

Vasopressin in the Lateral Septum Regulates Pair Bond Formation in Male Prairie Voles (*Microtus ochrogaster*)

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Male prairie voles (*Microtus ochrogaster*) form a pair bond with a female partner after mating, and this behavior is regulated by the neuropeptide vasopressin (AVP). The authors report that AVP in the lateral septum is important for pair bond formation. Administration of an AVP V1a receptor antagonist in the lateral septum blocked mating-induced pair bonding, whereas administration of AVP induced this behavior in the absence of mating. In addition, administration of an oxytocin (OT) receptor antagonist in the lateral septum also blocked pair bond formation induced by either mating or AVP administration, suggesting that the OT receptor blockade may have interfered with the AVP regulation of behavior. Together, these data provide evidence suggesting that AVP in the lateral septum regulates pair bond formation in male prairie voles and that this process requires access to both AVP and OT receptors.

Monogamous social organization is usually characterized by social behaviors including selective affiliation with a partner, high levels of biparental care, and intense aggression toward unfamiliar conspecifics (Clutton-Brock, 1991; Dewsbury, 1987). Although monogamy is a common form of social organization in birds, less than 3% of mammalian species have been found to be monogamous (Kleiman, 1977). Extensive data have demonstrated that the prairie vole (*Microtus ochrogaster*) is a monogamous species that forms long-term bonds in the field (Getz, Carter, & Gavish, 1981; Getz & Hofmann, 1986) and mates preferentially with one partner in the laboratory (Carter & Getz, 1993; Dewsbury, 1987; Getz & Carter, 1996). After mating, male and female prairie voles remain together during gestation and display biparental care throughout lactation (McGuire & Novak, 1984; Oliveras & Novak, 1986; Thomas & Birney, 1979). Twenty-four hours of mating are found to induce pair bond formation between males and females and selective aggression by males toward intruders (Insel, Preston, & Winslow, 1995; Williams, Catania, & Carter, 1992; Winslow, Hastings, Carter, Harbaugh, & Insel, 1993). In males, mating seems to be essential for the induction of pair bonding and selective aggression, because cohabitation with a female without mating does not induce these behaviors (Insel et al., 1995; Winslow et al., 1993). In females, long-time cohabitation without mating can also induce pair bonding (Williams et al., 1992). Finally, a pair bond, once formed, is enduring, lasting for at least 1 week even in the

absence of continuous exposure to a partner (Insel & Hulihan, 1995; Insel et al., 1995; Winslow et al., 1993).

What are the neuronal and hormonal mechanisms underlying pair bond formation in prairie voles? Vasopressin (AVP; Cho, De Vries, Williams, & Carter, 1999; Winslow et al., 1993), oxytocin (OT; Cho et al., 1999; Insel & Hulihan, 1995; Williams, Insel, Harbaugh, & Carter, 1994), dopamine (Gingrich, Liu, Cascio, Wang, & Insel, 2000; Wang, Yu, Cascio, Liu, Gingrich, & Insel, 1999), and glucocorticoids (A. C. De Vries, De Vries, Taymans, & Carter, 1995, 1996) have all been implicated in the regulation of pair bond formation in prairie voles. To investigate pair bond formation in male prairie voles, an early study demonstrated that intracerebroventricular injections of the AVP V1a receptor antagonist blocked mating-induced pair bonding and selective aggression, whereas intracerebroventricular infusions of AVP induced these behaviors in the absence of mating (Winslow et al., 1993). Although this study indicates the importance of endogenous AVP release during mating on pair bond formation, it is by no means clear where in the brain AVP regulates social behaviors in male prairie voles.

Previous studies have demonstrated that septal AVP is involved in learning, memory, and social recognition in rats (Dantzer, Koob, Bluthé, & Le Moal, 1988; Engelmann & Landgraf, 1994). In male prairie voles, mating and cohabitation with a female for 3 days induce a decrease in AVP immunoreactive staining in the lateral septum (Bamshad, Novak, & De Vries, 1994) and an increase in AVP messenger RNA (mRNA) labeling in the bed nucleus of the stria terminalis (BST, the presumed source of septal AVP [Wang, Smith, Major, & De Vries, 1994]), suggesting increased septal AVP release. The present study was undertaken to test the hypothesis that AVP release within the lateral septum during mating is important for pair bond formation in male prairie voles. A pair bond was defined as subjects spending significantly more time with the familiar partner versus a conspecific stranger (partner preference). In Experiment 1, we examined whether AVP V1a receptor blockade in the lateral septum or the cingulate cortex blocked mating-induced pair bonding. In Experiment 2, we examined whether exogenous AVP administration in the lateral septum

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could induce pair bonding in the absence of mating. In Experiment 3, we tested whether OT receptors in the lateral septum were also essential for mating-induced pair bonding. Finally, in Experiment 4, we examined whether OT receptor blockade interfered with the effects of exogenous AVP on behavior. Together, our data suggest that, during mating, the lateral septum is a brain area in which AVP acts, in concert with the activation of both AVP and OT receptors, to regulate pair bond formation in male prairie voles.

General Method

Subjects

Subjects were sexually naive male and female prairie voles (*Microtus ochrogaster*) that were the F3 generation of a laboratory breeding colony originally derived from field-captured voles. After weaning at 21 days of age, subjects were housed in single-sex sibling pairs in plastic cages (20 cm high \times 50 cm long \times 40 cm wide) that contained cedar chips as bedding. Water and food were provided ad libitum. All cages were maintained on a 14:10-hr light–dark cycle, with lights on at 0700. The temperature was about $20 \pm 1^\circ\text{C}$. Males at 70–90 days of age were used as the subjects, and females of a similar age were used as stimulus animals.

Stereotaxic Cannulation and Injection

Male prairie voles were anesthetized with sodium pentobarbital (1 mg/10 g body weight), and 26-gauge stainless steel guide cannulas (Plastics One Inc., Roanoke, VA) were implanted bilaterally, aimed at the lateral septum (nose bar at -2 mm; 0.8 mm rostral, ± 0.6 mm bilateral, and 4.1 mm ventral to the bregma; Figure 1A) or the cingulate cortex (nose bar at -2 mm; 0.8 mm rostral, ± 0.6 mm bilateral, and 2.25 mm ventral to bregma). After 1 week of recovery, each male vole received bilateral injections of either artificial cerebrospinal fluid (CSF) carrier or CSF containing varying concentrations of AVP, the V1a receptor antagonist, or the OT receptor antagonist (OTA), respectively. The injection volume was 200 nl per side (total = 400 nl per vole). Injections were performed by inserting a 33-gauge needle that extended 1 mm below the guide cannula. The needle was connected through PE20 tubing to a 1- μl Hamilton syringe that was controlled by a manual injector (Fisher Scientific, Houston, TX). The plunger was slowly depressed, requiring about 10 s per injection. After the behavioral test, the subjects were injected with 200 nl of 2% india ink per side before being killed. Their brains were harvested and cut into 30- μm sections on a cryostat to verify injection sites histologically.

Microdialysis Probe Implantation and Perfusion

Male prairie voles were anesthetized and then implanted stereotaxically with a concentric style microdialysis probe, which has an active membrane length of 2 mm, aimed at the septum (nose bar at -2 mm; 0.9 mm rostral, 1.1 mm lateral, and 5.4 mm ventral to bregma). The probe was angled at 19 degrees to avoid the midsagittal sinus and thus crossed the midline of the septum (Figure 1B). Probes were constructed as previously described (Sved & Curtis, 1993), except the membrane had a molecular cutoff at 13 kD. The microdialysis probes were implanted 1 day before testing, and the input and outlet tubes were threaded through a light spring tether supported by a counterbalanced swivel (Instec, Plymouth, PA). Both the probe and the spring were cemented to the skull with dental acrylic. After surgery, the individual voles were placed in clean cages with a bottom area of the same dimensions as their home cages. The sides of these cages were 40 cm high to contain the vole while allowing free movement of the spring tether. Voles had ad-lib access to food and water and had 19–24 hr to recover from surgery and adapt to the presence of the tether. A constant flow of 1.0 $\mu\text{l}/\text{min}$ of CSF was maintained throughout the implantation procedure and recovery period. Before beginning the exper-

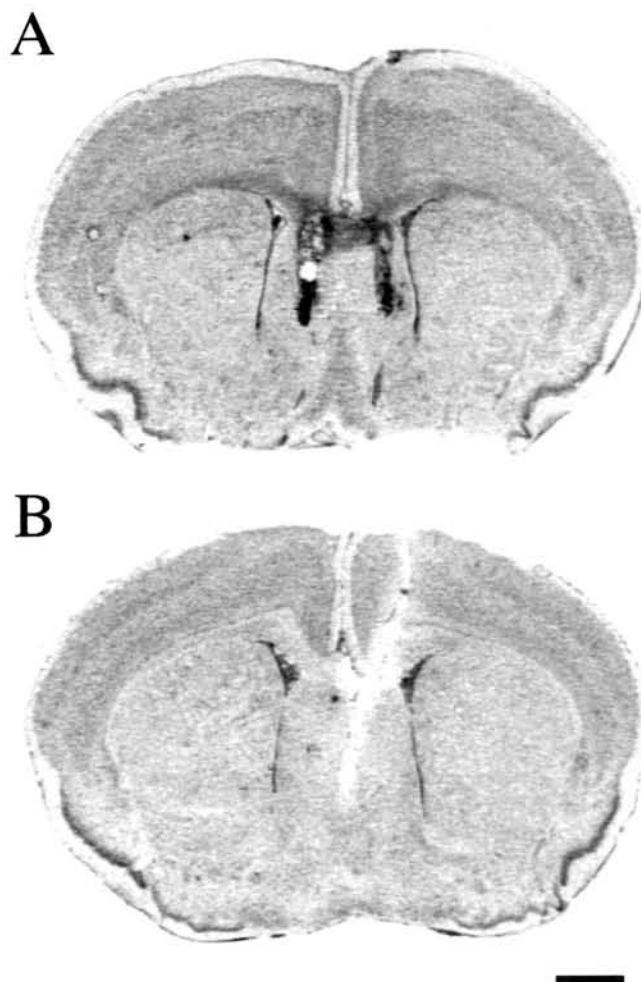


Figure 1. Photomicrographs of brain sections displaying the site for the bilateral injections (A) or the microdialysis probe perfusion (B) in the lateral septum of the male prairie vole. Scale bar = 2 mm.

iment, the pump supplying the probe was paused briefly (2–3 min) to allow a changeover to fresh CSF, AVP, AVP with the V1a antagonist, or AVP with OTA, as indicated by the group assignment. The tubing for the input side of the probe was calibrated such that it took 90 min for the solution to reach the active area of the probe, at which point the female was introduced into the male's cage.

Microdialysis probes were continuously perfused at a flow rate of 1.0 $\mu\text{l}/\text{min}$ during the 6 hr of cohabitation, and the dialysate from the outlet tube was checked periodically to ensure the proper functioning of the probe. After cohabitation, the spring and tubing were carefully cut to release the vole. The remainder of the probe did not impede the vole's movements. Subjects were then tested for a partner preference followed by histological verification of the probe placement. Voles with a probe extending beyond the dorsoventral level of the anterior commissure or with the probe track contacting the ventricle were excluded from data analysis.

Chemicals and Solutions

All peptide compounds were injected or perfused with the CSF (BioFluids, Inc., Rockville, MD). AVP was obtained from Sigma Chemical (St. Louis, MO). Both the selective AVP V1a receptor antagonist, $\text{d}(\text{CH}_2)_5[\text{Tyr}(\text{Me})]\text{AVP}$, and the OTA, $\text{d}(\text{CH}_2)_5\text{-}[\text{Tyr}(\text{Me})_2, \text{Thr}^4, \text{Try-NH}_2]$,

were obtained from Peninsula Labs (Belmont, CA). The estradiol benzoate was obtained from Sigma Chemical.

Partner Preference Test

The partner preferences were assessed in a three-chamber apparatus as described previously (Williams et al., 1992; Winslow et al., 1993). Briefly, the testing apparatus consisted of a neutral cage (20 cm high \times 50 cm long \times 40 cm wide) joined by plastic tubes (7.5 \times 16 cm) to two parallel identical cages, each housing a stimulus animal. The male voles were free to move throughout the apparatus, and the stimulus animals were tethered within their cages, providing no direct contact with each other. The familiar partner (the female that previously mated or cohabited with the subject) and a conspecific stranger (a female that had not previously encountered the subject) were used as stimulus animals. After the subjects were placed into the neutral cage, their behavior was recorded for 3 hr with a time-lapse VCR.

Data Quantification and Analysis

Although each treatment group originally started with 10–15 voles, some were excluded from data analysis because they failed to mate during the 24 hr of pairing or they had misplaced cannulas or microdialysis probes, as determined by histological verification. Therefore, the number of voles in each group mentioned in the experimental design (see later discussion) represents the number used for data analysis. To determine partner preferences, the following behavioral measures were recorded: duration and frequency of the subject's side-by-side contact with either the partner or with the stranger; time that the subjects spent in each cage; and frequency of cage entry. A partner preference was defined as subjects spending significantly more time in side-by-side contact with the partner versus the stranger, as determined by a *t* test ($p < .05$). Group differences for the time that subjects spent in each cage within each experiment were analyzed by a one-way analysis of variance (ANOVA), followed by a Student–Newman–Keuls (SNK) post hoc test. Because the effects of AVP on partner preferences could be secondary to its effects on locomotor activity during the preference test, the frequency of the subject's entries into the partner's or stranger's cage was subsequently used as an index of locomotor activity and was analyzed using a one-way ANOVA. Furthermore, male prairie voles that mate typically mount females within 3 hr and continue to mount every 40–60 min throughout the 24 hr (Insel et al., 1995). Therefore, the frequency of mating and duration of social contact between the subject and the female partner were also quantified throughout the first 6 hr of mating or the 6 hr of cohabitation to assess their possible effects on partner preferences. These data were also analyzed by a one-way ANOVA. Finally, in this study it was not possible to identify clearly the specific aspects of mating behavior, such as intromissions and ejaculations, and thus such analysis was not performed.

Experiment 1: Does Septal Administration of the V1a Receptor Antagonist Block Mating-Induced Partner Preferences?

Method

To test whether the blockade of the AVP V1a receptors in the lateral septum inhibited the formation of mating-induced partner preferences, sexually naive male prairie voles were stereotaxically implanted with guide cannulas bilaterally aimed at the lateral septum. One week later, they were divided into six groups that received bilateral injections of one of the following: CSF (200 nl per side; $n = 7$) or CSF containing 0.005 ng ($n = 6$), 0.05 ng ($n = 8$), 0.5 ng ($n = 7$), 5 ng ($n = 7$), or 50 ng ($n = 7$) of the AVP V1a receptor antagonist d(CH₂)₅[Tyr(Me)]AVP. Immediately after the injection, each prairie vole was exposed to a sexually receptive female for 24 hr of mating. Sexually receptive females were ovariectomized at

least 2 weeks before being injected with estradiol benzoate (1 μ g sc) for 3 days and then tested briefly with a sexually active male to ensure lordosis before being used as a mating partner. Pairs were videotaped throughout the 24 hr of pairing with a Panasonic light-sensitive camera, time-lapse VCR, and red light to accommodate recording during the dark cycle. At the end of the 24 hr of mating, subjects were tested for partner preferences. Thereafter, they were anesthetized and killed and their brains sectioned to verify injection sites histologically.

To test whether the blockade of the V1a receptor antagonist on partner preferences is specific to the lateral septum, in Experiment 1b male subjects were implanted bilaterally with guide cannulas aimed at the cingulate cortex. They were then divided into two groups that received injections of either CSF (200 nl per side; $n = 9$) or CSF containing 0.05 ng of the V1a antagonist (a dosage that in the lateral septum blocked partner preferences; $n = 7$). After 24 hr of mating, subjects were tested for the formation of partner preferences. The cingulate cortex was chosen because this area has been implicated in learning and memory in general and contains AVP receptors in prairie voles. After the behavioral test, subjects were killed and their brains sectioned to verify injection sites.

Results

As predicted, after 24 hr of mating, control males that received injections of CSF in the lateral septum spent more time in side-by-side contact with the partner than with the stranger, $t(1) = 5.48$, $p < .001$ (see Figure 2A). A similar partner preference was also found in males injected with the low dose (0.005 ng) $t(1) = 2.49$, $p < .05$, or the high doses (5 ng), $t(1) = 3.48$, $p < .01$; (50 ng), $t(1) = 4.24$, $p < .01$, of the V1a antagonist. However, injections of the V1a antagonist at the concentrations of 0.05 ng or 0.5 ng blocked mating-induced partner preference formation: Males spent approximately equal amounts of time with the partner or the stranger (Figure 2A). Although no significant group differences were detected in the time the subjects spent in each cage, injections of either 0.05 ng or 0.5 ng of the V1a antagonist tended to reduce the subjects' time in the partner's cage and to increase their time in the stranger's cage (Table 1). No group differences were detected in the frequency of cage entries during the preference test. In addition, injections of the V1a antagonist did not influence the frequency of mating or duration of social contact with the female during the first 6 hr of mating.

Subjects injected with CSF, $t(1) = 4.44$, $p < .001$, or with 0.05 ng of the V1a antagonist, $t(1) = 4.55$, $p < .001$, in the cingulate cortex both spent significantly more time in side-by-side contact with the partner than with the stranger after 24 hr of mating (Figure 2B). For both groups, the subjects also tended to spend more time in the partner's cage relative to the stranger's cage. Group differences were not found in the frequency of cage entries during the preference test nor in the duration of social contact during the first 6 hr of mating. However, subjects injected with the V1a antagonist mated (10.7 ± 2.0 over the 6-hr period) more than those injected with CSF alone (3.4 ± 1.2), $t(1) = 3.30$, $p < .01$.

Experiment 2: Does Septal Administration of AVP Induce Partner Preferences Without Mating?

Method

This experiment was designed to test the hypothesis that septal administration of AVP induces partner preferences in the absence of mating. In an initial experiment, male prairie voles were implanted bilaterally with

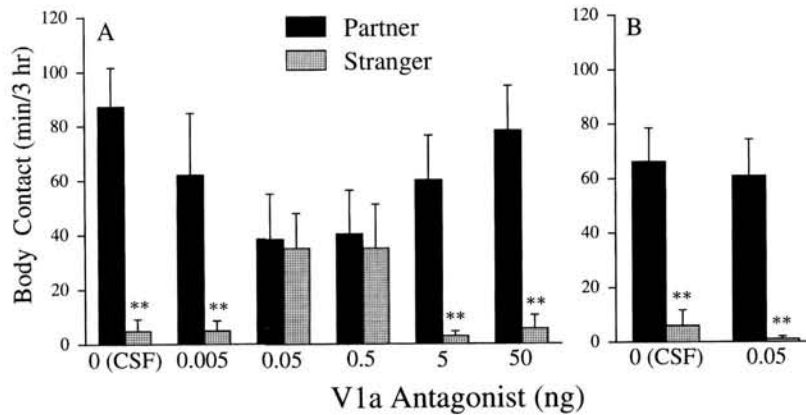


Figure 2. Effects of the vasopressin V1a receptor antagonist on partner preferences in male prairie voles. A: Control males injected with cerebrospinal fluid (CSF) into the lateral septum before 24 hr of mating had significantly more contact with the partner than with a stranger, as did males injected with the low (0.005 ng) or high (5 ng or 50 ng) concentrations of the V1a antagonist into the same brain area. However, males injected with 0.05 ng or 0.5 ng of the V1a antagonist in the lateral septum spent approximately equal amounts of time with either female during the preference test. B: Males injected with CSF or CSF containing 0.05 ng of the V1a antagonist into the cingulate cortex had more contact with the partner than with a stranger after 24 hr of mating. ** $p < .01$. Error bars indicate SEM.

guide cannulas aimed at the lateral septum. After 1 week of recovery, they were divided into four groups that received one of the following injections (200 nl per side): CSF ($n = 12$) or 0.5 ng ($n = 8$), 5 ng ($n = 8$), or 50 ng ($n = 7$) of AVP. After 6 hr of cohabitation with a sexually unreceptive female without mating (behaviors were videotaped to verify the absence of mating), subjects were tested for partner preferences. The sexually unreceptive females were ovariectomized at least 2 weeks before being used as stimulus animals. Although our behavioral data indicated that AVP-treated voles tended to spend less time with the stranger, no partner preferences were found. We hypothesized that the failure to induce partner preferences may have been due to a rapid clearance of AVP when injected into the tissue (Jones & Robinson, 1982). Therefore, in a second experiment we modified our procedures by continuously administering AVP via a microdialysis probe throughout the 6 hr of cohabitation. Subjects implanted with

a microdialysis probe in the septum were divided into three groups that received one of the following: probe perfusion of CSF ($n = 8$) or CSF containing AVP at concentrations of 0.08 ng/ μ l ($n = 6$) or 0.8 ng/ μ l ($n = 8$), respectively. The perfusion was conducted at a flow rate of 1.0 μ l/min throughout the 6 hr of cohabitation. Thereafter, subjects were tested for the formation of partner preferences and killed, and then probe placement was verified histologically.

Results

In an initial experiment in which AVP was injected into the lateral septum, our data did not demonstrate any significant treatment effects, although the subjects injected with AVP tended to

Table 1
Effects of the Vasopressin V1a Receptor Antagonist on Social Behavior of Male Prairie Voles

Behavior	V1a antagonist					
	0 (CSF) ($n = 7$)	0.005 ng ($n = 6$)	0.05 ng ($n = 8$)	0.5 ng ($n = 7$)	5 ng ($n = 7$)	50 ng ($n = 7$)
Partner cage						
Frequency	36.6 \pm 10.4	63.3 \pm 14.1	38.1 \pm 7.8	40.1 \pm 5.7	47.4 \pm 12.3	61.4 \pm 12.5
Duration	110.0 \pm 15.4	90.1 \pm 23.7	63.3 \pm 17.1	62.8 \pm 18.8	92.2 \pm 13.6	109.0 \pm 17.0
Stranger cage						
Frequency	39.3 \pm 9.4	55.8 \pm 14.1	38.0 \pm 6.1	45.6 \pm 6.8	47.7 \pm 7.6	59.3 \pm 12.1
Duration	47.2 \pm 14.2	62.8 \pm 22.6	92.6 \pm 17.4	91.1 \pm 19.6	51.4 \pm 11.0	43.2 \pm 15.6
Partner contact						
Frequency	8.0 \pm 1.8	5.0 \pm 2.1	3.4 \pm 1.4	4.0 \pm 1.4	6.6 \pm 1.2	7.3 \pm 1.4
Stranger contact						
Frequency	1.1 \pm 0.9	2.3 \pm 1.6	6.4 \pm 2.4	7.1 \pm 2.8	1.7 \pm 1.4	0.6 \pm 0.4
Mating bouts						
Frequency	9.0 \pm 1.7	12.3 \pm 1.7	10.8 \pm 1.5	9.5 \pm 0.9	5.3 \pm 2.1	9.1 \pm 0.8
Social contact						
Duration	205.2 \pm 24.1	208.8 \pm 14.8	159.1 \pm 19.8	182.3 \pm 38.2	196.0 \pm 19.0	186.1 \pm 15.1

Note. Duration is measured in minutes (mean \pm SEM). Mating bouts and social contact were measured during the first 6 hr of cohabitation with a female. CSF = cerebrospinal fluid.

spend less time in contact with the stranger (Table 2). In the follow-up experiment, subjects that received continuous probe perfusion of the high concentration of AVP (0.8 ng/1 μ l) spent significantly more time with the partner than with the stranger in the preference test, $t(1) = 2.68, p < .05$ (see Figure 3). In contrast, subjects that received probe perfusion of CSF alone or the low concentration of AVP (0.08 ng/1 μ l) showed no differences in side-by-side contact with the partner or with the stranger. Group differences were not found in the time that the subjects spent in each cage during the preference test. Furthermore, no differences were found between the CSF-dialyzed subjects and those dialyzed with the high concentration of AVP (0.8 ng/1 μ l) in their locomotor activity during the preference test or in the duration of social contact throughout the 6 hr of cohabitation with a female. Subjects treated with the low concentration of AVP (0.08 ng/1 μ l) appeared to be more active, scoring higher in the frequency of cage entries, $F(2, 19) = 9.53, p < .01$ for the partner cage; $F(2, 19) = 9.20, p < .01$ for the stranger cage, and lower in the duration of social contact $F(2, 19) = 6.0, p < .05$, relative to the other two groups.

Experiment 3: Does Septal OT Receptor Blockade Inhibit Mating-Induced Partner Preferences?

Method

Cho et al. (1999) reported that OT may also regulate partner preference formation in male prairie voles. Because the lateral septum in prairie voles contains OT receptors (Insel & Shapiro, 1992; Wang & Young, 1997), the present experiment was performed to test whether blockade of OT receptors in the lateral septum inhibited mating-induced partner preferences. Male prairie voles were implanted bilaterally with guide cannulas aimed at the lateral septum and were divided into three groups that received injections of one of the following: CSF ($n = 10$) or CSF containing 0.05 ng ($n = 12$) or 0.5 ng ($n = 10$) OTA. The subjects then mated for 24 hr and were

tested for a partner preference. The injection sites were verified histologically after the behavioral test.

Results

Administration of OTA into the lateral septum also blocked mating-induced partner preferences. Subjects that received septal injections of CSF spent more time in contact with the partner than with the stranger, $t(1) = 2.45, p < .05$ (see Figure 4). However, such behavior was not found after the subjects were injected with 0.05 ng or 0.5 ng OTA before mating. No treatment effects were found in the time that subjects spent in each cage or in the frequency of cage entries. In addition, no group differences were detected in the frequency of mating or duration of social contact during the first 6 hr of mating.

Experiment 4: Does Septal Administration of the V1a Antagonist or OTA Block AVP-Induced Partner Preferences?

Method

Because data from Experiment 3 indicated that the blockade of OT receptors in the lateral septum inhibited mating-induced partner preferences, the present experiment was performed to investigate whether blockade of the OT receptors interfered with the effects of AVP on behavior. Male prairie voles were stereotaxically implanted with a microdialysis probe aimed at the septum. They were then divided into three groups that received probe perfusion of one of the following: AVP (0.8 ng/1 μ l; $n = 10$), AVP with the same dose of the V1a receptor antagonist ($n = 8$), or AVP with the same dose of the OT receptor antagonist ($n = 9$). All perfusions were performed at a flow rate of 1.0 μ l/min throughout the 6 hr of cohabitation with the female in the absence of mating. Thereafter, subjects were tested for partner preference and killed, and probe placement was verified histologically.

Table 2
Vasopressin Effects on Behavior During a Partner Preference Test

Behavior	Vasopressin			
	0 (CSF) ($n = 12$)	0.5 ng ($n = 8$)	5 ng ($n = 8$)	50 ng ($n = 7$)
Body contact				
Partner				
Frequency	5.2 \pm 1.3	5.8 \pm 1.4	33.5 \pm 27.9	4.4 \pm 3.0
Duration	50.2 \pm 10.3	59.1 \pm 13.2	43.7 \pm 17.7	58.9 \pm 15.5
Stranger				
Frequency	4.8 \pm 1.0	2.6 \pm 1.3	4.1 \pm 2.0	3.3 \pm 2.0
Duration	32.9 \pm 11.7	26.5 \pm 13.3	16.0 \pm 9.7	19.8 \pm 11.9
In cage				
Partner				
Frequency	33.4 \pm 6.9	30.6 \pm 5.0	54.3 \pm 12.0	28.6 \pm 3.3
Duration	82.4 \pm 12.9	110.0 \pm 14.1	78.3 \pm 15.9	92.9 \pm 18.2
Stranger				
Frequency	35.7 \pm 5.7	34.3 \pm 5.2	44.4 \pm 6.4	32.7 \pm 4.4
Duration	76.8 \pm 12.5	48.8 \pm 15.0	63.0 \pm 18.1	67.5 \pm 19.1
Neutral				
Duration	19.0 \pm 1.9	21.3 \pm 2.8	34.7 \pm 6.7	22.3 \pm 3.7
Cohabitation				
Social contact				
Duration	190.2 \pm 15.3	182.1 \pm 17.0	173.0 \pm 25.4	161.8 \pm 18.2

Note. Duration is measured in minutes (mean \pm SEM). CSF = cerebrospinal fluid.

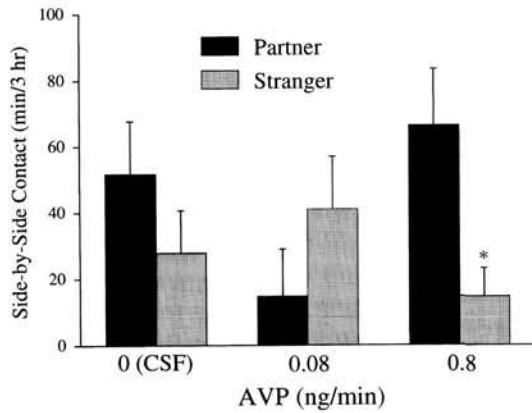


Figure 3. Effects of septal administration of vasopressin (AVP) on partner preferences in male prairie voles. AVP was perfused via a microdialysis probe continuously throughout the 6 hr of cohabitation with a female, without mating. Males dialyzed with cerebrospinal fluid (CSF; control) or with the low concentration of AVP ($0.08 \text{ ng} \cdot \mu\text{l}^{-1} \cdot \text{min}^{-1}$) had similar side-by-side contact with either female during the preference test. However, males dialyzed with the high concentration of AVP ($0.8 \text{ ng} \cdot \mu\text{l}^{-1} \cdot \text{min}^{-1}$) had more contact with the partner than with a stranger. * $p < .05$. Error bars indicate SEM.

Results

Subjects that received microdialysis administration of AVP in the septum spent significantly more time in contact with the partner than with the stranger, $t(1) = 2.28, p < .05$ (see Figure 5), consistent with previous observations in Experiment 2. However, the effect of exogenous AVP on behavior was blocked when the subjects received AVP treatment concurrent with the V1a antagonist or with OTA (see Figure 5). No group differences were found in the time that subjects spent in each cage or in the frequency of cage entries during the preference test or in the duration of social contact during the first 6 hr of mating.

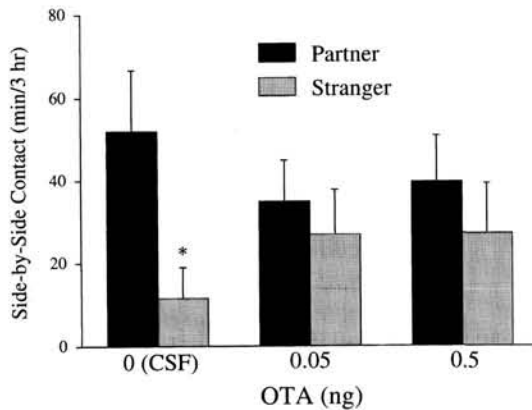


Figure 4. Effects of the oxytocin receptor antagonist (OTA) injected into the lateral septum on partner preferences in male prairie voles. Control males (cerebrospinal fluid; CSF) had more contact with the partner than with a stranger after 24 hr of mating. However, males injected with OTA (0.05 ng or 0.5 ng) in the lateral septum before mating spent approximately equal amounts of time in contact with either female during the preference test. * $p < .05$. Error bars indicate SEM.

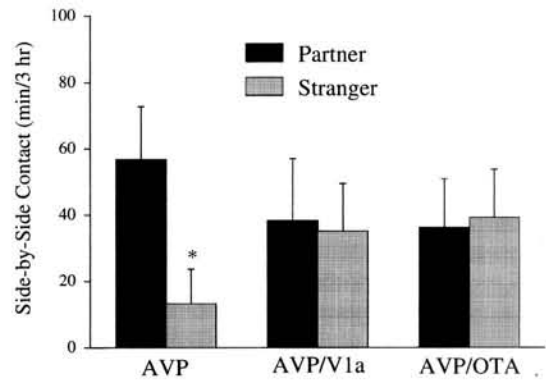


Figure 5. Administration of the V1a receptor antagonist or the oxytocin receptor antagonist (OTA) blocked vasopressin (AVP)-induced partner preferences in male prairie voles. Males dialyzed with AVP ($0.8 \text{ ng} \cdot \mu\text{l}^{-1} \cdot \text{min}^{-1}$) in the septum during the 6 hr of cohabitation had more contact with the partner than with a stranger in the preference test. However, males dialyzed with AVP concurrently with the same dose of the V1a antagonist or OTA spent approximately equal amounts of time in contact with either female. * $p < .05$. Error bars indicate SEM.

General Discussion

Previous studies have implicated the neuropeptide AVP in the regulation of pair bonding in the male prairie vole (Cho et al., 1999; Winslow et al., 1993). Results from the present study confirm and extend these findings, demonstrating that the lateral septum is an important brain area in which AVP regulates pair bond formation in the male prairie vole. In addition, we have found that administration of OTA in the lateral septum blocked pair bond formation induced by either mating or AVP administration. Finally, with few exceptions, administration of AVP, the V1a antagonist, or OTA generally had no effects on the prairie vole's locomotor activity during the preference test nor on the frequency of mating or duration of social contact during cohabitation with a partner, suggesting that the treatment effect on pair bond formation was not an artifact of hyper- or hypoactivity, nor was it the residual effect on mating or social contact. Taken together, these data suggest that AVP is essential, and the activation of both AVP and OT receptors in the lateral septum is necessary for pair bond formation in male prairie voles.

Septal AVP Regulates Pair Bond Formation

In the present study, administration of the AVP V1a receptor antagonist in the lateral septum blocked mating-induced partner preference, whereas administration of AVP induced this behavior in the absence of mating. These data provide evidence not only supporting the previous finding that AVP is essential for pair bond formation (Cho et al., 1999; Winslow et al., 1993) but also demonstrating, for the first time, that the lateral septum is important for the AVP regulation of pair bond formation in the male prairie vole. Furthermore, administration of the V1a antagonist in the cingulate cortex did not block mating-induced partner preference, indicating that the effects of AVP on behavior seem to be site specific. It needs to be pointed out, however, that the AVP effects on partner preferences are dose dependent. Therefore, our results with only one dose of the V1a antagonist being injected into the cingulate

cortex cannot exclude the possibility that this brain region may respond to different doses of the V1a antagonist or AVP.

In our study, acute injections of AVP in the lateral septum did not have significant effects on partner preferences. A similar phenomenon was also found in a previous study in which only continual administration of AVP by an osmotic minipump, but not acute injections of AVP, into the lateral ventricle induced pair bonding in male prairie voles (Winslow et al., 1993). Because the cohabitation was more than 6 hr and the clearance of centrally injected AVP has been reported to be only minutes (Jones & Robinson, 1982), it is likely that the AVP administered acutely before cohabitation did not last long enough to significantly affect behavior. Therefore, we chose to use microdialysis administration to deliver AVP locally into the extracellular space of the septum continuously throughout the 6 hr of cohabitation, a method that has the advantage of mimicking the pattern of AVP release in the septum more accurately than other types of administration (Engelmann, Ludwig, & Landgraf, 1992). We have found that prairie voles dialyzed with AVP (0.8 ng/ μ l) in the septum developed a partner preference without mating.

The data also indicate the feasibility of using the microdialysis technique to administer AVP concomitantly with a behavioral test on voles. A problem with our study, however, was that the precise effective dose of AVP in the septum was unknown. Taking into account the length of the membrane, the concentration of AVP in the perfusate, and the delivery rate of the probe as determined in a previous study using [3 H]AVP (Engelmann et al., 1992), we estimate that approximately 0.5–1 ng of AVP, the V1a antagonist, or OTA was delivered into the septum during the 6 hr of cohabitation.

Another issue that needs to be addressed is the possible diffusion of AVP into surrounding areas. Given the short half-life of AVP in the brain, it is likely that the majority of the administered AVP remained in the septum (Engelmann et al., 1992). Finally, it is unknown why prairie voles dialyzed with the low concentration of AVP (0.08 ng/ μ l) had less contact with the partner during cohabitation and a higher level of locomotor activity during the preference test than did those in the other two groups. Nevertheless, no differences were detected in these behaviors between the CSF-dialyzed prairie voles and those dialyzed with the high concentration of AVP (0.8 ng/ μ l), indicating that their differences on partner preferences were not due to the alteration in social contact or locomotor activity.

A large body of evidence from studies in rats has demonstrated that AVP in the lateral septum plays an important role in enhancing learning and memory and social recognition. For example, in the study of olfactory-mediated social memory and memory consolidation, microinjections of AVP in the lateral septum enhanced social memory, whereas injections of the V1a antagonist diminished it (Bluthe, Schoenen, & Dantzer, 1990; Dantzer et al., 1988; Le Moal, Dantzer, Michaud, & Koob, 1987). In addition, castration, which reduces the density of AVP immunoreactive projections in the lateral septum and the AVP mRNA expression in the BST (G. J. De Vries, Buijs, & Sluiter, 1984; Miller, De Vries, Al-Shamma, & Dorsa, 1992), diminished social memory (Dantzer, Bluthe, & Kelley, 1991). In contrast, physiological challenges such as injections of hypertonic saline, which induce septal AVP release, enhanced social memory (Demotes-Mainard, Chauveau, Rodriguez, Vincent, & Poulain, 1986). Together, these data suggest

that septal AVP may enhance memory formation and social recognition, a process that is presumably involved in the formation and expression of partner preferences (Cho et al., 1999).

Several neurochemical, neuroanatomical, and behavioral studies in voles have also provided evidence supporting the notion that septal AVP regulates social behavior. The lateral septum contains AVP projections (Bamshad, Novak, & De Vries, 1993; Wang, 1995) and AVP V1a receptors in the vole brain (Insel, Wang, & Ferris, 1994; Wang & Young, 1997; Young, Winslow, Nilsen, & Insel, 1997). Cohabitation or mating with a female induces a decrease in the density of AVP immunoreactive projections in the lateral septum, along with an increase in the AVP mRNA expression in the BST, suggesting a mating-induced AVP release within the lateral septum in the male prairie vole (Bamshad et al., 1994; Wang, Smith, et al., 1994). In addition, increased neuronal activation in the lateral septum, measured by *c-fos* activity, was also found to be associated with mating-induced selective aggression (Wang, Hulihan, & Insel, 1997). Finally, septal injections of AVP enhanced, whereas the V1a receptor antagonist diminished, paternal behavior in male prairie voles (Wang, Ferris, & De Vries, 1994). These data, together with those from our current study, suggest that the lateral septum is a brain region in which AVP regulates social behaviors in the male prairie vole.

Activation of OT Receptors Is Required for the AVP Regulation of Pair Bonding

Although OT has been found to regulate pair bond formation in female prairie voles (Insel & Hulihan, 1995; Williams et al., 1994), administration of OT or OTA into the lateral ventricle did not significantly alter mating- or AVP-induced pair bonding in male prairie voles (Winslow et al., 1993). It has been hypothesized that the pair bond formation in prairie voles is regulated by gender-specific mechanisms: AVP regulates pair bonding in males, whereas OT regulates a similar behavior in females (Carter, De Vries, & Getz, 1995; Insel, 1997; Insel & Hulihan, 1995). However, discrepancies have been reported. An intracerebroventricular administration of OTA blocked, whereas administration of OT induced, partner preferences in male prairie voles (Cho et al., 1999). In the present study, septal administration of OTA blocked mating-induced pair bonding, suggesting that the activation of the OT receptors is also necessary for pair bond formation in the male prairie vole. In addition, septal administration of OTA blocked pair bonding induced by exogenous AVP, indicating that AVP requires access to OT receptors to affect behavior. It is worthwhile to note that the same receptor antagonists and agonists were used in the present and aforementioned studies in voles.

Why are there discrepancies among reported data of the AVP-OT regulation of pair bonding? Different paradigms incorporating different amounts of cohabitation and social stimulation necessary for pair bond formation might contribute to the discrepancies reported in previous studies (Cho et al., 1999; Winslow et al., 1993). In addition, OT and OTA were administered into the lateral ventricle in the previous study (Winslow et al., 1993), whereas OTA was administered directly into the lateral septum in the present experiment. It is possible that the intracerebroventricular administration of OT or OTA (Winslow et al., 1993) did not result in a sufficient amount of drug concentration in the lateral septum to interfere with social behavior in male prairie voles.

It is interesting to note that the OT receptor blockade in the lateral septum inhibited pair bond formation induced either by mating or by AVP administration, suggesting that released AVP during mating interacts with the OT system to regulate behavior. The lateral septum contains both AVP and OT receptors in voles (Insel & Shapiro, 1992; Insel et al., 1994; Wang & Young, 1997; Young, Huot, Nilsen, Wang, & Insel, 1996; Young et al., 1997). Among all conceivable alternatives for AVP and OT interactions, one possible explanation may be that AVP and OT act concurrently and that activation of both receptors is essential for pair bond formation. Therefore, blockade of either receptor will block the behavior. Alternatively, it is also possible that AVP and OT act sequentially. If OT acts downstream from the AVP receptors, blockade of either receptor will also block AVP-induced behavior. However, this notion is refuted by the results from a study in which intracerebroventricular administration of the V1a antagonist blocked OT-induced pair bonding (Cho et al., 1999). Could it be possible that OT acts upstream from the AVP receptors? If so, we would expect that the OT receptor blockade should not interfere with the AVP-induced behavior, which is disproved by the results from our present study. Finally, AVP, OT, and their antagonists may have complex interactions with each other's receptors (Barberis & Tribollet, 1996; Engelmann, Wotjak, Neuman, Ludwig, & Landgraf, 1996). Although previous studies demonstrated that both the V1a antagonist and OTA are selective for the V1a and OT receptors, respectively, in prairie voles (Insel & Shapiro, 1992; Insel et al., 1994), and that each ligand has much higher affinity for its own versus the counterpart receptors in rats (Barberis & Tribollet, 1996), we cannot exclude the possibility that administration of either receptor antagonist may act on both AVP and OT receptors. In addition, although a concurrent activation of both AVP and OT receptors seems to be required for pair bond formation, it is still not clear whether such coactivation is due to AVP acting concurrently on both receptors or AVP and OT acting on their own receptors. Although our data show that administration of AVP alone induces pair bonding, the possibility that OT is released endogenously during cohabitation, which, in turn, acts on the OT receptors, cannot be excluded.

If AVP acts in the lateral septum to regulate pair bonding, then why do prairie voles have fewer AVP receptors in this brain area in relation to promiscuous montane voles (*Microtus montanus*), which do not display mating-induced pair bonding (Insel et al., 1994, 1995; Wang, Young, Liu, & Insel, 1997; Young et al., 1997)? It is not clear how to interpret this paradox, and several possibilities exist. First, species differences in the number of AVP receptors in the lateral septum may not represent a functional difference. Prairie voles may have a sufficient number of AVP receptors in the lateral septum, although at a lower level than that of montane voles. Second, the effects of AVP on pair bonding may be regulated at the presynaptic level: Mating may induce a species-specific pattern of AVP release within the lateral septum. This notion is supported by the finding that mating and reproduction induce changes in AVP immunoreactivity in the lateral septum and AVP mRNA labeling in the BST only in monogamous, but not in promiscuous, voles (Bamshad et al., 1993; Wang, Smith, et al., 1994). A third possibility is that AVP may interact with other neurotransmitter systems to regulate pair bonding, and species differences in the second neurotransmitter system may account for the differences in behavior.

Possible Mechanisms of AVP Actions

Although our data indicate that the blockade of AVP or OT receptors in the lateral septum diminishes mating-induced pair bonding, the underlying mechanisms are still unknown. Because both AVP and OT affect social recognition (Engelmann et al., 1996; Popik, Vetulani, & van Ree, 1992; Renelli et al., 1995) and individual recognition is presumably essential for the expression of partner preferences in prairie voles (Cho et al., 1999), it is possible that the blockade of either receptor may block the onset of social memory or individual recognition necessary for pair bond formation. In contrast, administration of AVP in the lateral septum induces partner preferences, mimicking the effects of mating. It is likely that administered AVP may facilitate memory formation for a partner by hastening the processes important for individual recognition, as has been demonstrated in rats (Dantzer et al., 1988; Engelmann et al., 1996). Another possibility is that the blockade of AVP or OT receptors may decrease the rewarding value of copulation. Earlier studies demonstrated that copulation may serve as a potent reinforcer. Male rats work to engage in copulation (Sheffield, Wulff, & Backer, 1955) or to gain access to stimuli associated with copulation (Everitt, Cador, & Robbins, 1989). In addition, both male and female rats display mating-induced place preferences (Everitt, 1990; Oldenburger, Everitt, & de Jonge, 1992). It is possible that with repeated mating an association may form between the partner and the rewarding attributes of mating. The released AVP (or OT) during mating may sustain this association. Therefore, the blockade of AVP (or OT) receptors before mating may disrupt this association and decrease the rewarding value of copulation. Nevertheless, these scenarios need to be further studied.

Summary

AVP is a neuropeptide that has been implicated in learning and memory and in the regulation of social behaviors in a variety of species (Dantzer & Bluthé, 1993; Engelmann et al., 1996). We have shown that, in the male prairie vole, administration of AVP in the lateral septum induces pair bond formation in the absence of mating, whereas administration of the V1a receptor antagonist or OT receptor antagonist blocks the induction of this behavior either by mating or by AVP treatment. Taken together, these data suggest that AVP in the lateral septum regulates pair bond formation in male prairie voles and that this process requires access to both AVP and OT receptors. It should be noted that this study focused on the AVP regulation of pair bonding, and thus examination of the effects of OT was limited only to its interference with the effects of AVP on behavior. A previous study showed that OT also regulates pair bonding in male prairie voles, presumably by acting on both OT and AVP receptors (Cho et al., 1999). Therefore, septal OT induction on pair bonding and its interaction with AVP on the regulation of behavior should not be ignored and are currently being investigated in our ongoing research.

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