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Both oxytocin and vasopressin may influence alloparental behavior in male prairie voles

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Abstract

Neuropeptides, especially oxytocin (OT) and arginine vasopressin (AVP), have been implicated in several features of monogamy including alloparenting. The purpose of the present study was to examine the role of OT and AVP in alloparental behavior in reproductively naïve male prairie voles. Males received intracerebroventricular (ICV) injections of artificial cerebrospinal fluid (aCSF), OT, an OT receptor antagonist (OTA), AVP, an AVP receptor antagonist (AVPA), or combinations of OTA and AVPA and were subsequently tested for parental behavior. Approximately 45 min after treatment, animals were tested for behavioral responses to stimulus pups. In a 10-min test, spontaneous alloparental behavior was high in control animals. OT and AVP did not significantly increase the number of males that showed parental behavior, although more subtle behavioral changes were observed. Combined treatment with AVPA and OTA (10 ng each) significantly reduced male parental behavior and increased attacks; following a lower dose (1 ng OTA/1 ng AVPA), males were less likely to display kyphosis and tended to be slower to approach pups than controls. Since treatment with only one antagonist did not interfere with the expression of alloparenting, these results suggest that access to either OT or AVP receptors may be sufficient for the expression of alloparenting.

Keywords: Oxytocin; Vasopressin; Alloparental behavior

Introduction

Maternal behavior is a reliable feature of the postpartum period in most mammals. However, pup-directed positive behaviors, including retrieval and kyphosis (huddling), also may occur in nonparturient animals including males. Male parental behavior and alloparenting by reproductively naïve animals are special characteristics of socially monogamous or cooperative breeding species (Carter and Roberts, 1997).

The mechanisms that facilitate the response to offspring have been well studied in females and to a lesser extent in males (reviewed in Carter and Keverne, 2002; Lonstein and De Vries, 2000; Marler et al., 2003; Numan and Insel, 2003). Hormones implicated in parturition and lactation are likely candidates for a role in maternal behavior. For example, the neuropeptide oxytocin (OT) has been linked to the onset of maternal care in rats (Pedersen and Prange, 1979; Pedersen et al., 1982) and sheep (Kendrick et al., 1987).

Arginine vasopressin (AVP) is a peptide that is structurally similar to OT. Extrahypothalamic AVP systems are sexually dimorphic and androgen-dependent within the central nervous system (De Vries and Simerly, 2002). Because testosterone promotes the synthesis of AVP, the latter peptide has been the focus of studies of male parental care (Carter and Roberts, 1997; Marler et al., 2003). However, both males and females synthesize both OT and AVP (Wang et al., 1996). In rats, AVP also may facilitate maternal behavior, albeit more slowly than OT (Pedersen et al., 1982). At the receptor level, interactions between OT and AVP are abundant (Carter, 1998; Cho et al., 1999; Engelmann et al., 1996). Thus, it is possible that both OT and AVP influence social behaviors including pup-directed behaviors in both sexes.

Prairie voles (*Microtus ochrogaster*) are socially monogamous rodents that typically show high levels of paternal and alloparental behavior (Carter and Roberts, 1997; Roberts et al., 1998a,b; Wang and Novak, 1994). In male prairie voles, AVP-immunoreactive fiber density decreased in the

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lateral septum and lateral habenular nucleus after mating (Bamshad et al., 1994). AVP gene expression in the hypothalamus increased postpartum in both male and female prairie voles, while OT gene expression and receptor binding increased only in females (Wang et al., 2000). Administration of AVP directly into the lateral septum enhanced paternal behavior in male prairie voles, while administration of an AVP antagonist (AVPA) blocked paternal behavior in this species (Wang et al., 1994). In socially monogamous deer mice (*Peromyscus californicus*), the density of AVP immunoreactive (IR) fibers within the bed nucleus of the stria terminalis also was correlated positively with retrievals and a paternal behavior score (Bester-Meredith and Marler, 2003). Administration of AVP also facilitated paternal behavior in meadow voles (*Microtus pennsylvanicus*), a species that although not usually considered monogamous does display facultative paternal behavior (Parker and Lee, 2001).

Despite these findings, the role of AVP in paternal care is still controversial. Castrated voles displayed reductions in AVP-ir cells in the bed nucleus of the stria terminalis and the medial amygdaloid nucleus (Wang and De Vries, 1993). In the above study, males tested 4 weeks postcastration displayed reduced alloparental care, while those that were castrated and implanted with a silastic capsule containing testosterone did not. However, in another study, castration did not influence paternal behavior (Lonstein and De Vries, 1999). Males castrated 8 weeks earlier, although retaining almost no AVP-ir fibers in the lateral septum and the lateral habenula, were still highly alloparental. A third study, from the same laboratory that originally reported a role for AVP in paternal behavior, failed to replicate the effects of AVP and AVPA injected into the lateral septum (Bucknell-Pogue et al., 2001). Finally, neonatal castration reduced alloparental care in adult males (Lonstein et al., 2002). Lonstein and De Vries (1999) suggest that differences in the geographical origins of the test subjects, or litter effects in infanticidal behavior, might account for the differences in results. The causes of these discrepancies remain unresolved, but leave open the possibility that hormones other than AVP play a role in male parental or alloparental behaviors.

OT also has been indirectly implicated in the regulation of paternal care. In sexually and parentally experienced (versus inexperienced) male meadow voles, Parker et al. (2001) found increases in OT receptor binding in the accessory olfactory nucleus, bed nucleus of the stria terminalis, lateral septum, and lateral amygdala. However, in California mice (*P. californicus*), plasma OT levels in expectant fathers did not differentiate in parental versus nonparental males (Gubernick et al., 1995).

In laboratory tests, about 60–80% of sexually naïve male prairie voles are spontaneously parental when first exposed to pups, while adult females that have not experienced pregnancy or parturition typically display much lower levels of infant care and may even attack pups (reviewed in Carter and Roberts, 1997; Lonstein and De Vries, 2000). There are no published accounts of the effects of centrally injected OT on parental behavior in this species, although in female prairie voles, central injections of OT reduced aggression toward males (Witt et al., 1990). At doses comparable to those used here, central administration of either OT or AVP can facilitate social contact and the development of a partner preference in both male and female prairie voles; in addition, blocking either the OT or AVP receptors interfered with partner preferences, although these animals remained highly social (Cho et al., 1999).

The purpose of the present study was to investigate the capacity of either OT or AVP to influence alloparental behavior in male prairie voles. Reproductively naive male prairie voles received intracerebroventricular (ICV) injections of either AVP or OT or their antagonists; these males subsequently were observed during pup encounters to examine the hypothesis that either AVP or OT might increase parental care in males, while blocking either the AVP or OT receptor might reduce male parental care.

Materials and methods

Subjects were laboratory-bred male prairie voles, F3-4 descendants of a wild stock originally captured near Champaign, IL. Animals were maintained on a 14:10-h light/dark cycle and provided with food (Purina rabbit chow) and water ad libitum. Breeding pairs were maintained in large polycarbonate cages (44 × 22 × 16 cm), with cotton for nesting material. At 21 days of age, offspring were removed and housed in same-sex sibling pairs in smaller (27 × 16 × 13 cm) cages. Males used in this study were sexually inexperienced and had no exposure to pups after weaning.

At approximately 60 days of age, male prairie voles were randomly assigned to groups that were uncannulated controls (NC) or underwent ICV cannulation and received one of the treatments described below. The day before parental care testing, voles were anesthetized with a mixture of ketamine and xylazine. The skull surface was exposed and a small opening was drilled through the skull. Using a stereotaxis apparatus, a 26-gauge stainless steel guide cannula (Plastics One, Roanoke, VA) was implanted into the right lateral cerebral ventricle and fixed in place with superglue and dental enamel. Animals were allowed to recover for 24 h before behavioral testing. A 24-h recovery period was chosen based on previous studies in this laboratory (Cho et al., 1999) and to minimize the chance that the cannula would become dislodged (screws are not used to secure the cannula in voles). In addition, following cannulation, animals must be housed singly because a cage-mate might remove the cannula through grooming; as voles are very social, we wished to avoid the stress from prolonged social isolation. At the conclusion of the experiment, voles were sacrificed and the placement of the cannula verified by the injection of India ink. Only successful hits were used in the analysis of behavioral data.

Cannulated males were given one of nine ICV treatments: artificial cerebrospinal fluid (aCSF, the vehicle for the other treatments), 100 ng OT (Peninsula Laboratories, San Carlos, CA), 1 ng oxytocin antagonist (OTA), 10 ng OTA, 100 ng AVP (Sigma-Aldrich, St. Louis, MO), 1 ng AVPA, 10 ng AVPA, a combined dose of 1 ng OTA/1 ng AVPA, or a combined dose of 10 ng OTA/10 ng AVPA. All injections were 1 μl in volume and were administered slowly from a 23-gauge gas-tight Hamilton syringe. The mode of administration and doses of OT or AVP (100 ng) and OTA or AVPA (1 or 10 ng) selected here have been shown to be behaviorally active in male prairie voles (Cho et al., 1999). All groups consisted of 9–11 animals. Only one animal from each litter was given a specific treatment.

The OT receptor antagonist ([d(CH_2)$_5$, Tyr(Me)^2, Orn^8]-Vasotocin) used here was designed by Bankowski et al. (1980) and is commercially available from Peninsula Laboratories. This compound has been tested extensively in behavioral studies of rodents, particularly sexual (Argiolas et al., 1987), social (Cho et al., 1999), and feeding behaviors (Arletti et al., 1989; Olson et al., 1991). The AVPA used here ([b-Mercapto-b,b-cyclopentamethylenepropionyl^1, O-me-Tyr^2, Arg^8]-Vasopressin; Sigma Laboratories) is a selective V_{1a} antagonist (Cotte et al., 2000) also used in behavioral research (Cho et al., 1999; Winslow et al., 1993). These antagonists have been shown to be approximately 10–100 times more effective in receptor binding than the natural ligands (Barberis and Tribollet, 1996; Witt and Insel, 1991); therefore, a lower dose of antagonist than peptide was used here.

Hormone treatments were administered and followed immediately by 45 min of habituation to a two-cage testing arena with a short connecting tube, allowing test animals to elect to engage or avoid pups (as described in Roberts et al., 1998b). The 45-min habituation period was chosen based on a number of studies (Pedersen et al., 1982; Wang et al., 1994) and could potentially have affected the results. Test animals then were exposed to two nonrelated pups, aged 1–3 days, for 10 min. Stimulus pups were placed in the cage furthest from the experimental animal. If pups were attacked (usually by biting them), the test was immediately stopped and the pups returned to their parents. Attacks rarely resulted in severe injury to the pup, and if it did, the pup was euthanized immediately. Tests were recorded on videotape and pup-directed behaviors were scored by an experimentally blind observer on Behavior Tracker 1.0 software (http://www.behaviortracker.com). Behaviors scored included the following: first approach to pup (latency); sniff pup (duration); lick or groom pup (duration); huddling or kyphosis, as defined by Stern and Johnson (1990) (latency and duration); nonkyphotic contact including lying next to the pup, lying flat on the pup, or resting the head on the pup (latency and duration); retrievals (frequency); and attacks (frequency). Behaviors as scored were mutually exclusive. Latencies were scored as well as durations for some behaviors because latency to kyphosis, in particular, has been previously described as particularly representative of full alloparental responsiveness in this species (Roberts et al., 1998a). In addition, a long latency to approach the infant often appears indicative of fear on the part of the test animal. OT- and aCSF-treated males were also rescored for autogrooming to address possible explanations for behavioral differences in these groups.

Data were analyzed in two different ways: an overall assessment of parental and nonparental behaviors per treatment, as well as quantitative comparisons of individual behaviors such as kyphosis and aggressive behavior. Parental behaviors were defined as licking or grooming, retrieving, kyphosis, and nonkyphotic contact. Nonparental behaviors were defined as avoidance or attacks. Fisher's exact probability tests (Sokal and Rohlf, 1981) were used to

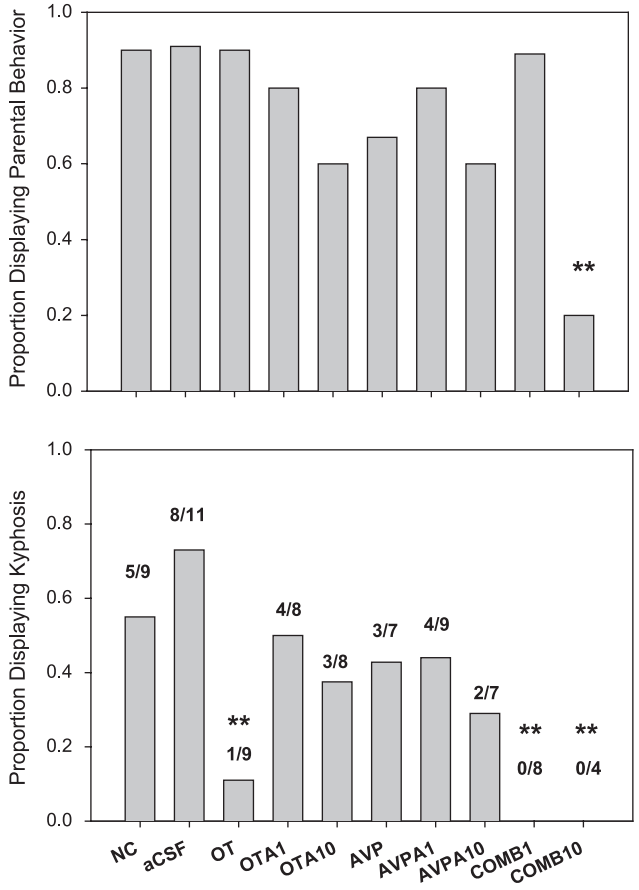

Fig. 1. (Top) Proportion of male voles responding parentally (displaying kyphosis, nonkyphotic contact, retrieving, or licking or grooming) towards infants. Significance was assessed in comparison to the aCSF group. Treatments are as follows: control (NC—no cannulation), aCSF (artificial cerebrospinal fluid), OT (oxytocin), OTA1 (1 ng oxytocin antagonist), OTA10 (10 ng OTA), AVP (arginine vasopressin), AVPA1 (1 ng AVP antagonist), AVPA10 (10 ng AVPA), COMB1 (combination treatment, 1 ng AVPA and 1 ng OTA), and COMB10 (combination treatment, 10 ng AVPA and 10 ng OTA). Only the COMB10 group differed significantly from the aCSF control (Fisher's exact probability test, $^{**}P = 0.002$). All group sizes are equal to 9–11 males. (Bottom) Proportion of male voles (out of those not attacking) that displayed kyphosis. Significance was assessed in comparison to the aCSF group. **Different from aCSF group at $P < 0.05$.

compare parental and nonparental behaviors for each treatment to the aCSF control. Individual behaviors were examined by one-way ANOVA for males that were not aggressive towards infants, and latencies for behaviors not displayed were set to 600 s. Residuals were checked for normality, and when necessary, data were transformed using the square root, quad root, or natural log transformations (Sokal and Rohlf, 1981) or compared using nonparametric tests (Wilcoxon's two-sample test; Sokal and Rohlf, 1981). Data were analyzed in SAS 8.0 (SAS Institute, Cary, NC). All tests were two sided and significance level was set to $P < 0.05$. A power analysis performed beforehand indicated a power of 0.8 at this sample size.

Results

Overall tendencies in behavior

Only males treated with 10 ng AVPA/10 ng OTA displayed a higher percentage of nonparental behavior when compared to aCSF controls (Fisher's exact probability test, $P = 0.002$; Fig. 1; called COMB10 in figure). In both control groups, 90–91% of the males showed parental behavior (Fig. 1). Control males that were cannulated and given aCSF were not significantly less parental than the control group that was not cannulated or otherwise treated. In fact, on more subtle behavioral measures (Table 1), aCSF-treated animals that displayed kyphosis actually spent more time doing so than did untreated controls (see below). For statistical comparisons to other treatment groups, the aCSF group was used throughout this paper. Below, individual behaviors are examined for males that did not attack infants.

Approaching pups

Males in the AVPA/OTA 1 ng group (COMB1 in Fig. 1) tended to have a longer approach latency to pups than aCSF males [$F(1,17) = 3.82$, $P = 0.067$; log-transformed; Table 1], while males in the AVPA/OTA 10 ng group had a significantly longer latency to approach pups [$F(1,13) = 7.42$, $P = 0.017$] than aCSF males.

Parental behaviors

Parental behaviors were observed in 60–90% of the males in all groups except those receiving a combined treatment with 10 ng AVPA/OTA (Fig. 1, top). Parental behavior, as defined in our methods, included four behaviors: kyphosis, nonkyphotic contact, licking or grooming, and retrieving.

Three groups differed significantly from aCSF males in overall proportion of kyphosis (Fig. 1, bottom). OT-treated males (Fisher's exact probability test, $P = 0.007$) were significantly less likely to display kyphosis than aCSF males. The reduced kyphosis in this group appears to have been a side effect of increased autogrooming in OT-treated males [$F(1,18) = 5.00$, $P = 0.038$; quad root transformed]. Kyphosis was especially affected by the combined antagonist treatments and none of the AVPA/OTA males displayed this behavior. When compared to aCSF males, the overall proportion showing kyphosis (Fig. 1) was lower in males receiving either 1 ng (Fisher's exact probability test, $P = 0.003$) or 10 ng AVPA/OTA (Fisher's exact probability test, $P = 0.026$).

Latencies to display kyphosis also were examined. Those that did not display kyphosis were assigned a latency of 600 s. These data would not transform to normality and were therefore analyzed nonparametrically. In comparison to aCSF males, 1 ng AVPA/OTA males had a significantly longer latency to kyphosis than aCSF males (Wilcoxon's test; $\chi^2_1 = 8.65$, $P = 0.003$), as did 10 ng AVPA/OTA males (Wilcoxon's test; $\chi^2_1 = 4.85$, $P = 0.028$).

When males that did not display kyphosis were excluded, group sizes were not sufficient for statistical analysis of latency to or duration of kyphosis in most cases. However, the data are presented in Table 1. Sample sizes were sufficient to compare aCSF and control males; control males

Table 1
Means (±SE) of approach and kyphosis by treatment group

	n	Approach latency	Latency to kyphosis (including nondisplayers) from beginning of observation	Latency to kyphosis (not including nondisplayers)	Percentage of time in kyphosis after initial onset (not including nondisplayers)
Control—NC	9	6.33 ± 1.78	416.11 ± 69.29	269.0 ± 71.49	8.26 ± 5.8%**
Control (aCSF)	11	8.78 ± 2.79	396.63 ± 61.01	320.38 ± 65.3	50.91 ± 11.99%
OT, 100 ng	9	37.67 ± 30.24	598.67 ± 1.33**	588.0 ± 0	91.67 ± 0
OTA, 1 ng	8	5.75 ± 2.23	538.12 ± 38.09	435.0 ± 70.59	53.89 ± 23.9%
OTA, 10 ng	8	13.38 ± 6.8	573.0 ± 17.36	528.0 ± 34.53	8.8 ± 5.69%
AVP, 100 ng	7	12.29 ± 6.54	521.71 ± 46.19	417.33 ± 73.53	34.0 ± 39%
AVPA, 1 ng	9	5.45 ± 2.49	466.78 ± 63.38	360.2 ± 89.85	50.31 ± 15.44%
AVPA, 10 ng	7	39.71 ± 27.57	523.29 ± 49.81	331.5 ± 24.5	33.97 ± 0.01%
AVPA/OTA, 1 ng (COMB1)	8	89.12 ± 67.92*	600 ± 0**	0 displayed kyphosis	0 displayed kyphosis
AVPA/OTA, 10 ng (COMB10)	4	35.75 ± 15.53**	600 ± 0**	0 displayed kyphosis	0 displayed kyphosis

Nonattackers only; see Fig. 1 for abbreviations. The final column was statistically analyzed only for NC and aCSF groups.
* $P < 0.1$ (trend only).
** $P < 0.05$ (statistically significant).

Table 2
Means (±SE) of individual behaviors by treatment group

	n	Percentage of time sniffing, licking, and grooming after initial approach	Time from approach to nonkyphotic contact (s)	Percentage of time in nonkyphotic contact after initial approach	Number of retrievals	Proportion attacking
Control (NC)	9	49.77 ± 6.33%	24.22 ± 4.5	24.15 ± 2.98%**	2.44 ± 0.73	10%
Control (aCSF)	11	42.5 ± 8.84%	134.18 ± 69.16	14.07 ± 3.12%	2.64 ± 0.91	0%
OT, 100 ng	9	44.08 ± 5.32%	88.44 ± 35.09	28.22 ± 5.65%**	2.0 ± 1.17	10%
OTA, 1 ng	8	56.29 ± 5.45%	38.12 ± 14.17	28.85 ± 4.43%**	2.25 ± 1.08	20%
OTA, 10 ng	8	30.0 ± 7.77%	218.62 ± 89.19	30.24 ± 7.86%**	1.12 ± 0.61	20%
AVP, 100 ng	7	46.02 ± 10.17%	138.0 ± 79.28	19.63 ± 6.13%	0.29 ± 0.18*	22%
AVPA, 1 ng	9	37.6 ± 8.31%	112.33 ± 68.11	22.46 ± 5.91%	2.78 ± 1.3	10%
AVPA, 10 ng	7	49.6 ± 18.63%	152.14 ± 74.45	14.86 ± 4.44%	1.71 ± 1.28	30%*
AVPA/OTA, 1 ng (COMB1)	8	50.0 ± 7.42%	68.87 ± 18.95	13.3 ± 5.77%	0.87 ± 0.29	11%
AVPA/OTA, 10 ng (COMB10)	4	24.87 ± 13.2%	177.5 ± 120.42	12.31 ± 8.23%	6.5 ± 5.2	60%**

Nonattackers only, except for far right column; see Fig. 1 for abbreviations.
* $P < 0.1$ (trend only).
** $P < 0.05$ (statistically significant).

[$F(1,11) = 7.03$, $P = 0.022$] spent significantly less time in kyphosis after initiation than aCSF males.

There were no significant differences in the percentage of time that males spent sniffing or licking or grooming after an initial approach to the pup (Table 2).

There were no significant differences between groups in the amount of time nonparental males took from the initial approach to the infant to the initiation of nonkyphotic contact (data in Table 1). Control males spent significantly more time in nonkyphotic contact than aCSF males [$F(1,18) = 5.42$, $P = 0.032$], as did OT-treated males [$F(1,18) = 5.28$, $P = 0.034$]. Treatment with 1 ng of OTA [$F(1,17) = 7.81$, $P = 0.012$] or 10 ng OTA [$F(1,17) = 4.54$, $P = 0.048$] was also associated with high levels of nonkyphotic pup contact, though not with statistically significantly reduced amounts of kyphosis; these animals also rarely attacked or avoided pups.

AVP-treated males tended to retrieve less than aCSF males [$F(1,16) = 3.47$, $P = 0.081$; Table 2]. However, no groups were significantly different from aCSF males in retrieval behaviors.

Nonparental behavior

Nonparental behavior, as defined above, includes both avoidance and attacks. Only a small percentage of males were avoidant. When only males that attacked infants were considered (Table 2), once again the only group that differed significantly from aCSF males was the AVPA/OTA 10 ng group (Fisher's exact probability test, $P = 0.004$). There was a nonsignificant trend for AVPA 10 ng males to attack infants more often than aCSF males (Fisher's exact probability test, $P = 0.090$).

Discussion

In the present study, only combined treatments with OTA and AVPA significantly inhibited parental care in male prairie voles. Specifically, treatments with 1 or 10 ng of AVPA plus OTA abolished kyphosis and inhibited other indices of parental behavior. Sixty percent of males receiving the higher dose (10 ng) of combined antagonist AVPA/OTA treatment showed pup attacks, which are uncommon in males of this species (Roberts et al., 1998a,b). This is significant given that previous results for administration of AVPA alone have shown inconsistent results (see Introduction).

Prior research has associated AVP with male parental care and OT with female parental care. In the present study, control animals exhibited high levels of parental behavior. Thus, it was difficult to examine the hypothesis that either OT or AVP could facilitate alloparenting behavior. However, when administered individually, antagonists for either peptide (OTA and AVPA) also did not significantly inhibit the proportion of animals that showed some aspect of alloparenting.

It is also possible that results could have been affected by the 45-min time interval between the administration of drugs and the onset of testing; optimal timing may differ for each drug. The studies that have reported effects of AVP on social behavior have had intervals between injection and testing including 5 min (Wang et al., 1994); 90 min (Parker and Lee, 2001); 15 min (Cho et al., 1999); and 60–120 min (for optimal effect; Pedersen et al., 1982). We attempted to choose an intermediate time period; however, the possibility exists that a different interval between treatment and testing might have affected the outcome of this study.

Because a high percentage of control (NC and aCSF) males in the present study showed alloparental behavior (Fig. 1), a more detailed analysis of pup-directed patterns was undertaken. While the lower combined dose (1 ng AVPA/OTA) of both antagonists did not significantly affect the proportion of animals showing positive infant-directed behavior, the combined AVPA/OTA (1 or 10 ng) treatments were associated with a reluctance to approach infants and to display kyphosis. Even the animals in these groups that showed no attacks had a longer latency to approach infants and reduced kyphosis.

In contrast, the OT-treated group, which also displayed kyphosis less than the aCSF group, displayed no reluctance to approach infants. OT-treated males spent significantly more time engaged in autogrooming than aCSF males, which is a previously described consequence of central OT administration (reviewed in Argiolas and Gessa, 1991). In the OT-treated males, the lower rate of kyphosis was due apparently to a competing behavior, that is, self-grooming, rather than fear of or aversion to the infant. The OTA-treated groups show increased nonkyphotic contact with infants (Table 2). While not showing statistically significant decreases in the proportion of males displaying kyphosis, it is apparent from the data that there is a tendency to less kyphosis in these groups (Fig. 1 and Table 1). Due to the small number of males displaying kyphosis in the OTA 10 ng group, the duration of time spent in kyphosis after the initial onset was not analyzed; however, the three OTA 10 ng animals that displayed this behavior appear to have done so considerably less than the aCSF males (Table 1). There is evidence that kyphosis behavior is mediated via cells in the periaqueductal gray area of the midbrain (Salzberg et al., 2002), and it is possible that blocking OT receptors interferes with this behavior (see below).

Although inherently complex, the role of anxiety or stressful experiences may be essential to understanding the effects of peptide hormones on various social behaviors. Sociality can be protective and beneficial (Carter, 1998), but social interactions, especially those with novel stimuli and especially infants, also may provoke anxiety (Fleming and Leubke, 1981; Fleming et al., 2002). Overcoming neophobia or anxiety is critical to the subsequent expression of parental behavior, especially in reproductively naïve animals. It is common for nonparturient rodents and sheep to show an aversion to and even a fear of neonates (Fleming and Leubke, 1981; Maestripieri and D'Amato, 1991). There also is evidence that both OT (Uvnas-Moberg, 1998) and AVP (Appenrodt et al., 1998; Dharmadhikari et al., 1997) can be anxiolytic, and this capacity to reduce anxiety could be related to the behavioral effects of both of these peptides.

Administration of OT has been shown to facilitate maternal care in rats (Pedersen and Prange, 1979) and reduce infanticide in house mice (McCarthy, 1990). Several methods for blocking the effects of OT also may interfere with the onset of maternal behavior in rats (Fahrbach et al., 1985; Pedersen et al., 1985). In fact, the beneficial effects of OT on maternal behavior may only be apparent when animals have experienced some degree of additional stress, such as exposure to a novel environment (Fahrbach et al., 1985).

In the present study, it is possible that when receptors for either OT or AVP were blocked, the effects of the alternative peptide or other related systems might have been sufficient to permit relatively normal pup-directed behaviors, possibly by overcoming fear of the young. If both the OT and AVP systems were inactivated by receptor antagonists, then animals might have had difficulty in overcoming the fear or anxiety associated with pup stimuli, possibly accounting for the increased avoidance or attack behaviors, especially in animals receiving the 10-ng AVPA/OTA treatment.

The analysis of more subtle behavioral patterns also revealed a difference between the nontreated control animals and the aCSF group. The noncannulated control animals spent a greater proportion of time in nonkyphotic contact and a lower proportion in kyphosis than the aCSF-treated males. This difference could be a consequence of the surgery, perhaps due to lingering hormonal or behavioral effects of the surgery itself or the anesthetic (Bentson et al., 2003). The periaqueductal gray area of the midbrain is known to be involved in the neurobiology of both nociception and pup-directed kyphosis (Salzberg et al., 2002); it is possible that residual effects of anesthetic or stress associated with cannulation affected kyphosis through a mechanism involving this area. Alternatively, cannulation may have produced a lasting increase in hormones of the hypothalamic–pituitary–adrenal axis, such as corticosterone. In male prairie voles, increases in corticosterone are associated with higher levels of pair-bonding behavior (DeVries et al., 1996). In rats, there is evidence that binding to OT receptors (Liberzon and Young, 1997) and AVP release (Ebner et al., 1999) can be enhanced by stressful experiences and/or corticosterone. The findings from the present study suggest the possibility that male alloparenting might be enhanced by prior stressful experiences. It is also possible that this stress masked effects of the OTA and AVPA treatments administered singly.

It is possible that some behavioral effects of peptides, including those following AVP, might be mediated via changes in hypothalamic–pituitary–adrenal (HPA) axis. This notion is supported by research in rats showing that AVP can be released following exposure to a stressor and that AVP acts in concert with corticotrophin-releasing factor CRF to activate the HPA axis (for a recent review, see Carrasco and Van de Kar, 2003). However, in prairie voles, the relationship between AVP and the HPA axis remains to be defined. In prairie voles, ICV administration of AVP in doses similar to those used here produced increases in social behavior (Cho et al., 1999) but did not significantly influence plasma levels of corticosterone (A.C. DeVries and Carter, unpublished data).

A previous study in prairie voles, employing the lower doses of antagonists (1 ng) used here, showed that either OTA or AVPA could inhibit the expression of a selective partner preference; however, in that study, animals receiving only OTA or AVPA continued to be highly social (Cho et al., 1999). It has been suggested that alloparental behavior and nonselective sociality share neural substrates (Kirkpatrick et al., 1994), a hypothesis that may be supported by the present study.

Results from studies of the hormonal basis of male parental care have not always been consistent within species or even within laboratories. Baseline levels of paternal behaviors often vary widely between studies (Lonstein and

De Vries, 1999; Lonstein et al., 2002; Wang et al., 1994; this study). Recent work from our laboratory has revealed that although male prairie voles are typically spontaneously parental, variations in early social experience or hormonal environment can dramatically reduce the incidence of male parental care (Bales et al., 2003, 2004). Research in female rats indicates that the developmental history of an individual also can contribute to the later parental behavior, as well as the sensitivity of an animal to OT (Pedersen and Boccia, 2002). For example, female rat pups that experienced high levels of licking and grooming in early life produce more OT receptors in brain regions known to be associated with social behavior and may later be more parental (Champagne et al., 2001; Francis et al., 2000, 2002; Pedersen and Boccia, 2002). We have also found in prairie voles that extra handling, probably mediated by maternal stimulation, can influence the later endogenous production of OT (Carter et al., 2003). Thus, variation in responsiveness to infants may be part of a more general pattern of reactivity to novel stimuli and also may vary among individuals, in part due to difference in peptide hormones or sensitivity to these hormones.

The present study offers some support for early research implicating AVP in male parental behavior. These data also suggest the hypothesis that OT can influence alloparental behavior in males. However, the most striking behavioral changes were observed following treatments that blocked both AVP and OT receptors. Whether OT and AVP act on the same or divergent neural systems remains to be determined.

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