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BRIEF COMMUNICATIONS

Developmental Exposure to Oxytocin Facilitates Partner Preferences in Male Prairie Voles (*Microtus ochrogaster*)

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The authors investigated the effects of postnatal manipulations of oxytocin (OT) on the subsequent tendency to form a partner preference in male prairie voles (*Microtus ochrogaster*). Neonatally, males received either an injection of OT, an oxytocin antagonist (OTA), 0.9% saline vehicle, or handling without injection. As adults, males were tested for partner preference following 1 hr of cohabitation with a nonestrous female. In a 3-hr preference test, males neonatally exposed to exogenous OT exhibited a significant partner preference, not seen in males receiving OTA or saline. Both OT and OTA voles had significantly higher levels of social contact than saline controls. A single neonatal injection of OT increased both total and selective social behaviors in male prairie voles.

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Monogamy in mammals is rare (3% of species; Kleiman, 1977) and especially uncommon in rodents. A primary characteristic of mammalian monogamy is the emergence of a selective and long-lasting social preference for a familiar partner of the opposite sex. Prairie voles (*Microtus ochrogaster*) are small arvicoline rodents that live in stable male–female pairs in the field and laboratory (Getz, Carter, & Gavish, 1981). Because they can be studied in the laboratory, prairie voles have provided a unique opportunity to examine the neural and molecular basis of pair bond formation (Carter & Keverne, 2002; Insel & Young, 2001).

Various hormones and neurotransmitters have been linked with the formation of partner preferences in voles, including dopamine (Wang et al., 1999), glucocorticoids (DeVries, 2002; DeVries, DeVries, Taymans, & Carter, 1995, 1996), corticotropin-releasing hormone (DeVries, Guptaa, Cardillo, Cho, & Carter, 2002), arginine vasopressin (AVP; Cho, DeVries, Williams, & Carter, 1999; Pitkow et al., 2001; Wang, Young, De Vries, & Insel, 1998; Winslow, Hastings, Carter, Harbaugh, & Insel, 1993), and oxytocin (OT; Cho et al., 1999; Insel & Hulihan, 1995; Williams, Insel, Harbaugh, & Carter, 1994).

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In adult animals, the relationship between OT and the onset of partner preferences is well-documented. OT is released during mating in several species (Carter, 1992), and sexual behavior can hasten partner preference formation in prairie voles (Williams, Catania, & Carter, 1992). However, mating is not essential for pair bond formation, at least as indexed by partner preferences; nonsexual cohabitation also leads to the subsequent development of a partner preference (DeVries & Carter, 1999). In female prairie voles, centrally administered OT (intracerebroventricular), given either as an infusion over a 24-hr period (Williams et al., 1994) or administered acutely just prior to testing (Cho et al., 1999), can facilitate the onset of a partner preference in female prairie voles. Acute OT treatment, especially in high doses, also can facilitate the onset of a partner preference in male prairie voles (Cho et al., 1999). An OT receptor antagonist (OTA) administered prior to OT treatment prevented partner preference formation in both female and male prairie voles (Cho et al., 1999).

There is increasing evidence that social behaviors are affected by early experiences and that these experiences may be mediated by hormonal processes, including changes in the same peptides that have been implicated in social monogamy (Carter, 1998; Champagne, Diorio, Sharma, & Meaney, 2001). Although OT has been implicated in the formation of partner preferences in adulthood, comparatively little is known regarding the developmental consequences of OT.

In the present study, we examined the hypothesis that neonatal exposure to OT would facilitate adult pair bond formation and that neonatal treatment with an OTA would have the opposite effect. This hypothesis was tested in male prairie voles by administering on Day 1 postpartum a single injection of OT or a selective OTA or the saline vehicle control; voles were tested in adulthood for partner preferences following a brief, 1-hr exposure to a previously unfamiliar female (Williams et al., 1992). In earlier studies with male prairie voles, neither 1 nor 6 hr of cohabitation was sufficient to form a reliable partner preference (DeVries & Carter, 1999; DeVries et al., 1996).

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Method

Subjects were laboratory-bred male prairie voles (*Microtus ochrogaster*), descendants of a wild stock originally caught near Champaign, Illinois. Stock was systematically outbred. Prairie voles were maintained on a 14:10-hr light–dark cycle and allowed food (Purina 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, offspring were removed and housed in same-sex sibling pairs in smaller (27 cm long × 16 cm wide × 13 cm high) cages. The same-sex sibling pairs were then kept in single-sex colony rooms.

Within 24 hr of birth, experimental subjects were identified by sex, and all voles were toe-clipped for identification. Voles were randomly assigned to groups, receiving either a 3.0 μg injection of OT, a 0.3 μg injection of OTA, or an injection of 0.9% saline (SAL), or they were handled without injection (HAN). All injections were 50.0 μl in volume and administered in 250.0 μl gas-tight Hamilton syringes. The OTA ([d(CH_2)$_5$, Tyr(Me)^2, Orn^8]-vasotocin) used here was selected from the compounds designed by Bankowski, Manning, Seto, Haldar, and Sawyer (1980) and is commercially available from Peninsula Laboratories (San Carlos, CA). This compound has been tested extensively in behavioral studies, including sexual (Argiolas, Melis, Vargiu, & Gessa, 1987) and feeding behavior research (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 up to at least 6 hr after administration (Witt & Insel, 1991). Numerous studies have shown that OT and OTA cross the blood–brain barrier in small amounts, even in adults whose blood–brain barrier would be more fully developed than those of the infants studied here (1.3% in Ermisch, Ruhle, Landgraf, & Hess, 1985; see also Banks & Kastin, 1985; Jones & Robinson, 1982). The blood–brain barrier in other rodent species is not fully formed until approximately 14 days after birth (Vorbrodt, 1993). The dosages used here were based on previous studies in prairie voles (Cho et al., 1999; Stribley & Carter, 1999) and rats (Sohlstrom, Carlsson, & Uvnas-Moberg, 2000).

Prairie voles in this study were part of a larger project on the developmental effects of OT, in which they previously had received two alloparental care tests (10 min in duration), one elevated plus-maze test (5 min in duration), and an aggression test (5 min in duration; Bales & Carter, in press); the last aggression test was approximately 1 day prior to the partner preference test. Males did not differ significantly by treatment in the aggression test (Bales & Carter, in press), nor did the level of same-sex aggression displayed correlate with the subsequent partner preference behavior (i.e., total aggressive acts did not correlate with time spent in side-to-side contact with partner, $r = -.22$, $p = .14$; nor with time spent in side-to-side contact with stranger, $r = .08$, $p = .57$). In addition, a separate study that assessed male partner preference behavior after a 10-min parental care test and a 1-hr period of cohabitation found no difference between males that had undergone the parental care test and males that had not ($F = 0.0$, $p = .98$). Prairie voles were tested for partner preferences between 60 and 90 days of age.

In the present study, the experimental male was exposed to a previously unfamiliar, randomly selected female for a 1-hr period. Cohabitation began between 9 and 10 a.m. near the onset of the light cycle. Randomly assigned male and female partners were paired in a neutral cage during this period.

Immediately following the 1-hr period of cohabitation, experimental voles 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 (Williams et al., 1992). Testing was conducted using an apparatus consisting of three identical polycarbonate cages (27 × 16 × 13 cm) attached by Plexiglas tubes (7.5 × 16.0 cm). The experimental vole was free to move throughout the apparatus while the 2 stimulus voles were loosely tethered within their separate chambers.

Tests were recorded in time-lapse video and scored later by an experimentally blind observer for the location of the experimental vole (alone or in the cage with the partner or the stranger), as well as the time spent in physical side-to-side contact with the partner and stranger (The Observer 3.0; Noldus Information Technologies, Wageningen, the Netherlands). Total social contact was measured by the summed time spent in side-to-side contact with the partner and the stranger, whereas activity levels were measured by the number of entries into all cages. Data were analyzed by mixed-model analyses of variance (ANOVAs; Littell, Milliken, Stroup, & Wolfinger, 1996) in SAS 8.0 (SAS Institute, Cary, NC). Dependent variables included time spent in side-to-side contact with the partner, time spent in side-to-side contact with the stranger, overall levels of social contact, and activity levels. The animal's litter and parents were included as random factors. Data for most variables were transformed with the square- or quad-root transformations (Sokal & Rohlf, 1981) because of non-normality of the residuals. If random effects were determined to have a covariance of zero, they were dropped from the model. Post hoc analyses were carried out by least squared means. All significance levels were set at $p < .05$, and all tests were two sided. Partner preference within groups was analyzed by paired *t* tests.

Results

Total Social Contact and Between-Groups Partner Preferences

The overall ANOVA for total social contact was significant: $F(3, 33) = 5.33$, $p < .01$ (see Figure 1). All other groups differed significantly from the SAL group, which was low, but not from each other.

An overall ANOVA for treatment effect on physical side-to-side contact with partner did not reach significance: $F(3, 56) = 1.95$, $p = .13$ (see Figure 2). However, a planned comparison of OT-treated males with those from the SAL group revealed a difference between the two, $t(28) = 2.05$, $p = .05$. Treatment differences in side-to-side contact with the stranger were not significant, nor were differences in activity levels. Differences in time spent in the partner's cage, the stranger's cage, and alone were not signifi-

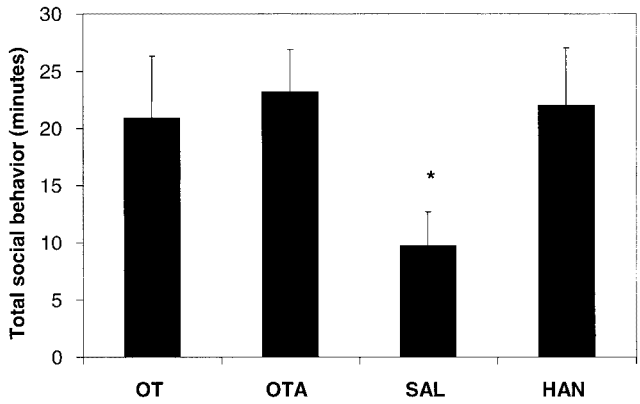

Figure 1. Total social contact in adult male prairie voles (minutes; *M* + *SEM*) as a function of neonatal treatment. On Day 1 postpartum, voles received oxytocin (OT; $n = 13$), an oxytocin antagonist (OTA; $n = 14$), or the 0.9% saline vehicle (SAL; $n = 17$), or they were handled but not injected (HAN; $n = 16$). * $p < .01$.

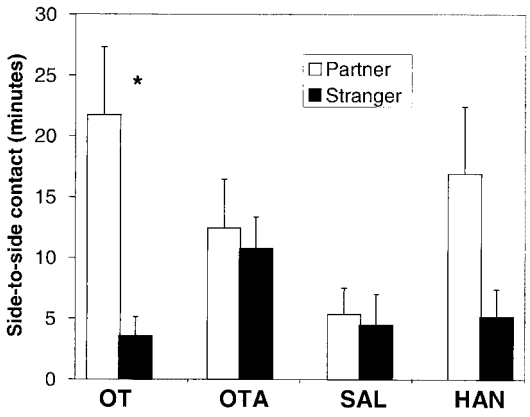

Figure 2. Partner preference in adult male prairie voles as a function of neonatal treatment. On Day 1 postpartum, voles received either oxytocin (OT; $n = 13$), an oxytocin antagonist (OTA; $n = 14$), or the 0.9% saline vehicle (SAL; $n = 17$), or they were handled but not injected (HAN; $n = 16$). Partner preference was measured as minutes ($M + SEM$) of side-to-side contact with a familiar partner or a comparable unfamiliar stranger during a 3-hr test. * $p < .05$, significant within-group partner preference.

cantly different but followed the same patterns as side-to-side contact.

Within-Group Partner Preferences

Only OT males showed a statistically significant preference for the partner after 1 hr of cohabitation, $t(12) = 2.23$, $p = .05$ (see Figure 2). The HAN group showed a similar but nonsignificant tendency to prefer the familiar vole. However, both OTA-treated males and SAL males were equally likely to select either the familiar partner or an unfamiliar stranger.

Discussion

Data from the present study, as well as other observations (Bales, Abdelnabi, & Carter, 2001; Bales & Carter, in press; Uvnas-Moberg, Alster, Petersson, Sohlstrom, & Bjorkstrand, 1998), support the hypothesis that developmental manipulations of OT can have long-lasting consequences for physiology and behavior. Female but not male prairie voles exposed to OT on Day 1 and sacrificed on Day 21 postpartum showed an increase in the number of neurons showing OT-immunoreactivity (OT-IR; Yamamoto, Cushing, Hoffman, et al., 2002). In contrast, a comparable analysis of AVP-IR neurons in males has revealed that early exposure to OTA but not OT is followed by a subsequent reduction in the number of neurons expressing AVP (Yamamoto, Cushing, Kramer, et al., 2002). However, both the behavioral and physiological consequences of OT or OTA must be interpreted in the context of other developmental factors, including those associated with handling and the saline vehicle used to deliver these compounds.

Developmental Consequences of Handling and Saline Injections

The importance of early experience, including handling and maternal separation, for later behavioral and endocrine response patterns is well documented (reviewed in Levine, 2001; Meaney, 2001; Plotsky, Sanchez, & Levine, 2001). Although it is rarely the major aim of behavioral studies, work in our laboratory and others also suggests that saline injections can have neural (Smith, Kim, Oers, & Levine, 1997) and behavioral consequences (Stribley & Carter, 1999).

In the present study sterile physiological saline, obtained from a commercial biomedical supplier, was used as the carrier for OT and OTA. We do not know if the saline treatment is actually isotonic in prairie voles. Furthermore, the volume of the injection (50 μl) might have been a stressor. Because the two control groups in this study differed significantly both in side-to-side contact with a familiar partner (see Figure 2) and total social contact (see Figure 1), we conclude that the saline treatment had long-term consequences beyond those of our routine handling procedures. In an earlier study in which we repeated daily injections of saline for 1 week, we observed an increase in aggression in female voles (Stribley & Carter, 1999) and a reduction in the tendency to show a partner preference in males (Stribley, 1998). These effects were no longer apparent when an AVP antagonist was administered in the saline treatment, suggesting that endogenous AVP might have been responsible for the effects observed after saline treatment.

Saline injections are a potential stressor, although most studies of the stressful effects of saline are based on hypertonic solutions. Saline injections may affect both OT and AVP neurons as well as the hypothalamic–pituitary–adrenal (HPA) axis (Harbuz, Jessop, Lightman, & Chowdrey, 1994; Kiss & Aguilera, 1993; Xiong & Hatton, 1996). There is some evidence in both adult (Sharp, Sagar, Hicks, Lowenstein, & Hisanaga, 1991) and infant rats that isotonic saline injections induce c-Fos expression in brain regions, including the paraventricular nucleus of the hypothalamus, where OT is produced. In addition, isotonic saline injections in rats have been shown to reduce OT content of the pituitary, hypothalamus, and spinal cord within 15 min of injection (Lukic & Haldar, 1993).

The effects of being handled as an infant on subsequent HPA axis reactivity have been well studied in rats. In general, handling or brief maternal separation acts to reduce reactivity in measures of behavior and HPA axis activity, perhaps mediated by maternal grooming (Levine, 2001; Meaney, 2001; Plotsky et al., 2001). Changes in central OT or its receptors have been implicated in the beneficial effects of maternal grooming (Champagne et al., 2001).

Developmental Consequences of Oxytocin

In the context of a background of neonatal saline treatment described above, we have observed a different pattern of adult social behavior in prairie voles treated with OT. In general, OT seems to ameliorate the inhibitory effects of saline injection. OT is known for its calming, antinociceptive effects and is associated with positive social interactions both during development and in adulthood (Carter, 1998; Insel & Winslow, 1991; Uvnas-Moberg, 1998). OT also facilitates the onset of partner preferences when administered ICV (Cho et al., 1999). Recent studies in rats also have implicated maternal care in the later expression of OT and AVP receptor binding (Francis, Young, Meaney, & Insel, 2002), and OT administered postnatally increased pup-directed grooming (Pedersen & Boccia, 2002).

OT binding has been observed and is presumed functional in infant rats (Boer, 1993; Shapiro & Insel, 1989). In prairie voles,

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OT receptor binding appears neonatally and increases during development until adult levels are achieved at weaning (Wang & Young, 1997; Witt, Carter, & Insel, 1991). Csaba, Ronai, Laszlo, Darvas, and Berzetei (1980) found that neonatal injections of OT in infant rats resulted in long-lasting changes in sensitivity to AVP and noradrenaline. However, another study found few differences of neonatal OT injections in behavior, brain weight, and only a transient increase in body weight (Boer, Quak, de Vries, & Heinsbroek, 1994).

In the current study, although both OT and OTA prairie voles were more contact prone than animals in the SAL group, only OT voles showed the selective behaviors indicative of a partner preference. It is possible that the effects observed here are mediated through differing behavior of the mother toward differently treated pups, as has been demonstrated in handling studies (Meaney, 2001). A study of infant rat pups indicated that those injected with OT reduced their rate of vocalization (Insel & Winslow, 1991). However, OT knockout mice also vocalized less than wild-type counterparts (Winslow et al., 2000). Generalizations are difficult because both the short-term and long-term effects of OT and OTA on behavior and physiology remain poorly described.

Alternatively, the observed effects might be accounted for by changes in glucocorticoid or androgen production secondary to the OT system. Neonatal OT injection has been shown to affect adult stress reactivity in rats (Noonan, Continella, & Pedersen, 1989). In the present study, corticosterone and androgen levels were measured in a random subset of the males as adults (6 to 8 months of age). There were differences in baseline levels of corticosterone, with OTA prairie voles exhibiting higher levels than any other group. However, the differences in corticosterone were not correlated with differences in time spent in side-to-side contact with a familiar partner ($n = 39$, $r = .08$, $p = .607$) nor in time spent in side-to-side contact with a stranger ($r = .216$, $p = .186$). No treatment differences were found in circulating levels of androgens (Bales et al., 2001). Therefore, we do not have any evidence that the changes observed were due to differences in hormones of the HPA or gonadal axis, although more thorough analyses would be useful.

Finally, it is possible that differences in behavior during the cohabitation period could have affected subsequent partner preference behavior. In general, the responses of sexually naive males to a potential female partner are highly stereotyped and involve immediate investigation of the female, followed by high levels of social contact (DeVries, Johnson, & Carter, 1997; Gavish, Carter, & Getz, 1981). Prairie voles were monitored during the cohabitation period for overt fighting, and no indications of aggression were observed in any group. In addition, a separate quantitative study of male–male interactions in these animals (Bales & Carter, in press) did not reveal overall group differences in the tendency of males from the different neonatal treatment groups to be aggressive. However, in the present study we did not measure positive social behaviors during the cohabitation period and thus cannot exclude the possibility that more subtle differences might have influenced the outcome of this study.

Developmental Consequences of OTA

In comparison to SAL-treated prairie voles, those receiving neonatal OTA showed an increase in total social contact, similar to that seen in OT-treated and untreated males. However, OTA-treated males showed no tendency toward a preference for a familiar partner. In contrast, in adult prairie voles OTA treatment was associated with marked reductions in total social contact (Cho et al., 1999).

The OTA used in this study was selected for its documented effects on OT receptors. However, this OTA may bind to both OT and AVP receptors (Bankowski et al., 1980). In addition, our recent finding of a reduction in AVP-IR in OTA-treated but not OT-treated males suggests that changes in AVP could have contributed to the observed behavioral differences. AVP has been previously implicated in pair bonding in male prairie voles (Winslow et al., 1993). In addition, access to both OT and AVP receptors appeared to be necessary for formation of a partner preference, although stimulation of either OT or AVP receptors may be sufficient to facilitate social contact (Cho et al., 1999). Furthermore, there is considerable cross-communication between OT and AVP; compounds capable of affecting one system often influence the other as well (Carter, 1998; Legros, 2001). More selective OTAs have become available (Manning, Stoev, Cheng, Wo, & Chan, 2001) and will be used in subsequent studies.

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