

The Effects of Oxytocin and Vasopressin on Partner Preferences in Male and Female Prairie Voles (*Microtus ochrogaster*)

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This study compared the effects of centrally administered oxytocin (OT) and arginine vasopressin (AVP) on partner preference formation and social contact in male and female prairie voles (*Microtus ochrogaster*). After 1 hr of cohabitation and pretreatment with either AVP or OT, both males and females exhibited increased social contact and significant preference for the familiar partner. After pretreatment with either an OT receptor antagonist (OTA) or an AVP (V1a) receptor antagonist (AVPA), neither OT nor AVP induced a partner preference. In addition, treatment with OT+OTA or AVP+AVPA was associated with low levels of social contact in both sexes. Either AVP or OT is sufficient to facilitate social contact if either the OT or AVP receptor is available. However, the formation of partner preferences may require access to both AVP and OT receptors.

Monogamous social systems are rare among mammals, occurring in less than 3% of mammalian species (Kleiman, 1977). Prairie voles (*Microtus ochrogaster*) exhibit several traits associated with monogamy, including high levels of social contact and the capacity to form pair bonds (Carter, DeVries, & Getz, 1995; Dewsbury, 1987; Dewsbury, Baumgardner, Evans, & Webster, 1980). In the laboratory, the selection of a familiar partner in preference to a stranger has been used as an index of pair bonding. Partner preferences can form during nonsexual cohabitation, and the onset of partner preferences is facilitated by mating (Williams, Catania, & Carter, 1992; Winslow, Hastings, Carter, Harbaugh, & Insel, 1993).

Two neuropeptides, arginine vasopressin (AVP) and oxytocin (OT), are released during sexual behavior (reviewed by Carter, 1992) and have been implicated in social behavior in several different species, including prairie voles (Insel, 1997; Insel & Hulihan, 1995; Williams, Insel, Harbaugh, & Carter, 1994; Winslow et al., 1993). These two peptides are structurally similar, differing by only two amino acids, and may have complex interactions with each other's receptors (Barberis & Tribollet, 1996; Engelmann, Wotjak, Neumann, Ludwig, & Landgraf, 1996).

In female prairie voles, OT is capable of increasing sociality in general (Witt, Carter, & Walton, 1990) and hastens the onset of partner preferences (Insel & Hulihan, 1995; Williams et al., 1994). AVP has been implicated in the

induction of several monogamous traits, including the formation of partner preferences. AVP synthesis, especially in the limbic system, is greater in males than in females and declines following castration in males (Bamshad, Novak, & De Vries, 1993, 1994; G. J. De Vries & Villalba, 1997). It has been hypothesized that pair bonding in male prairie voles is regulated by AVP, whereas in females, pair bonding may rely on OT (Carter et al., 1995; Insel, 1997; Insel & Hulihan, 1995). However, evidence testing this hypothesis is at present incomplete because different paradigms, which incorporate differing amounts of cohabitation and social stimulation and varying methods for applying either the peptides or their antagonists, have been used in males and females.

The purpose of the present study was to compare the effects of OT and AVP on social behavior in general, and more specifically on the development of selective social behaviors. These experiments differed from previous studies in that they used an acute intracerebroventricular method for administering the peptides or their antagonists. In addition, a comparatively brief 1-hr period of cohabitation with a member of the opposite sex was used to establish familiarity. In the absence of peptide treatments or other manipulations, 1 hr of nonsexual cohabitation is not sufficient to induce a partner preference in either males or females (A. C. DeVries & Carter, in press).

Experiment 1 compared the effects of OT or AVP on the development of partner preferences in male and female prairie voles. The peptides were administered prior to a 1-hr cohabitation. In Experiment 2, selective antagonists for the OT receptor (OTA) or the V1a AVP receptor (AVPA) were used in an attempt to specify the receptors that are essential to permit the behavioral effects of OT or AVP. OTA or AVPA were administered prior to treatment with either OT or AVP (100 ng); as in Experiment 1, prairie voles were allowed a 1-hr cohabitation. In both experiments, social contact and partner preferences were subsequently measured during a 3-hr test in which the experimental prairie vole could elect to

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spend time either with a partner made familiar by cohabitation or with a comparable stranger.

that small doses were highly effective in this paradigm. For these reasons, we used doses of 1 ng of each antagonist.

General Method

Subjects

The prairie voles (*Microtus ochrogaster*) that were used in these studies were from the F₃ generation of wild stock, trapped near Urbana, Illinois. They were housed in polycarbonate cages (20 × 25 × 45 cm) with pine chip bedding and maintained on a 14:10-hr light-dark cycle (lights on at 0600, EST). Purina Rabbit Chow (Ralston-Purina, St. Louis, MO) and tap water were provided ad libitum. Breeding pairs lived in family groups consisting of sire, dam, and a litter. Prairie voles were weaned and segregated into unisex sibling groups at 21 days of age and remained housed with their siblings until randomly assigned to experimental groups at 60 to 90 days of age. All prairie voles were reproductively naive at the onset of this study. To control possible changes in ovarian hormones, which follow exposure to a male (Carter et al., 1995), experimental females were ovariectomized 2 weeks prior to testing. Ovariectomy was conducted under sterile conditions with sodium pentobarbital anesthesia (50 mg/kg). All procedures were approved by the University of Maryland Animal Care and Use Committee and were within National Institutes of Health guidelines.

Intraventricular Cannulation

One day prior to testing, prairie voles were anesthetized with sodium pentobarbital (50 mg/kg); the skull surface was exposed, and a small opening was drilled through the skull. A 26-gauge stainless steel guide cannula (Plastics One, Roanoke, VA) was implanted stereotaxically into the right lateral cerebral ventricle. The cannula was attached to the surface of the skull with cranioplastic cement. Prairie voles were returned to their home cages and randomly assigned to an experimental group. At the conclusion of the experiment, they were killed, and cannula patency and placement were verified by means of a postmortem injection of dilute India ink through the cannula. Prairie voles without staining in the lateral ventricles were neither scored nor used in data analysis; injection success was greater than 90%, and misses were randomly distributed among the groups.

Peptides and Antagonists

OT or AVP or peptide receptor antagonists—AVPA [$d(\text{CH}_2)_5$ -Tyr(Me)]AVP] or a selective OT receptor antagonist, OTA [$d(\text{CH}_2)_5$ [Tyr(Me)²-Thr⁴-Tyr-NH₂]⁹OV¹⁰T] (Sigma, St. Louis, MO), or the artificial cerebrospinal fluid vehicle (CSF; Biofluids, Rockville, MD)—were administered intracerebroventricularly.

Doses were selected on the basis of a pilot study that revealed that 100 ng of either peptide was highly effective in affecting social behavior. Injections were 2 μ l in volume and were given slowly over a period of 10 s with a Hamilton microsyringe. Doses and sequences of treatments for each experiment are described below. In binding studies in rats, these antagonists have been shown to be at least 100 times more effective in receptor binding than the natural ligands (Barberis & Tribollet, 1996). Pilot studies suggested

Cohabitation and Social Preference Tests

Fifteen minutes after the last injection, the experimental prairie voles were placed with a reproductively naive member of the opposite sex (designated the partner) for a 1-hr period of cohabitation. After the cohabitation, social preferences were assessed using a Y-shaped apparatus that consisted of three identical, clear, polycarbonate cages (20 × 25 × 45 cm; Williams et al., 1992). Two parallel cages housed the stimulus prairie voles. The partner (made familiar by cohabitation) and the stranger (conspecifically similar to the partner in sex, age, and weight, but unfamiliar to the experimental prairie vole) were tethered with plastic collars that restricted their movements to a single cage. Tethering has been reported to cause minimal disturbance to the stimulus and experimental animals (Webster, Williams, & Dewsbury, 1982). At the beginning of the preference test, the experimental subject was placed in the third (empty) cage and was free to move throughout the apparatus. Each test lasted 3 hr (180 min), which gave a reliable assessment of social preference in this species (Williams et al., 1992). Preference tests were monitored using a Panasonic time-lapse video system, with a 12:1 temporal reduction; tests were scored by experimentally blind observers using the S & K Computerized Event Recorder System (Buffalo, NY). Observers were trained to at least 95% reliability prior to scoring data. Behaviors that were scored included the following: time in each cage, time in physical contact with each stimulus animal, and aggression frequency.

Data Analysis

Total social contact was assessed as the number of seconds (later converted to minutes) that an experimental prairie vole spent in physical contact with both stimulus animals during the preference test. Differences among the groups regarding the duration of total social contact were assessed using an analysis of variance (ANOVA). Following a significant *F* value, post hoc comparisons to the control (CTL) group were made using Fisher's protected least significant difference test. Time in physical contact with either the familiar partner or a comparable stranger (mean \pm SEM min) also was measured. Partner preferences within each treatment group were measured with paired *t* tests by comparing the time spent in physical contact with the partner versus the stranger. Additional ANOVAs for time spent with the familiar partner among groups and time in each cage also were assessed. The latter variables mirrored the patterns seen in physical contact and, therefore, are not reported here. Aggression was too infrequent for statistical analysis. Comparisons were considered statistically significant at $p < .05$.

Experiment 1: Effects of AVP or OT on Social Behavior and the Development of Partner Preferences

Method

The effects of AVP or OT were compared in males (Experiment 1A) and females (Experiment 1B) and as a function of the dose of each peptide. Male and female prairie voles were randomly assigned to groups receiving intracerebroventricular injections of

either artificial CSF (vehicle CTL) or 1 ng, 10 ng, or 100 ng of either OT or AVP. Fifteen minutes after the injection, each prairie vole was housed for 1 hr with a partner of the opposite sex and tested immediately after cohabitation for social behavior in a 3-hr partner preference test. All groups contained 10 prairie voles.

Results

Experiment 1A: Males

Total physical contact. An overall analysis revealed that treatment with AVP or OT produced significant changes in total social contact, $F(6, 63) = 13.38, p < .01$. As shown in Figure 1A, males receiving 100 ng OT showed significantly higher levels of physical contact than did CTL males. In addition, CTL males spent more time in contact than did males treated with 10 ng OT. There also was some evidence of a biphasic effect of AVP; males receiving 1 ng AVP showed lower levels of social contact than did CTL males or males receiving higher doses of AVP.

Partner preference. After 1 hr of cohabitation, CTL males did not exhibit a significant preference for either stimulus animal, $t(9) = 1.88, p > .05$ (see Figure 2A). However, treatment with any of the three doses of AVP resulted in a significant partner preference: 1 ng AVP, $t(9) = 4.68, p < .01$; 10 ng AVP, $t(9) = 3.59, p < .01$; or 100 ng AVP, $t(9) = 4.43, p < .01$. In males, treatment with the two highest doses of OT also resulted in significant partner preferences: 10 ng OT, $t(9) = 4.61, p < .01$, and 100

ng OT, $t(9) = 6.15, p < .01$. Males treated with 1 ng OT, $t(9) = 0.99, p > .05$, did not exhibit a significant partner preference.

Experiment 1B: Females

Total physical contact. AVP and OT affected the total amount of time the experimental females spent in physical contact, $F(6, 63) = 13.68, p < .01$ (see Figure 1B). Females treated with 100 ng AVP or 100 ng OT spent significantly more time in physical contact than did animals that received the lower doses of the peptides or CTL.

Partner preference. As shown in Figure 2B, females exhibited a preference for the familiar partner following treatment with the highest dose (100 ng) of AVP, $t(9) = 4.73, p < .01$, or OT, $t(9) = 12.37, p < .01$. Significant social preferences were not evident in females treated with 1 ng AVP, $t(9) = 0.99, p > .05$; 10 ng AVP, $t(9) = 0.61, p > .05$; 1 ng OT, $t(9) = 1.34, p > .05$; 10 ng OT, $t(9) = 0.79, p > .05$; or the vehicle CTL, $t(9) = 1.57, p > .05$.

Experiment 2: Effects of Peptide Antagonists on Social Behavior and the Development of Partner Preferences Induced by AVP and OT

Method

In Experiment 2, males (Experiment 2A) and females (Experiment 2B) were pretreated with 1 ng/2.0 μ l of either AVPA or OTA.

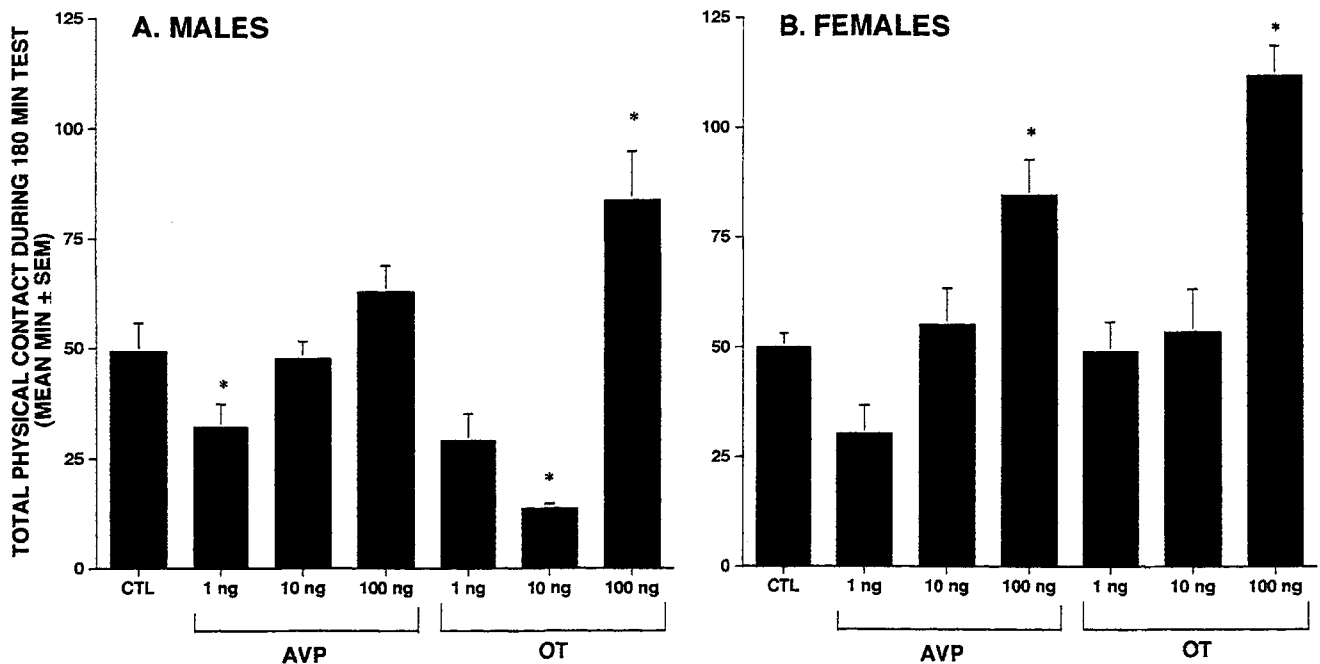


Figure 1. Total social contact as a function of peptide pretreatment in male (A) or female (B) prairie voles. Each experimental animal was injected intracerebroventricularly with either 1, 10, or 100 ng oxytocin (OT) or arginine vasopressin (AVP) or with a vehicle control (CTL). Each prairie vole then spent 1 hr of cohabitation with a prairie vole of the opposite sex designated as the familiar partner. Total time in physical contact with both the partner and a comparable stranger (mean minutes \pm SEM) was measured during a 180-min preference test. Asterisk indicates a significant difference in the amount of time spent in total contact when compared with CTL animals ($p < .05$).

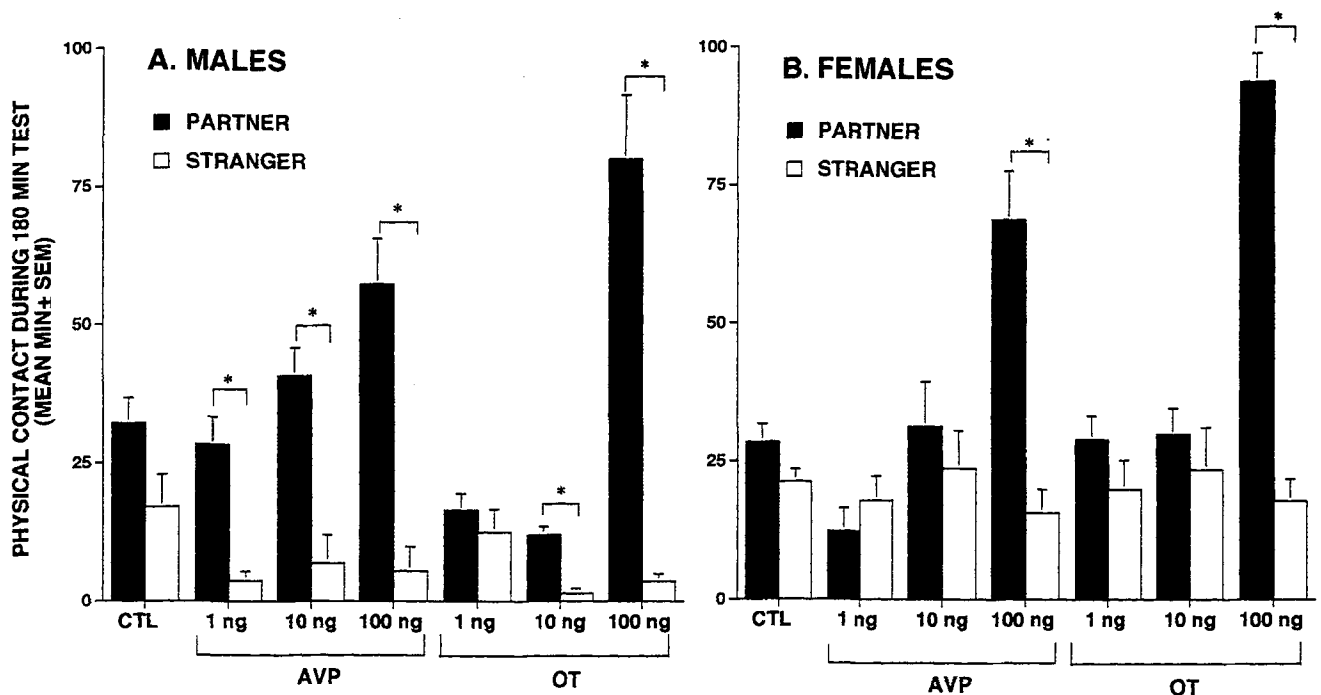


Figure 2. Partner preferences as a function of peptide pretreatment in male (A) or female (B) prairie voles. Time in physical contact with either the familiar partner or a comparable stranger (mean minutes \pm SEM) was measured during a 180-min preference test. Prairie voles were treated as in Figure 1. Asterisk indicates a significant preference for the partner (paired t test, $p < .05$). CTL = vehicle control; AVP = arginine vasopressin; OT = oxytocin.

Fifteen minutes after the first injection, the voles received a second intracerebroventricular injection of either AVP or OT (100 ng/2.0 μ l). Experimental groups thus consisted of prairie voles receiving OTA+OT (male $n = 13$; female $n = 10$), OTA+AVP (male $n = 11$; female $n = 10$), AVPA+OT (male $n = 13$; female $n = 9$), or AVPA+AVP (male $n = 12$; female $n = 8$). Additional CTL groups that did not receive an antagonist received an injection of CSF and were then treated with either AVP or OT (CTL+OT, male $n = 14$ and female $n = 10$; CTL+AVP, male $n = 12$ and female $n = 8$). Fifteen minutes after the second injection, as in Experiment 1, each experimental prairie vole was housed for 1 hr with a partner of the opposite sex and then tested immediately after cohabitation in a 3-hr partner preference test.

Results

Experiment 2A: Males

Total physical contact. Group comparisons were made among males receiving AVP and among those receiving OT as shown in Figure 3A. Differences in physical contact among treatment groups in males treated with AVP were not significant, $F(2, 32) = 1.60$, $p > .05$. However, there were significant differences among treatment groups of males receiving OT, $F(2, 37) = 3.93$, $p < .05$. Males treated with OTA+OT spent less time in physical contact than did those treated with AVPA+OT or CTL+OT.

Partner preference. Males receiving a control injection of artificial CSF followed by either AVP or OT showed significant preferences for the familiar partner, whereas

groups that received either antagonist (OTA or AVPA) prior to treatment with OT or AVP did not exhibit partner preferences, as shown in Figure 4A. Preferences were seen in males treated with CTL+AVP, $t(11) = 3.30$, $p < .01$, or CTL+OT, $t(13) = 3.30$, $p < .01$, whereas preferences for the partner were not present in males treated with AVPA+AVP, $t(11) = 0.80$, $p > .05$; OTA+AVP, $t(10) = 0.90$, $p > .05$; AVPA+OT, $t(12) = 0.70$, $p > .05$; or OTA+OT, $t(12) = 1.30$, $p > .05$.

Experiment 2B: Females

Total physical contact. In females, significant differences were observed among treatment groups regarding the total amount of time that the experimental females spent in physical contact with the stimulus animals (see Figure 3B). There were significant differences in total contact among females receiving AVP, $F(2, 23) = 5.86$, $p < .05$. Females treated with AVPA+AVP spent significantly less time in physical contact than did those treated with OTA+AVP. A similar trend in the comparison of the CTL+AVP versus AVPA+AVP did not reach statistical significance. In groups of females receiving OT, there also were significant differences in the amount of time spent in physical contact, $F(2, 26) = 11.58$, $p < .05$. Females treated with OTA+OT spent significantly less time in total contact than did those treated with AVPA+OT or CTL+OT.

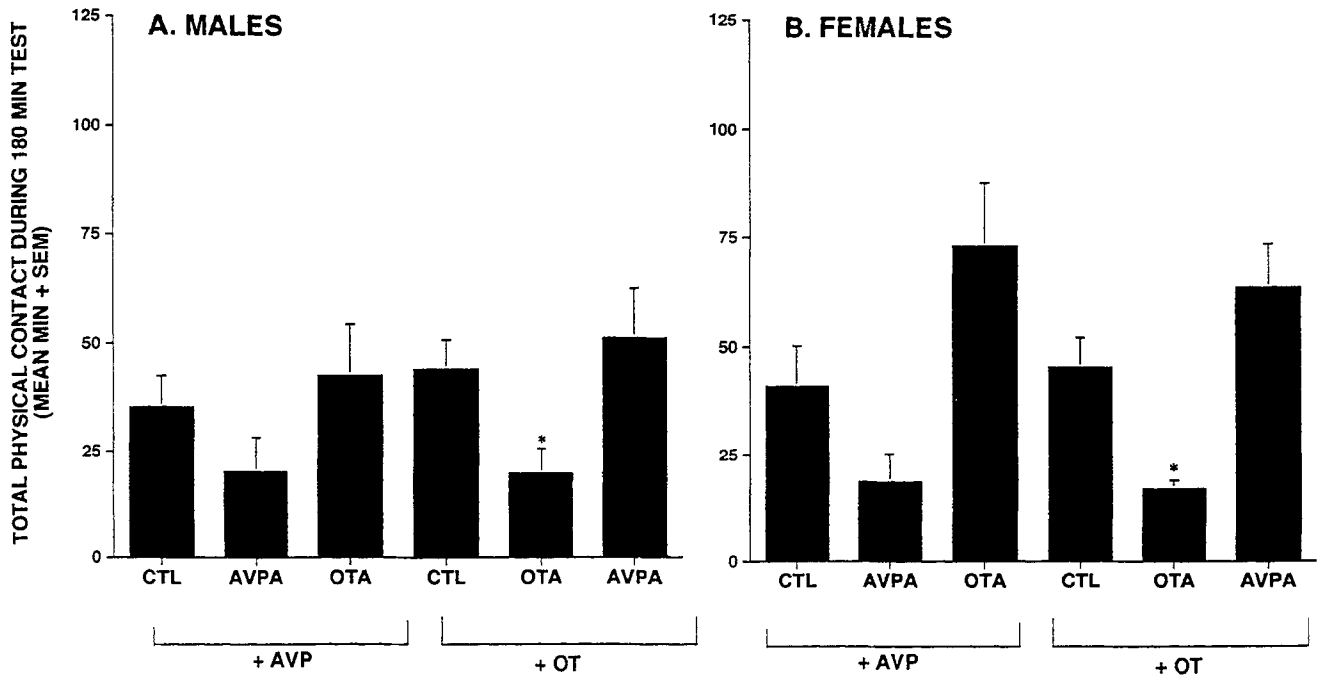


Figure 3. Total social contact as a function of peptide plus antagonist pretreatment in male (A) or female (B) prairie voles. Prior to testing, prairie voles were injected intracerebroventricularly with either an oxytocin antagonist (OTA), an arginine vasopressin V1a antagonist (AVPA), or a vehicle control (CTL), followed by either 100 ng oxytocin (OT) or arginine vasopressin (AVP). Each prairie vole then spent 1 hr of cohabitation with a prairie vole of the opposite sex designated as the familiar partner. Total time in physical contact with both the partner and a comparable stranger (mean minutes \pm SEM) was measured during a 180-min preference test. Asterisk indicates a significant difference in the amount of time spent in total contact when compared with CTL prairie voles ($p < .05$).

Partner preference. As shown in Figure 4B, female prairie voles treated with CTL+AVP, $t(7) = 2.98$, $p < .05$, or CTL+OT, $t(9) = 5.13$, $p < .01$, exhibited a significant preference for the partners. Females did not exhibit a significant partner preference when treated with AVPA+AVP, $t(7) = 0.43$, $p > .05$; AVPA+OT, $t(8) = 0.12$, $p > .05$; OTA+AVP, $t(9) = 1.13$, $p > .05$; or OTA+OT, $t(9) = 0.71$, $p > .05$.

Discussion

AVP and OT are released in response to specific stimuli, including social contact (Cunningham & Sawchenko, 1991; Uvnas-Moberg, 1997), which may, in turn, result in behavioral changes, including the formation of partner preferences. Rapid endocrine changes accompany the interactions that normally lead to pair bonding. The conditions of the present study were designed to describe the behavioral effects of acute changes in peptides. We used a paradigm that mimicked hormonal changes that might occur during pair bond formation in prairie voles.

The present data confirm and extend earlier studies indicating that OT and AVP are capable of facilitating both social contact and the development of partner preferences in prairie voles (Insel & Hulihan, 1995; Williams et al., 1994; Winslow et al., 1993; Witt et al., 1990). Prior studies have

used different paradigms to suggest that gender differences may exist in the behavioral effects of OT and AVP (Carter et al., 1995; Insel, 1997) and to implicate OT in pair bonding in females and AVP in males. In the present study, high doses (100 ng) of OT or AVP were associated with high levels of social contact and with the development of partner preferences in both sexes, although males were apparently sensitive to lower doses of peptides. In both sexes, pretreatment with an OT or AVP antagonist interfered with peptide-induced partner preferences, although high levels of social behavior continued to occur when the heterologous receptor remained accessible. Together, these results suggest that access to both AVP and OT receptors may be necessary to allow the induction of a partner preference, whereas access to either AVP or OT receptors is sufficient to permit high levels of social contact.

Procedural Issues

Various paradigms have been used to examine the role of exogenous peptides, including OT or AVP, in the induction of a preference for a familiar partner. Preferences have been defined by the amount of time that the treated animal spends with a familiar partner versus an otherwise comparable unfamiliar stranger. Comparatively long tests, usually 3 hr in duration, yield the most reliable index of social preference

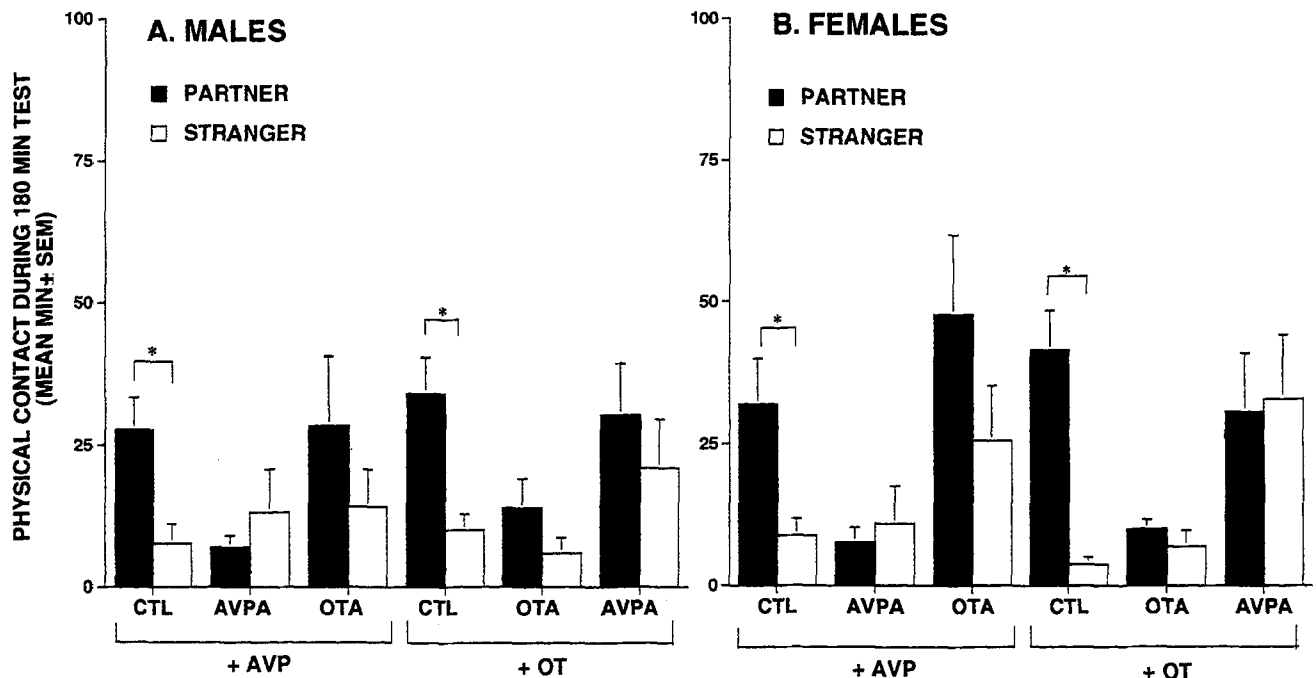


Figure 4. Partner preferences as a function of peptide plus antagonist pretreatment in male (A) or female (B) prairie voles. Time in physical contact with either the familiar partner or a comparable stranger (mean minutes \pm SEM) was measured during a 180-min preference test. Prairie voles were treated as shown in Figure 1. Asterisk indicates a significant preference for the familiar partner (paired *t* test, $p < .05$). CTL = vehicle control; AVPA = arginine vasopressin V1a antagonist; OTA = oxytocin antagonist; AVP = arginine vasopressin; OT = oxytocin.

(Williams et al., 1992). In addition, the measurement of time in physical contact with a stimulus animal produces a particularly sensitive index of social preference and sociality in general.

In females, prior attempts to examine the role of peptides in pair bonding have used a period of 6 or more hours of cohabitation to establish familiarity (Insel & Hulihan, 1995; Williams et al., 1994). In males, longer periods of cohabitation, typically 24 hr, have been used to determine familiarity (Winslow et al., 1993). In addition, peptides have usually been applied with an osmotic minipump, which was implanted approximately 24 hr prior to the introduction of a partner. These extended periods of cohabitation and the chronic application of the peptides complicate the interpretations of prior studies and present problems for attempts to identify the minimal neuroendocrine and social conditions necessary for pair bonding. For example, a prolonged period between treatment and testing offers opportunities for intervening endocrine or behavioral processes to affect behavior. The half-life of these peptides is short, and the effects of chronic treatments differ from those observed following acute exposure.

The present experiments were designed to examine the capacity of OT or AVP to induce social preferences under conditions that minimized both the social and peptide history of the prairie vole being tested. In the present paradigm, an acute injection of either OT or AVP, followed by a brief (1-hr) cohabitation, preceded tests for social

behavior and preferences. Using this paradigm, we also examined the effects of a prior treatment with antagonists for OT or AVP (V1a) receptors. These antagonists have been successfully used in earlier studies to block the development of partner preferences that typically follow prolonged cohabitation, mating, or both in either male (Insel, Preston, & Winslow, 1995; Winslow et al., 1993) or female prairie voles (Insel & Hulihan, 1995) and to antagonize the effects of exogenous peptides on pair bonding (Williams et al., 1994).

In Experiment 2, there was a tendency for prairie voles to show overall lower levels of social contact than those observed in Experiment 1. It is possible that the use of a two-injection procedure in the second study accounted for this difference. Stressful experiences can influence pair bond formation in prairie voles. However, earlier studies have indicated that males and females tend to respond differently to stress (A. C. DeVries, DeVries, Taymans, & Carter, 1996), whereas in the present study, the differences between Experiments 1 and 2 were similar for both males and females. It is also possible that other yet unidentified differences may have influenced these data.

Sex Differences

In the present study, partner preferences in female prairie voles were observed following treatment with a comparatively high dose (100 ng) of either peptide, whereas in males, significant partner preferences were significant in

prairie voles receiving lower dose treatments. However, in post hoc comparisons of social contact, males receiving low doses of peptides did not differ from CSF controls and did not show the very high levels of social contact that were characteristic of groups receiving high doses (100 ng) of peptides. There are indications, especially in males receiving OT, that in some cases, prairie voles that received lower doses of peptides were less social than controls. This effect was not seen in females, suggesting that OT can have sexually dimorphic actions. In general, however, males and females showed a markedly similar pattern of response to the different doses of peptides. Thus, it is not possible to reliably conclude that males are more sensitive than females to either OT or AVP.

Comparisons between males and females in the present study are complicated by the fact that females were ovariectomized, whereas males remained gonadally intact. Gonadectomized male or female prairie voles are capable of forming pair bonds that are apparently identical to those observed in reproductively intact animals (A. C. DeVries, Johnson, & Carter, 1997). Presently there is no direct evidence for an activation role for gonadal hormones in pair bonding in prairie voles. In addition, prairie voles are reproductively quiescent in the family group, and the presence of an unfamiliar member of the opposite sex is necessary to fully activate reproduction. Female prairie voles do not experience estrous cycles; however, after exposure to a novel male, the ovary is activated, producing rapid increases in estrogen that eventually lead to estrus induction (Carter et al., 1995). Because these complex hormonal fluctuations in ovarian hormones could influence social interactions or peptide sensitivity, females (in Experiments 1 and 2) were ovariectomized prior to testing. Although social experience may also produce hormonal changes in males, measurements of serum levels of gonadal hormones within the time frame used in this study have not revealed reliable changes in testosterone in prairie voles from our laboratory. Within the relatively short time span of the present study, we did not anticipate that sex differences would occur as a result of differential responses to gonadal steroids. However, the results of the present study leave open the possibility that subtle sex differences in peptide sensitivity may exist, and that such differences are due to the activational or organizational effects of reproductive hormones.

Differences in AVP and OT receptor binding patterns have been described in monogamous versus polygamous species (Insel, 1997; Insel & Shapiro, 1992) and between the sexes in other species of mammals (Barberis & Tribollet, 1996). In general, however, male and female prairie voles show similar patterns of peptide receptor binding and affinity (Insel, Wang, & Ferris, 1994). Effects of reproductive hormones on OT receptor binding in female prairie voles have been measured in the anterior olfactory nucleus, but have not been seen in hypothalamic nuclei, where such effects are common in rats (Barberis & Tribollet, 1996; Witt, Carter, & Insel, 1991).

It has been suggested that OT is primarily involved in the induction of social preferences in female prairie voles and

that AVP plays a major role in pair bonding in male prairie voles (Insel, 1997; Insel & Hulihan, 1995; Insel et al., 1995; Winslow et al., 1993). However, there also are indications in the previous data (Insel & Hulihan, 1995), as well as clear support from the present study, for the hypothesis that both exogenous AVP and OT are capable of facilitating the development of partner preferences in both sexes. We initially hypothesized that males would respond to AVP, whereas females would respond to OT. The results of the present study, however, reveal that both sexes are capable of responding to both peptides, at least at high doses.

The present findings with exogenous peptides do not discount the hypothesis that the endogenous mechanisms underlying pair bonding are sexually dimorphic. Endogenous AVP production is androgen dependent and male prairie voles have higher levels of AVP than do females (Bamshad et al., 1993; G. J. De Vries & Villalba, 1997; Wang, Ferris, & De Vries, 1994). AVP has been implicated in postcopulatory aggression (Insel et al., 1995; Winslow et al., 1993) and parental care (G. J. De Vries & Villalba, 1997; Wang, Zhou, Hulihan, & Insel, 1996) in male prairie voles. In male prairie voles, blocking the V1a (but not the V2) receptor for AVP selectively inhibits the development of partner preferences and stranger-directed aggression, and also reduces male parental care. In addition, the effects of stressful experiences are different in males than in females. The stress of swimming or injections of corticosterone inhibits the formation of heterosexual pair bonds in females and hastens pair bonding in males (A. C. DeVries et al., 1996).

The Effects of Peptide Antagonists

When AVP or OT were administered in the presence of the antagonist for the heterologous receptor (AVPA+OT or OTA+AVP), social contact remained high (see Figure 3), but social preferences were no longer evident (see Figure 4). In rats, both endogenous peptides are capable of binding to both AVP and OT receptors (Barberis & Tribollet, 1996). It is possible that partner preference formation in prairie voles requires binding to both types of receptors; this would explain the fact that either AVPA or OTA inhibited AVP-induced or OT-induced partner preferences. Alternatively, these antagonists could inhibit partner preferences with a nonspecific mechanism. For example, if the antagonist caused discomfort during the cohabitation period, this might induce a conditioned aversion to the partner. However, treatment with either AVPA+OT or OTA+AVP, although not associated with a partner preference, did not produce an aversion to the partner or reduce social contact in general.

In the present study, OT and AVP had different behavioral effects in the presence of either OTA or AVPA, supporting the assumption that OTA and AVPA do act on different receptors. Together, these data suggest that both OT and AVP receptors are necessary for the formation of partner preferences, whereas activity in either system is sufficient for the expression of nonselective social contact. Thus, although both OT and AVP are capable of affecting both selective and nonselective social behaviors, different receptor-based

mechanisms may mediate social contact versus social preferences.

These conclusions must be conditioned by awareness that the specificity of the antagonists that were used here is relative; both antagonists have some capacity (at least *in vitro* in rats) to bind to the heterologous receptor (OTA to AVP receptors and AVPA to OT receptors; Chan, Wo, Cheng, & Manning, 1996). The antagonists used here were selected because they were the most specific and best described of the receptor antagonists available when this study was conducted. Both antagonists have been used extensively in studies of receptor binding and behavior in various species, including prairie voles (Barberis & Tribollet, 1996; Insel & Hulihan, 1995; Insel et al., 1995; Insel & Shapiro, 1992; Winslow et al., 1993; Witt et al., 1991). Studies in rats suggest that both antagonists are effective in binding to the targeted receptor and in blocking the effects of the homologous antagonist. Analyses of the relative potencies of these antagonists are not available in prairie voles. However, both binding and behavioral studies, including the data presented here, have revealed different actions of the antagonists, which is consistent with the hypothesis that these peptides bind most strongly to the homologous receptors (OTA to OT receptors and AVPA to AVP receptors). Since this study was completed, more selective antagonists have been synthesized (Chan et al., 1996); future studies that use such compounds may provide more conclusive analyses of the relative importance of OT and AVP in social behavior.

Mechanisms Through Which Peptides May Affect Behavior

The present study does not specify the mechanisms through which AVP or OT affect behavior. Among the alternatives, however, is the possibility that these peptides influence social motivation. In both sexes, treatments with high doses of either OT or AVP were associated with high levels of social contact in general, as well as with a specific increase in contact with a familiar partner. It is possible that both peptides are rewarding, perhaps acting through interactions with dopaminergic systems. For example, monogamous, but not nonmonogamous voles, show intense OT and dopamine binding within the nucleus accumbens—an area that has a role in reward in other species (Insel & Shapiro, 1992).

Peptides also may affect social recognition (Engelmann et al., 1996). Individual recognition is presumably necessary for the expression of social preferences in prairie voles, and it is possible that OT and AVP induce partner preferences by facilitating the onset of social recognition or individual identification. Studies using rats indicate that both AVP and OT can facilitate social recognition (Dantzer, Koob, Bluthé, & LeMoal, 1988; Popik, Vos, & van Ree, 1992). In contrast, pharmacological doses of OT have been shown to attenuate memory (Dantzer, Bluthé, Koob, & LeMoal, 1987; Engelmann et al., 1996). On the basis of these and other data, OT has been considered an amnesic peptide. In prairie voles, however, the highest dose of OT used in the present experiments (100 ng) does not appear to be amnesic because

following treatment with OT, the experimental animals were able to differentiate between the stimulus animals and subsequently exhibited a strong social preference for their partners.

Sensory processes may also be influenced by these peptides. For example, OT and AVP receptors are located throughout the olfactory system. Steroid-peptide interactions could affect the olfactory or other sensory systems to reduce fear or otherwise facilitate social behaviors (Carter, 1998; Insel & Hulihan, 1995; Witt et al., 1991).

Summary

Both AVP and OT are capable of facilitating social contact and inducing partner preferences in male and female prairie voles. Although the patterns of response to either the peptide or its antagonists are remarkably similar in both sexes, males may be slightly more sensitive than females to the partner preference inducing effects of either AVP or OT. However, these experiments yielded no indication that females are more responsive than males to OT. A combined treatment with OTA+OT or AVPA+AVP produced a marked inhibition in social contact, suggesting that either peptide, when endogenously administered, could not act to increase either social contact or partner preference when its own receptor was blocked. The effects of AVP and OT on partner preferences were inhibited by either AVP or OT receptor antagonists, suggesting that these peptides act to facilitate partner preferences by binding to both types of receptors. Future experiments are needed to examine the neural mechanisms through which peptides influence selective and nonselective social behaviors in male and female prairie voles.

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