Pedersen and Boccia, 2002;Bales et al., 2004b). In addition, several studies have shown that exposure to these same peptides during development has long-term consequences for neuroendocrine and behavioral systems (Stribley and Carter, 1999;Carter, 2003;Bales and Carter, 2003a;Bales and Carter, 2003b;Bales et al., 2004a;Bales et al., 2004c). The present study is unique in showing long-lasting, dose-dependent changes in social behavior following neonatal OT exposure in female prairie voles.

The tendency to develop social preferences has been used as a defining feature of socially monogamous species (Kleiman, 1977). However, as shown here the expression of preference behavior also is sensitive to early hormonal experiences. In this study, we showed that developmental exposure to exogenous OT can alter female preferences for partners vs. strangers after a brief period of cohabitation (Figure 3). Although in this paradigm the 1 mg/kg dose did not significantly facilitate partner preference formation, exposure to 2 mg/kg was associated in later life with a statistically significant partner preference (although differences from saline controls were only at the trend level). Unexpected in this context is the finding that females treated to higher dosages of OT (4 mg/kg and 8 mg/kg dosages) did not show a preference for the familiar partner. Rather than showing this preference, females in the 8 mg/kg group spent significantly more time in contact with a stranger. Stranger preferences have

also been previously demonstrated in female voles which were given exogenous corticosterone (DeVries et al., 1996). In both cases it is possible that state changes in these females (such as changes in emotionality or anxiety) caused them to avoid the familiar partner; however, the partner preference test provides the experimental animal with the option of avoiding either partner, thus avoiding social contact in general. That is not the case here; in fact females from the 8 mg/kg group, in comparison to other groups, spent the least amount of time alone, suggesting that this group was not simply asocial. It is possible that these animals were seeking novelty, or lacked fear of a strange animal. Alternatively, exposure to early OT might have led to the development of neural systems associated with a tendency to seek variety in partners and polygamy.

In the present study, we observed that developmental exposure to OT was associated in adulthood with a dose-dependent tendency to show alloparenting behavior. Behavior in the EPM was not significantly affected. Both behavior in the EPM (Ramos and Mormede, 1998) and parental behavior may be indicative of a reduction in anxiety or fear (Fleming and Leubke, 1981;Fleming and Corter, 1995;Bales et al., 2004c). Although EPM behavior did not differ significantly by treatment, neonatal treatment with OT did affect measures of parental behavior (latency to approach infants, and frequency of retrievals). In another experiment, neonatal exposure to an OT antagonist (OTA, 0.1 mg/kg) affected neural activation to a stressor in later life, as measured by c-Fos expression in the central nucleus of the amygdala (Kramer et al., 2006). These findings support the hypothesis that reactivity to stressful experiences can be permanently altered by developmental manipulations of peptide systems.

In general, the central mechanisms for these changes remain to be investigated. There is considerable evidence from between species comparisons that monogamous and nonmonogamous mammals have differences in the distribution of peptide receptors including those for OT and AVP (Witt et al., 1991;Shapiro and Insel, 1992;Insel et al., 1994). In addition, we have observed that early manipulations of OT, even at the lowest dose used here (1 mg/kg), are capable of producing life-long, sexually-dimorphic changes in peptide receptors (Bales et al., 2007b) . In female prairie voles neonatal treatment with OT reduced vasopressin (V1a) receptor binding in several brain regions including the medial preoptic area, bed nucleus of the stria terminalis, lateral septum, and mediodorsal thalamus, without significantly affecting OT receptors or dopamine D2 receptors. Using, methods similar to those of the present study, Yamamoto et al. (2004) found that early exposure to a single dose (1 mg/kg) of OT produced a significant increase in OT immunoreactivity in the PVN; however, those measurements were taken at weaning (21 days of age) while the females in this study were adult. Another study (Kramer et al., 2006) found that neonatal OT exposure resulted in reduced AVP immunoreactivity in the SON in adult females; in addition, neonatal OTA resulted in increased cFos expression in the central amygdala after exposure to a male. It is possible that changes in either OT or AVP peptide or receptors (or other systems) could be affecting females at different dosages, and that the mechanisms underlying the changes observed here might be different for each dosage.

In summary, the data from this experiment support the hypothesis that early manipulations of OT can have long-lasting behavioral and neuroendocrine effects. The results of the present study revealed that even within an individual the effects of neonatal OT may differ across behavioral measures. In addition, exposure to OT in the neonatal period, although capable of facilitating pup-directed behavior at one dosage, appeared to interfere with – rather than facilitate - the development of preferences for familiar partners at another dosage.

In previous studies, neonatal OT, at 1 mg/kg, tended to either have no effect or to facilitate social behaviors in females (Bales and Carter, 2003a;Bales et al., 2004c). When compared to earlier studies in male prairie voles, the present study suggests that females may differ from

males in their sensitivity to neonatal peptide manipulations (Bales and Carter, 2003b;Bales et al., 2004c). The effects of higher doses of OT in males remain unknown. However, there are indications that male reproductive capacity and some aspects of sexual behavior may be disrupted by exogenous neonatal OT even at the 1 mg/kg dosage (Bales et al., 2004a). Earlier studies have revealed that a single exposure to an OTA has long-lasting behavioral and endocrine consequences (Bales et al., 2004a;Bales et al., 2004c). These effects were in general disruptive to behavior and physiology, with the most marked effects seen in males and on the AVP system (Yamamoto et al., 2004;Bales et al., 2007b). However, some negative consequences for sociality, such as a trend to be non-parental (Bales et al., 2004c), and more fearful following OTA (Kramer et al., 2006) also were seen in females.

OT (pitocin) is widely used in obstetrics to facilitate labor and reduce potential postpartum hemorrhage. In addition, OT antagonists are clinically available as tocolytics, for the prevention of prematurity (Husslein, 2002). In both cases the behavioral or neuroendocrine effects for the human infant do not appear to have been investigated (Rojas Wahl, 2004). It is generally assumed that OT does not cross the placenta, although the integrity of this barrier may be affected by the birth process (Laudanski and Pierzynski, 2003). OT also is present in breast milk (Leake et al., 1981), but probably not in formula, and may be lowered by other obstetric practices such as Caesarian section (Nissen et al., 1996). The consequences for infant development of such manipulations have in general not been examined. The results of the present studies support the need for a deeper understanding of the developmental consequences of OT and related peptides.

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REFERENCES

Bales KL, Abdelnabi M, Cushing BS, Ottinger MA, Carter CS. Effects of neonatal oxytocin manipulations on male reproductive potential in prairie voles. Physiology & Behavior 2004a;81:519–526. [PubMed: 15135025]

Bales KL, Carter CS. Sex differences and developmental effects of oxytocin on aggression and social behavior in prairie voles (*Microtus ochrogaster*). Hormones and Behavior 2003a;44:178–184. [PubMed: 14609540]

Bales KL, Carter CS. Developmental exposure to oxytocin facilitates partner preferences in male prairie voles (*Microtus ochrogaster*). Behavioral Neuroscience 2003b;117:854–859. [PubMed: 12931969]

Bales KL, Kim AJ, Lewis-Reese AD, Carter CS. Both oxytocin and vasopressin may influence alloparental behavior in male prairie voles. Hormones and Behavior 2004b;45:354–361. [PubMed: 15109910]

Bales KL, Kramer KM, Lewis-Reese AD, Carter CS. Effects of stress on parental care are sexually dimorphic in prairie voles. Physiology & Behavior 2006;87:424–429. [PubMed: 16360186]

Bales KL, Lewis-Reese AD, Pfeifer LA, Kramer KM, Carter CS. Early experience affects the traits of monogamy in a sexually dimorphic manner. Developmental Psychobiology 2007a;49:335–342. [PubMed: 17455224]

Bales KL, Pfeifer LA, Carter CS. Sex differences and effects of manipulations of oxytocin on alloparenting and anxiety in prairie voles. Developmental Psychobiology 2004c;44:123–131. [PubMed: 14994263]

Bales KL, Plotsky PM, Young LJ, Lim MM, Grotte ND, Ferrer E, Carter CS. Neonatal oxytocin manipulations have long-lasting, sexually dimorphic effects on vasopressin receptors. Neuroscience 2007b;144:38–45. [PubMed: 17055176]

Carter CS. Neuroendocrine perspectives on social attachment and love. Psychoneuroendocrinology 1998;23:779–818. [PubMed: 9924738]

Carter CS. Developmental consequences of oxytocin. Physiology & Behavior 2003;79:383–397. [PubMed: 12954433]

Carter CS. Oxytocin and sexual behavior. Neurosci.Biobehav.Rev 1992;16:131–144. [PubMed: 1630727]

Carter CS, Altemus M. Integrative functions of lactational hormones in social behavior and stress management. Annals of the New York Academy of Sciences 1997;807:164–174. [PubMed: 9071349]

Carter CS, Getz LL, Gavish L, McDermott JL, Arnold P. Male-related pheromones and the activation of female reproduction in the prairie vole (Microtus ochrogaster). Biology of Reproduction 1980;23:1038–1045. [PubMed: 7008851]

Carter CS, Witt DM, Schneider J, Harris ZL, Volkening D. Male stimuli are necessary for female sexual behavior and uterine growth in prairie voles (*Microtus ochrogaster)*. Hormones and Behavior 1987;21:74–82. [PubMed: 3549517]

Cho MM, DeVries AC, Williams JR, Carter CS. The effects of oxytocin and vasopressin on partner preferences in male and female prairie voles (*Microtus ochrogaster*). Behavioral Neuroscience 1999;113:1071–1079. [PubMed: 10571489]

DeVries AC, DeVries MB, Taymans SE, Carter CS. The effects of stress on social preferences are sexually dimorphic in prairie voles. Proceedings of the National Academy of Sciences 1996;93:11980–11984.

Devries GJ, Villalba C. Brain sexual dimorphism and sex differences in parental and other social behaviors. Annals of the New York Academy of Sciences 1997;807:273–286. [PubMed: 9071357]

Dharmadhikari A, Lee YS, Roberts RL, Carter CS. Exploratory behavior correlates with social organization and is responsive to peptide injections in prairie voles. Annals of the New York Academy of Sciences 1997;807:610–612. [PubMed: 9071412]

Diaz-Cabiale Z, Petersson M, Narvaez JA, Uvnas-Moberg K, Fuxe K. Systemic oxytocin treatment modulates alpha 2-adrenoceptors in telencephalic and diencephalic regions of the rat. Brain Research 2000;887:421–425. [PubMed: 11134634]

Fleming, AS.; Corter, CM. Psychobiology of maternal behavior in nonhuman mammals. In: Bornstein, MH., editor. Handbook of parenting, Volume 2: biology and ecology of parenting. Lawrence Erlbaum Associates; Mahwah, NJ: 1995. p. 59-85.

Fleming AS, Leubke C. Timidity prevents the virgin female rat from being a good mother: Emotionality differences between nulliparous and parturient females. Physiology & Behavior 1981;27:863–868. [PubMed: 7323193]

Husslein P. Development and clinical experience with the new evidence-based tocolytic atosiban. Acta Obstetricia et Gynecologica Scandinavica 2002;81:633–641. [PubMed: 12190838]

Insel TR, Hulihan TJ. A gender-specific mechanism for pair bonding - oxytocin and partner preference formation in monogamous voles. Behavioral Neuroscience 1995;109:782–789. [PubMed: 7576222]

Insel TR, Preston S, Winslow JT. Mating in the monogamous male: Behavioral consequences. Physiology & Behavior 1995:615–627. [PubMed: 7777594]

Insel TR, Wang ZX, Ferris CF. Patterns of brain vasopressin receptor distribution associated with social organization in microtine rodents. Journal of Neuroscience 1994;14:5381–5392. [PubMed: 8083743]

Kleiman DG. Monogamy in mammals. Quarterly Review of Biology 1977;52:39–69. [PubMed: 857268]

Kramer KM, Choe C, Carter CS, Cushing BS. Developmental effects of oxytocin on neural activation and neuropeptide release in response to social stimuli. Hormones and Behavior 2006;49:206–214. [PubMed: 16112115]

Laudanski T, Pierzynski P. Oxytocin and fetal membranes in preterm labor: current concepts and clinical implication. Gynecological Endocrinology 2003;17:261–267. [PubMed: 12857435]

Leake RD, Weitzman RE, Fisher DA. Oxytocin concentrations during the neonatal period. Biology of the Neonate 1981;39:127–131. [PubMed: 7295833]

Lipschitz DL, Crowley WR, Bealer SL. Central blockade of oxytocin receptors during late gestation disrupts systemic release of oxytocin during suckling in rats. Journal of Neuroendocrinology 2003;15:743–748. [PubMed: 12834434]

Littell, R.; Milliken, GA.; Stroup, WW.; Wolfinger, RD. SAS System for Mixed Models. SAS Institute Inc.; Cary, NC: 1996.

Liu Y, Wang ZX. Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female prairie voles. Neuroscience 2003;121:537–544. [PubMed: 14568015]

Nissen E, Uvnas-Moberg K, Svensson K, Stock S, Widstrom AM, Winberg J. Different patterns of oxytocin, prolactin but not cortisol release during breastfeeding in women delivered by caesarean section or by the vaginal route. Early Human Development 1996;45:103–118. [PubMed: 8842644]

Olazabal DE, Young LJ. Oxytocin receptors in the nucleus accumbens facilitate “spontaneous” maternal behavior in adult female prairie voles. Neuroscience 2006;141:559–568. [PubMed: 16725274]

Pedersen CA, Ascher JA, Monroe YL, Prange AJ Jr. Oxytocin induces maternal behavior in virgin female rats. Science 1982;216:648–650. [PubMed: 7071605]

Pedersen CA, Boccia ML. Oxytocin links mothering received, mothering bestowed, and adult stress responses. Stress 2002;5:267.

Pedersen CA, Prange AJ Jr. Induction of maternal behavior in virgin rats after intracerebroventricular administration of oxytocin. Proceedings of the National Academy of Sciences 1979;76:6661–6665.

Ramos A, Mormede P. Stress and emotionality: a multidimentional and genetic approach. Neuroscience and Biobehavioral Reviews 1998;22:33–57. [PubMed: 9491939]

Roberts RL, Williams JR, Wang AK, Carter CS. Cooperative breeding and monogamy in prairie voles: influence of the sire and geographical variation. Animal Behaviour 1998;55:1131–1140. [PubMed: 9632499]

Rojas Wahl RU. Could oxytocin administration during labor contribute to autism and related behavioral disorders? a look at the literature. Medical Hypotheses 2004;63:456–460. [PubMed: 15288368]

Shapiro LE, Insel TR. Oxytocin receptor distribution reflects social organization in monogamous and polygynous voles. Ann.N.Y.Acad.Sci 1992;652:448–451. [PubMed: 1320837]

Sohlstrom A, Carlsson C, Uvnas-Moberg K. Effects of oxytocin treatment in early life on body weight and corticosterone in adult offspring from ad libitum-fed and food-restricted rats. Biology of the Neonate 2000;78:33–40. [PubMed: 10878420]

Sokal, RR.; Rohlf, FJ. Biometry. W.H. Freeman and Company; New York: 1981.

Stribley JM, Carter CS. Developmental exposure to vasopressin increases aggression in adult prairie voles. Proceedings of the National Academy of Sciences 1999;96:12601–12604.

Uvnas-Moberg K. Oxytocin may mediate the benefits of positive social interactions and emotions. Psychoneuroendocrinology 1998:819–835. [PubMed: 9924739]

Wang ZX, Ferris CF, Devries GJ. Role of septal vasopressin innervation in paternal behavior in prairie voles (*Microtus ochrogaster*). Proceedings of the National Academy of Sciences 1994;91:400–404.

Wang ZX, Young LJ, De Vries GJ, Insel TR. Voles and vasopressin: A review of molecular, cellular, and behavioral studies of pair bonding and paternal behaviors. Progress in Brain Research 1998;119:483–499. [PubMed: 10074808]

Williams JR, Catania KC, Carter CS. Development of partner preferences in female prairie voles (*Microtus ochrogaster*): The role of social and sexual experience. Hormones and Behavior 1992;26:339–349. [PubMed: 1398553]

Williams JR, Insel TR, Harbaugh CR, Carter CS. Oxytocin centrally administered facilitates formation of a partner preference in female prairie voles (*Microtus ochrogaster*). Journal of Neuroendocrinology 1994:247–250.. [PubMed: 7920590]

Winslow JT, Hastings N, Carter CS, Harbaugh CR, Insel TR. A role for central vasopressin in pair bonding in monogamous prairie voles. Nature 1993;365:545–548. [PubMed: 8413608]

Witt DM, Carter CS, Insel TR. Oxytocin receptor binding in female prairie voles - Endogenous and exogenous estradiol stimulation. Journal of Neuroendocrinology 1991;3:155–161.

Yamamoto Y, Cushing BS, Kramer KM, Epperson PD, Hoffman GE, Carter CS. Neonatal manipulations of oxytocin alter expression of oxytocin and vasopressin immunoreactive cells in the paraventricular nucleus of the hypothalamus in a gender specific manner. Neuroscience 2004;125:947–955. [PubMed: 15120854]

Yamamoto Y, Cushing BS, Kramer KM, Epperson PD, Hoffman GE, Carter CS. Neonatal manipulations of oxytocin alter expression of oxytocin and vasopressin immunoreactive cells in the paraventricular

nucleus of the hypothalamus in a gender-specific manner. Neuroscience 2004;125:947–955. [PubMed: 15120854]

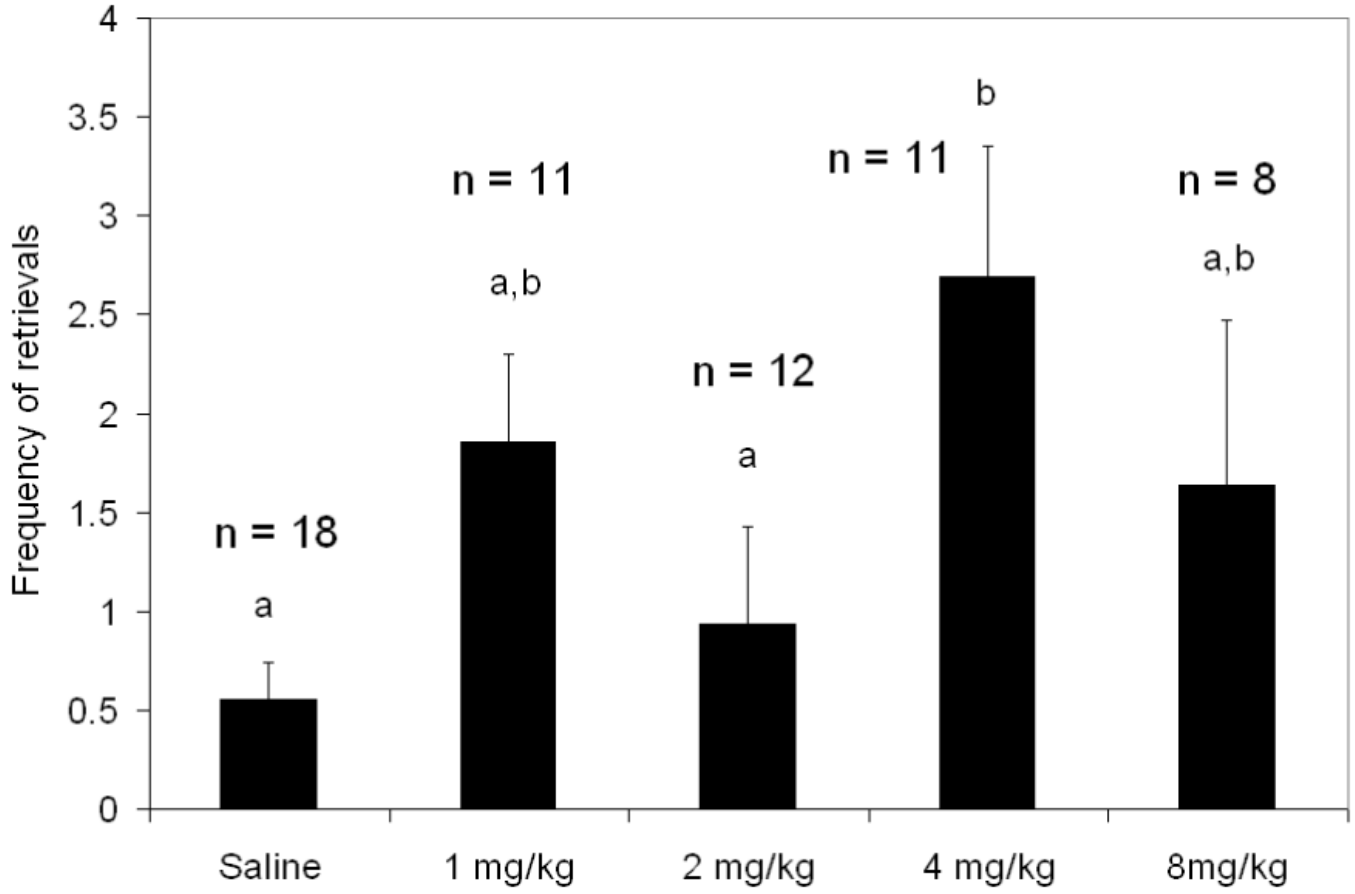

Figure 1.
Effects of early OT treatment on frequency of pup retrievals (overall ANOVA: $F(4) = 3.39$, $p = 0.015$). This includes only females that did not attack the pup. Groups which are statistically different according to post-hoc testing are marked with different letters; groups marked with the same letters are not statistically different.

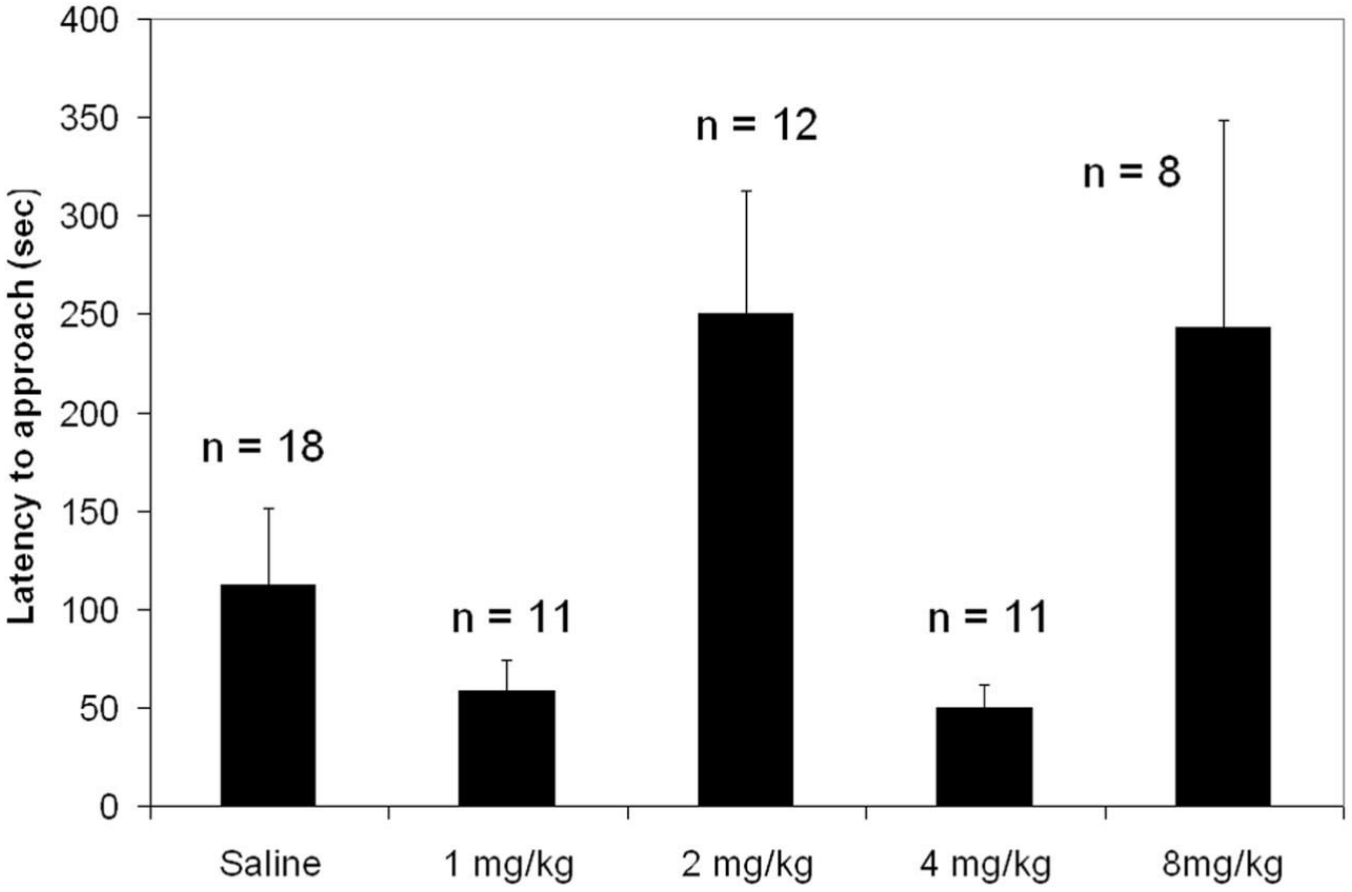

Figure 2.
Effects of early OT treatment on latency (in seconds) to approach within one inch of the pup (overall ANOVA: $F(4) = 2.81$, $p = 0.034$). This includes only females that did not attack the pup. Groups which are statistically different according to post-hoc testing are marked with different letters; groups marked with the same letters are not statistically different.

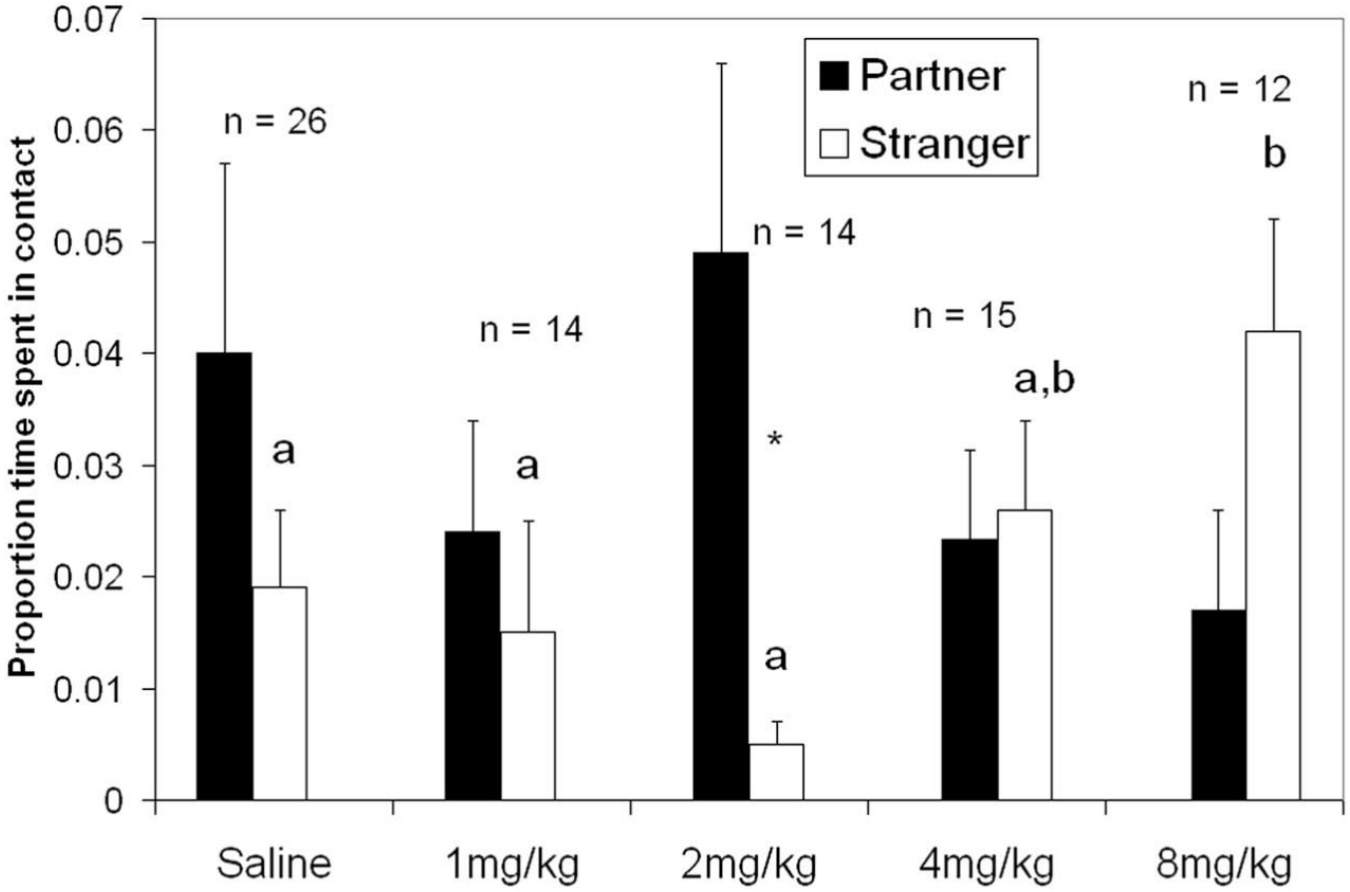

Figure 3.

Effects of early OT treatment on preferences for a partner vs. a stranger, measured as proportion of test time spent in side-to-side contact. Proportion of test time spent in side-to-side contact with a stranger differs significantly by treatment [χ^2 (4) = 10.76, p = 0.029], as does the difference between time spent with the partner and time spent with the stranger [χ^2 (4) = 10.05, p = 0.04].

Table 1

Means ± standard errors for behaviors displayed during the alloparenting test, for non-attacking females only (non-significant results only; for significant results see Figures 1 and 2).

	Saline n= 18	**1 mg/kg OT n = 11**	**2 mg/kg OT n = 12**	**4 mg/kg OT n = 11**	**8 mg/kg OT n = 8**
Sniff (sec)	12.4 ± 2.2	16.5 ± 4.2	11.3 ± 2.7	11.0 ± 2.3	8.3 ± 2.6
Lick (sec)	177.3 ± 44.3	206.5 ± 39.3	119.3 ± 37.8	174.0 ± 34.9	153.3 ± 51.2
Non-huddling contact (sec)	27.7 ± 13.9	45.2 ± 24.2	14.4 ± 6.8	24.1 ± 10.7	15.4 ± 9.9
Huddle (sec)	14.2 ± 8.8	3.5 ± 3.5	11.1 ± 11.1	49.7 ± 18.6	40.8 ± 27.5