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Sex Differences and Developmental Effects of Manipulations of Oxytocin on Alloparenting and Anxiety in Prairie Voles

ABSTRACT: *In adult animals, peptide hormones, including oxytocin and arginine vasopressin, have been implicated in both parental behavior and the modulation of anxiety. The purpose of this study was to examine the consequences of developmental manipulations of oxytocin for the later expression of alloparental behavior as well as behavioral responses to a novel environment, the elevated plus maze (EPM). Prairie voles (Microtus ochrogaster), a cooperatively breeding species, were selected for this study. On neonatal Day 1, pups received an ip injection of oxytocin or oxytocin antagonist, or were controls, receiving either saline or handling only. At 21 and approximately 60 days of age, each animal was tested for parental care toward novel stimulus pups. At approximately 67 days, an EPM test was administered. Control females at 60 days of age were more likely to attack pups and spent less time in the open arm of the EPM, both of which might reflect higher levels of anxiety in females than males. In males, neonatal treatment with oxytocin antagonist was associated with reductions in parental care, especially during the initial exposure to pups on Day 21. Female behavior was not significantly changed as a function of neonatal treatments. Findings to date implicate vasopressin in the behavioral changes in males, that in later life followed a single exposure to an oxytocin antagonist, and suggest caution in the clinical use of agents such as Atosiban, which may have the potential to influence infant development.*
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Keywords: *prairie vole; cooperative breeding; monogamy; alloparental care; oxytocin; vasopressin; anxiety*

In mammals, parental care is essential for survival. Studies of the biology of parental behavior traditionally have focused on the mother. However, biparental care is observed in several unrelated species including prairie voles (Roberts, Miller, Taymans, & Carter, 1998; Roberts, Williams, Wang, & Carter, 1998), and tamarin and marmoset monkeys (Goldizen, 1987) as well as humans (Fuentes, 1999). Each of these species exhibits a social system, sometimes described as cooperative breeding or social monogamy, consisting of a ''pair-bonded'' male and female and an extended family which may incorporate consecutive litters of offspring. Both the father and prepubertal juvenile offspring of these species tend to exhibit spontaneous care giving toward younger animals, also known as ''alloparenting'' (Solomon & French, 1997).

Research aimed at understanding the biology of parental behavior initially focused on steroid hormones,

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which fluctuate dramatically during pregnancy or parturition (Bridges, 1996). However, in many cooperative breeders, virgin females, fathers, and prepubertal animals are capable of high levels of spontaneous care giving, suggesting that hormonal changes associated with gestation or birth are not essential for these forms of parental behavior (Solomon & French, 1997).

Attempts to understand the mechanisms underlying parental behavior in nonparturient animals have directed attention to hypothalamic and pituitary hormones, including prolactin (Ziegler, 2000; Ziegler & Snowdon, 2000; Ziegler, Wegner, Carlson, Lazaro-Perea, & Snowdon, 2000; Ziegler, Wegner, & Snowdon, 1996), oxytocin (Pedersen, Ascher, Monroe, & Prange, 1982), and arginine vasopressin (AVP; De Vries & Villalba, 1997). Oxytocin (OT), in particular, is released during sexual behavior, labor, and lactation, and has been associated with the onset of maternal behavior (Fahrbach, Morrell, & Pfaff, 1985; Kendrick et al., 1997; Kendrick, Keverne, Hinton, & Goode, 1992; Pedersen, 1997; Pedersen et al., 1982; Pedersen, et al., 1995), pair bonding, and other positive social behaviors (Carter, 1998; Carter & Keverne, 2002). OT also may play a role in homeostatic responses to stressors (Uvnas-Moberg, 1998).

Most research on the role of neuropeptide hormones in parental behavior has involved adults (De Vries & Miller, 1998). However, the developmental history of an animal also can influence the tendency to be parental (Boccia & Pedersen, 2001; Meaney, 2001), possibly in part through effects on corticotropin-releasing-hormone (CRH) and OT and AVP receptors (Champagne, Diorio, Sharma, & Meaney, 2001; Francis, Young, Meaney, & Insel, 2002). In rats and mice, differential levels of parental behavior lead to different levels of OT and AVP receptor binding in adult offspring (Bester-Meredith & Marler, 2003; Francis et al., 2002). There also is evidence that the social behavior of young animals can be modified by OT (Boccia & Pedersen, 2001; Nelson & Panksepp, 1996, 1998). Furthermore, OT has been implicated in the regulation of the hypothalamic–pituitary–adrenal (HPA) axis, emotional reactivity, anxiety, and responses to novel stimuli, which could in turn influence the response to younger animals (Fleming, Cheung, Myhal, & Kessler, 1989; Fleming & Corter, 1995; Fleming et al., 2002; Fleming & Leubke, 1981; Uvnas-Moberg, 1998).

In addition to endogenous fluctuations in OT, this peptide also may be manipulated by medical or child-rearing procedures during the perinatal period. For example, synthetic OT (pitocin) is routinely used to induce uterine contractions, and Caesarean section or the duration of labor can influence maternal OT (Nissen, Gustavsson, Widstrom, & Uvnas-Moberg, 1998). OT is present in human milk (Leake, Wietzman, & Fisher, 1981), but probably not in infant formulas. An OT antagonist (Atosiban), intended to delay premature labor, is in use in Europe and in clinical trials in the United States (Husslein, 2002). Atosiban has been shown to cross the placental barrier relatively freely in nonhuman primate studies (Nathanielsz et al., 1997). Despite the widespread use of perinatal interventions that could affect exogenous OT or influence endogenous OT, the long-term consequences of such manipulations for the infant remain largely unexplored, even in animal models.

In the present study, we examined in prairie voles (*Microtus ochrogaster*) the possible long-term effects of neonatal manipulations of OT and an oxytocin receptor antagonist (OTA) on adult behavior. Prairie voles were chosen because of their unique social system, and because it is known that in adults of this species maternal behavior (Bales & Carter, 2002) and the regulation of the hypothalamic–pituitary–adrenal (HPA) axis (Carter, 1998) can be affected by OT. Although in this species alloparental behavior is observed in both males and females (Carter & Roberts, 1997), the physiological mechanisms underlying the behaviors of the two sexes may differ, with females more dependent on OT and males more reliant on the related peptide, AVP (Bales & Carter, 2002; De Vries & Villalba, 1997).

Male and female prairie voles received a single injection of OT or OTA or were assigned to one of two control groups (saline or handling only) on postnatal Day 1. The OTA selected for the present experiments has been used in several behavioral studies, and is known to block both OT and to some degree may serve as an antagonist at AVP receptors (Bankowski, Manning, Seto, Haldar, & Sawyer, 1980). Ongoing studies (e.g., Yamamoto et al., 2003) revealed that in males (but not females) a single neonatal treatment with the dose of OTA used here is capable of inhibiting AVP-immunoreactivity (IR) in hypothalamic tissue sampled on Day 21 postpartum. In contrast, in females (but not males) exposure to either OT or OTA on postnatal Day 1 is associated in later life with increases in OT-IR in the paraventricular nucleus of the hypothalamus (Yamamoto et al., 2002).

In the present study, males and females were tested twice for their response to pups: first on Day 21 and then at approximately Day 60. In nature, voles living in a communal group would be likely to be exposed to the birth of a new litter from their parents around postnatal Day 21 (McGuire, Getz, Hofman, Pizzuto, & Frase, 1993). By 60 days of age, animals may have offspring of their own (McGuire et al., 1993), but even unmated animals that remain in the natal nest may continue to engage in communal care of the young (Roberts, Williams, et al., 1998). As an independent assessment of emotionality, animals also were tested in an elevated plus maze (EPM) (Insel, Preston, & Winslow, 1995; Ramos & Mormède, 1998). In this test, a greater amount of time

spent in the closed, rather than open, arms of the maze is taken to indicate higher anxiety.

Based upon the known long-term effects of natural and pharmacological manipulations of perinatal peptides in rats (Francis et al., 2002; Sohlstrom, Carlsson, & Uvnas-Moberg, 2000), we hypothesized that early exposure to OT would enhance care-giving behavior and increase the tendency of animals to be exploratory in an EPM. At present, the commercially available OTAs (including the one used here and also Atosiban) can bind to both OT and AVP receptors. We predicted that OTA might have effects opposite to those of OT, possibly through long-lasting inhibitory effects on neural systems dependent on OT, AVP, or both. This experiment also examined possible sex differences in the response of prairie voles to pups and the EPM. We predicted that in comparison to males, females would display both lower levels of parental behavior and possibly higher levels of anxiety, and that the difference in parental behavior would increase with age.

METHODS

Subjects were laboratory-bred male and female prairie voles, descendants of a wild stock originally caught near Champaign, IL. Stock was systematically outbred. Animals were maintained on a 14:10 hr light:dark cycle and allowed food (Purina high-fiber rabbit chow) and water ad libitum. Breeding pairs were maintained in large, polycarbonate cages ($44 \times 22 \times 16$ cm) and provided with cotton for nesting material. At 21 days of age, offspring were removed and housed in same-sexed sibling pairs in smaller ($27 \times 16 \times 13$ cm) cages. Offspring were always removed before the birth of the next litter to prevent prior exposure to pups. The same-sex sibling pairs were then kept in single-sexed colony rooms.

Within 24 hr of birth, test subjects randomly received either a 3 μg injection of OT, a 0.3 μg injection of OTA, an injection of isotonic saline (SAL), or were handled without injection (HAN). All injections were 50 μl in volume and administered ip in 250 μl gas-tight Hamilton syringes. Litters of more than 6 pups were culled to 6. Within a litter, at least 1 pup of each sex received a control treatment (SAL or HAN) while another pup of each sex received a treatment (OT or OTA). No litter contained more than 1 pup of each sex that received the same treatment. Litters that did not contain at least 2 infants of each sex were not used.

The OT receptor antagonist ([d(CH_2)$_5$, Tyr(Me)^2, Orn^8]-Vasotocin) used here was selected from the compounds designed by Bankowski and associates (1980), and is commercially available from Peninsula Laboratories (Alameda, CA). This compound has been tested extensively in behavioral studies including sexual (Argiolas, Melis, Vargiu, & Gessa, 1987) and feeding behavior (Arletti, Benelli, & Bertolini, 1989; Olson, Drutarosky, Stricker, & Verbalis 1991). A lower dose of OTA than OT was used because in studies in rats, OTA has been shown to be approximately 10 to 100 times more effective in receptor binding than the natural ligand (Barberis & Tribollet, 1996). In one study, OTA blocked 75% of OT receptor binding for at least 6 hr after administration (Witt & Insel, 1991). The dosage of OT used here was based on previous studies in adult and infant prairie voles (Cho, DeVries, Williams, & Carter, 1999; Stribley & Carter, 1999) and rats (1 mg/kg body weight; Sohlstrom et al., 2000). Other studies have shown that neonatal OT and OTA as administered in this study can produce a rapid change in cFos expression (Cushing, Yamamoto, Hoffman, & Carter, 2003) and long-lasting changes in social behavior (Bales & Carter, 2003a, 2003b). OT has been shown to cross the blood–brain barrier in rodents, albeit in small amounts, when administered peripherally (Ermisch, Ruhle, Landgraf, & Hess, 1985; Jones & Robinson, 1982).

Each test subject received two parental care tests, one at weaning (approximately 21 days of age) and one in adulthood (approximately 60 days of age). All subjects were sexually inexperienced. Procedures for testing parental behavior were modeled on those of Roberts, Williams, et al. (1998). Animals were first given 45 min to adjust to an empty testing arena. The arena 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 test arena and 2 unrelated 1- to 3-day-old pups placed in the front cage. The subject then was placed into the arena, and behavior was recorded for 10 min on videotape. If the test subject displayed aggression towards the pups, the test was stopped immediately, and the pup was removed and returned to its parents. In most cases, pups were not injured, and this protocol resulted in a full recovery for the pup. Tapes were scored by an experimentally blind observer using Observer 3.0 behavioral software (Noldus, Inc., The Netherlands). Subjects were scored as either parental or nonparental, and as attacking or not attacking. A subject was said to be parental if it huddled over or retrieved an infant (without later attacking it; occasionally, an extremely short retrieval was a prelude to an immediate attack). Most ''parental'' subjects performed both huddling and retrieving.

Approximately 1 week after the second parental care test was administered, test animals were placed in an EPM. The EPM consisted of two open and two closed arms (each 67 cm long and 5.5 cm wide; Insel et al., 1995). Each vole was placed in the neutral area in the center of the EPM, and its behavior was recorded for 5 min using Observer 3.0. Any vole that jumped or fell off the EPM was eliminated from the dataset; differences in the percentage of animals removed for this reason were not significant across groups. Behaviors recorded included the total number of entries into any arm (referred to here as total activity) and the total time spent in the open or closed areas. We analyzed EPM behavior as time spent in the open arm divided by the total amount of time spent in the open arm and closed arms. It is possible that the EPM is less anxiogenic in voles than in rats; however, it is still responsive to changes in state. For instance, both treatment with AVP (Dharmadhikari, Lee, Roberts, & Carter, 1997) and neonatal handling (Bales, Lewis-Reese, & Carter, 2003) are associated with increased exploration in the EPM in male prairie voles.

Data Analysis

Sex Differences. At the beginning of all data analyses, data from the two control groups (HAN and SAL) were analyzed.

HAN and SAL groups did not show significant differences for any variables, and were therefore combined into one control group (CTL) for the remaining analyses. Data from CTL males and females were examined for possible sex differences in the tendency to show parental and anxiety behaviors. The percentages of animals displaying parental or attack behavior were analyzed using Fisher's exact test (Sokal & Rohlf, 1981). Sex differences in time spent in the open arm, divided by the total amount of time spent in the open and closed arms, were analyzed by ANOVA. Residuals were checked for normality, and if necessary, data were transformed using the square-root transformation (Sokal & Rohlf, 1981).

Treatment Differences. The percentages of animals of different treatments displaying parental or attack behavior were compared using Fisher's exact test (Sokal & Rohlf, 1981). Post hoc group comparisons also were carried out using Fisher's exact test. Individual behaviors such as huddling and licking also were scored and then compared for OT-treated and CTL males; no significant differences were found, and these data are therefore not presented here. Other groups, particularly OTA-treated males and adult females, had low numbers of responders, and individual behaviors were therefore not analyzed. Treatment differences in the plus-maze variables described earlier were analyzed by ANOVA, with post hoc comparisons carried out by least-squared means. All statistics were performed in SAS 8.0 (SAS Institute, Cary, NC), with a level of significance assigned at $p < 0.05$.

RESULTS

Sex and Age Differences

There were no sex differences in CTL animals in either parental or attack behavior on Day 21 ($n = 21$ males, 22 females), with both sexes highly responsive to infants. Seventy-three percent of CTL males and 64% of CTL females were responsive to infants, with 5% of CTL males and 9% of CTL females attacking.

However, on Day 60, males were more likely to be parental than females, due to a reduction in the tendency of females to care for young ($n = 21$ males, $n = 22$ females; $P = 0.013$, $p = 0.031$) (Figure 1). Day-60 females also tended to attack more than males ($P = 0.022$, $p = 0.054$). When analyzed by sex and treatment, the only group which showed a significant change by age (in attack behavior) was CTL females ($P = 0.003$, $p = 0.007$), which tended to attack more on Day 60 than on Day 21.

There was a sex difference in the use of the open and closed arms, with females apparently more ''anxious'' (Figure 2). Females from the CTL groups spent less time in the open arms (divided by total time in all arms) when compared to males, $F(1, 33) = 6.25$, $p = 0.02$; $n = 16$ males, 19 females.

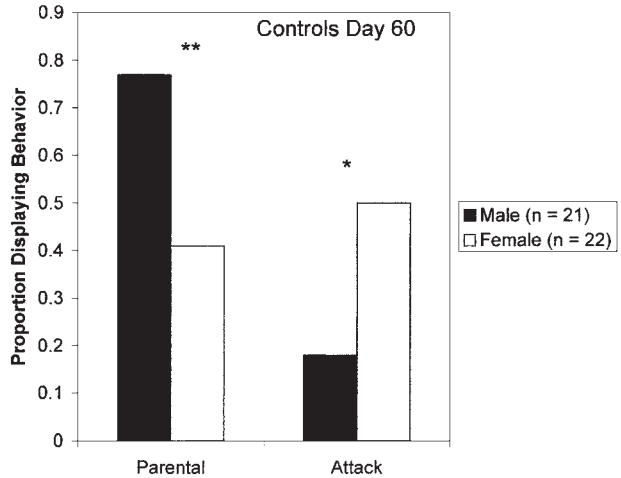

FIGURE 1 Parental and attack behavior (controls, Day 60) by sex. Males are significantly more likely to display parental behavior ($^{**}p = 0.031$) while females tend to attack more ($^{*}p = 0.054$).

Neonatal Treatment Effects

Parental Care. On Day 21, males showed a significant effect of treatment on parental behavior ($P = 0.002$, $p = 0.021$) (Figure 3a), with OTA-treated males displaying significantly lower rates of parental behavior than CTL males and tending to display less parental behavior than OT-treated males ($P = 0.051$, $p = 0.09$). There also was a treatment effect on attack behavior ($P = 0.004$, $p = 0.007$) (Figure 3a), with OTA-treated males displaying significantly higher rates of attack behavior than both OT-treated and CTL males. Female parental care ($P = 0.066$, $p = 0.932$) and attack behavior ($P = 0.044$,

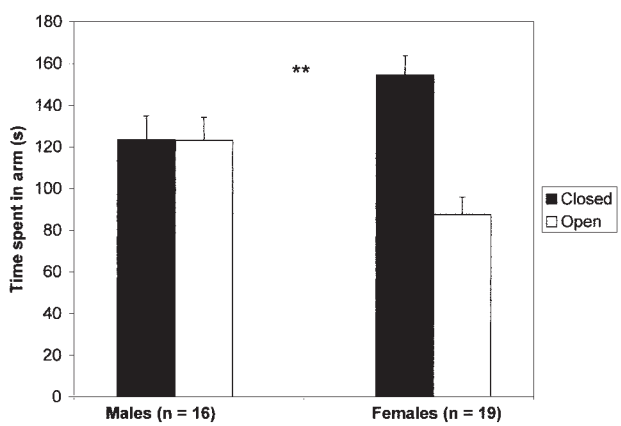

FIGURE 2 Sex differences in behavior in an elevated plus maze (analyzed as time spent in open arm/(total time in open + closed arm), but presented here as total time spent in each arm ($^{**}p = 0.02$).

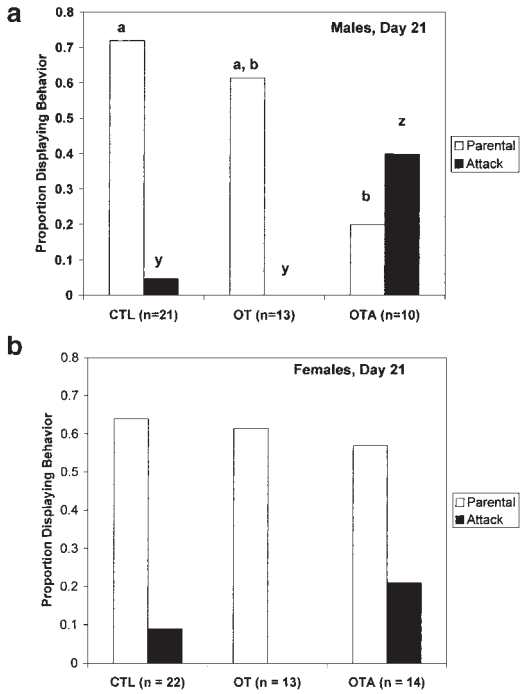

FIGURE 3 Effects of neonatal exposure to oxytocin (OT), an OT antagonist (OTA), or control (CTL) treatment on male parental and attack behavior, Day 21 (Fisher's exact test, $p = 0.021$). Different letters indicate groups that are different at $p < 0.05$. (a). Effects of neonatal exposure to OT, OTA, or CTL treatments on female parental and attack behavior, Day 21 (b).

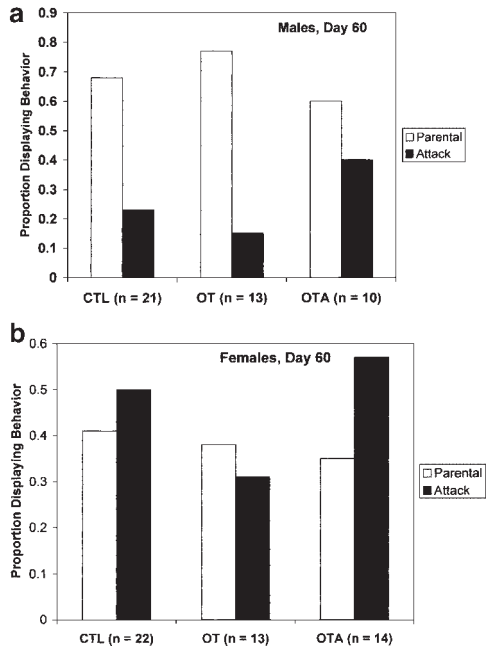

FIGURE 4 Effects of neonatal exposure to OT, OTA, or CTL treatment on male parental and attack behavior, Day 60. Treatment effects, while significant on Day 21, are no longer significant at Day 60 (a). Effects of neonatal exposure to OT, OTA, or CTL treatment on female parental and attack behavior, Day 60. Treatment effects were not statistically different (b).

$p = 0.155$) on Day 21 did not vary by group (Figure 3b). On Day 60, overall group differences were not significant for either males or females (Figures 4a and b).

Elevated Plus Maze. The overall effect of neonatal treatments on behavior in the EPM for either males, $F(2, 31) = 1.43$, $p = 0.256$, or females, $F(2, 37) = 0.59$, $p = 0.560$, was not statistically significant (males: CTL, $n = 16$; OT, $n = 9$; OTA, $n = 9$; females: CTL, $n = 19$; OT, $n = 10$; OTA, $n = 11$).

DISCUSSION

The results of the present study in prairie voles revealed sex differences in the expression of alloparental behavior and exploratory behavior in an EPM. In addition, early manipulations of peptide hormones had sexually dimorphic consequences for the later expression of alloparental behavior, but not behavior in the EPM. The most striking change attributed to early treatments was the tendency of males exposed to a single neonatal injection of OTA to be more likely to attack pups and less parental than CTLs when tested at 21 days of age.

Sex Differences

The present study confirms earlier observations indicating that as prairie voles mature, sex differences in alloparental behavior become apparent (Figure 1). These differences are primarily due to changes in female behavior, including a decline in spontaneous parental behavior and an increased tendency for reproductively naïve females to attack pups. In addition, adult females were less exploratory than males when tested in an EPM. Male voles in contrast were more consistently alloparental across the age range studied (Lonstein & De Vries, 1999, 2000; Roberts, Miller, et al., 1998) and more exploratory in the EPM. These responses are in contrast to those in rats, in which adult males are less parental than females or juvenile males (Lonstein & De Vries, 2000) and display more anxiety in the EPM (Fernandez, Gonzales, Wilson, & File, 1999).

The mechanisms responsible for these behaviors and in particular the sex differences in behavior observed here remain to be described. It has been proposed that the regulation of emotional states, including fear or anxiety in the presence of infants, may influence the capacity of naïve animals to show parental behavior (Fleming et al., 2002; Fleming & Leubke, 1981). It is possible, especially

in females, that common neural mechanisms relevant to reactivity to novel situations are reflected in the hesitance to respond to both pup stimuli and the open arm of the EPM.

Steroid and peptide hormones have been implicated in sexual differentiation and may influence both anxiety and parental behavior (Aikey, Nyby, Anmuth, & James, 2002; De Vries & Villalba, 1997; Dharmadhikari et al., 1997; Wang & Insel, 1996). It also is possible that different mechanisms, involving various neuroendocrine systems, regulate alloparental behavior or anxiety at different points in the life cycle. In male prairie voles, for example, pubertal increases in androgens facilitate the production of AVP; AVP in turn has been implicated in paternal behavior in voles (Bamshad, Novak, & De Vries, 1994; Wang & De Vries, 1993; Wang, Ferris, & De Vries, 1994; Wang, Liu, Young, & Insel, 2000; but also see Lonstein & De Vries, 1999).

As in most other mammals, virtually all recently parturient female prairie voles are parental, possibly due to hormonal priming associated with pregnancy and parturition (Numan & Insel, 2003). However, the physiological basis for alloparenting has received less attention. Ongoing studies in adult prairie voles have indicated that giving additional AVP does not facilitate female alloparental behavior in this species, although OT treatment (icv) was associated with an increase in female care giving (Bales & Carter, 2002). These data are consistent with a variety of studies, suggesting a sexual dimorphism in social behavior based on underlying sex differences in peptide hormones (De Vries & Villalba, 1997). Moreover, complex interactions between OT and AVP may be possible in part because both peptides are capable of binding to each other's receptors (reviewed Carter, 1998; Cho et al., 1999).

Developmental Effects of Oxytocin

A second goal of the present study was to examine the effects of a single neonatal exposure to OT or an OTA on subsequent alloparental behavior. We have previously observed in voles that treatments like those used here have long-lasting effects on the tendency to show intrasexual aggression, form a partner preference, or respond to stressful stimuli (Bales & Carter, 2003a, 2003b; Carter, 2003; Kramer, Cushing, & Carter, 2003). Based on the hypothesis that peptides (including OT, AVP, or both) may facilitate parental behavior, we specifically predicted that transient exposure to an OT antagonist might interfere with later behaviors that are modulated by either OT or related peptides, such as AVP.

In the present study, the disruptive effects of OTA treatment were especially obvious in males and during tests on Day-21 postpartum. OT or control treatments did not have this effect. However, as is typical of this species, levels of spontaneous alloparenting in 21-day-old males were very high, which precluded further increases in these groups.

Although the behavior of OTA-treated males was relatively consistent between tests, declines in alloparenting and increases in attacks in control groups obscured possible group differences on Day 60. The latter test also was a second exposure of the experimental animal to an infant, and repeated exposure could influence behavior. For example, pup exposure within the natal family around the age of weaning (Day 21) has been shown to increase alloparental behavior in prairie voles (Roberts, Miller, et al., 1998).

In the present study, female alloparental behavior tended to decline, rather than increase, between the first and second pup exposure. In females, especially on Day 60, pup attacks were relatively common, and group differences also were not significant. Therefore, it does not appear that prairie voles are always more parental upon a second exposure to an infant.

The general trends in attack behavior for females were similar to those in males, with the group that received neonatal OT being least likely to attack. However, the variance seen among these groups does not allow us to conclude that the neonatal manipulations of OT used here have long-term consequences for female parental behavior.

Based on studies implicating anxiety in the tendency to inhibit parental behavior in rats (Fleming et al., 2002; Fleming & Leubke, 1981), we also measured behavior in an EPM. Exploration, especially in the open arms of the EPM, is sometimes used to index anxiety in rodents (Ramos & Mormède, 1998). The EPM test, which was of secondary interest here, was administered at approximately 67 days of age after the completion of both alloparenting tests and was not affected by neonatal treatment. Behavior in the EPM, although sexually dimorphic, may simply be insensitive to such peptide manipulations. However, alloparenting tests on Day 21 were most influenced by treatment effects; thus, it is also possible that experience in the intervening period or maturational changes may have reduced the usefulness of the EPM test in indexing anxiety, obscured the effects of early peptide manipulations on both alloparenting and behavior in the EPM, or both.

The outcome of the present study suggests that OTA exposure on the first day of life may alter the development of neuroendocrine systems involved in social behavior, with the most prominent effects occurring in males. OTA has been shown to decrease maternal care when administered centrally in adult rats (Pedersen et al., 1982; Pedersen et al., 1995); however, there are no previous reports of either immediate or long-lasting effects of OTA

on male parental care. AVP, a hormone closely related to OT in structure, has been linked to male parental care (Bamshad et al., 1994; Wang et al., 1994; reviews in Wang & Insel, 1996; Ziegler, 2000); however, the exact role of AVP remains to be understood. Although several studies have shown relationships between AVP and male parental care, Lonstein and De Vries (1999) found that castrating male prairie voles (which should reduce androgen-dependent AVP) did not affect alloparental care.

Recent immunocytochemical data from our laboratory revealed that in neonatally OTA-treated males, but not females, AVP-IR was reduced in the paraventricular nucleus (PVN) at 21 days of age (Yamamoto et al., 2003). OT-IR was not significantly affected by either neonatal OTA or OT in 21-day-old males. It is therefore possible, and consistent with data implicating AVP in male parental behavior, that the long-term, negative effects on alloparenting of exposure to neonatal OTA in early life are mediated through changes in systems that rely on AVP.

These results may have implications for natural variation in parental care giving as well as pharmacological manipulations of OT, such as those currently used in medicine. In the natural mother–infant relationship, suckling and being licked and groomed has the potential to release OT in young animals (Uvnas-Moberg, 1998). In addition, maternal stimulation of pups has been associated in later life with higher levels of CNS OT receptors (Champagne et al., 2001; Francis et al., 2002) and lower levels of CRH receptors (Liu et al., 1997). We also have recently observed that handling during the first week of life can increase central OT-IR at 21 days of age (Carter et al., 2003). Thus, positive early experiences, including higher levels of some types of parental stimulation such as licking, might be capable of enhancing later sensitivity to OT and concurrently reducing reactivity to stressful experiences or anxiety. In contrast, drugs such as Atosiban, which are used to prevent premature labor, block receptors for both OT and AVP (Husslein, 2002; Manning, Stoev, Cheng, Wo, & Chan, 2001). The results of the present study also suggest caution in the use of OT antagonists in clinical practice and the need for careful observations of the potential behavioral effects of developmental manipulations that might influence OT or AVP.

NOTES

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