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Central Vasopressin Administration Regulates the Onset of Facultative Paternal Behavior in *Microtus pennsylvanicus* (Meadow Voles)

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Pharmacological experiments have implicated a role for central arginine vasopressin (AVP) in regulating paternal behavior in monogamous prairie voles. Although nonmonogamous meadow voles exhibit appreciable paternal care when housed under winter, short day lengths (SD), no research has examined whether the same neurobiological systems are involved in regulating paternal behavior in a nonmonogamous species when it behaves paternally. The goal of these experiments was to determine whether central administration of AVP, but not cerebrospinal fluid (CSF), affected the suppression of pup-directed aggression and/or the onset of paternal behavior in meadow voles. Data from experiment 1 implicated a role for AVP in facilitating changes in male behavior: central administration of 1 ng of AVP (but not 3 ng or CSF) inhibited pup-directed aggression in previously pup-aggressive males, and 3 ng of AVP (but not 1 ng or CSF) induced paternal behavior in previously nonpaternal males. In contrast, AVP (1 and 3 ng) did not enhance paternal behavior in already paternal males. Experiment 2 tested the specificity of AVP. Previous research indicated that 24 h of unmated cohabitation with a female reliably induced paternal behavior in SD males. Hence, experiment 2 examined whether administration of a V_{1a} AVP antagonist (AVPA), but not CSF, prior to 24 h of unmated cohabitation would block the onset of paternal behavior. Males that received CSF displayed paternal behavior faster and engaged in more investigatory and paternal behaviors than males that received AVPA. Thus, pharmacological experiments support the hypothesis that AVP likely regulates paternal behavior in both facultatively and consistently paternal vole species.

Key Words: **aggression; ICV; meadow vole; *Microtus;* paternal; photoperiod; vasopressin.**

In autumn, summer territories collapse, and reproductive adult male meadow voles may be found nesting and sleeping with females and preweanling young (Madison, Fitzgerald, and McShea, 1984). Because paternal care is often tied to social living and harsh breeding conditions (Kleiman and Malcolm, 1981), it has been argued that characteristically nonpaternal meadow voles may have evolved the ability to form selective partner preferences and display paternal care to offset fitness costs associated with the less favorable breeding conditions that characterize autumn and winter months (e.g., low-density populations and colder temperatures) (Storey and Snow, 1987; Parker and Lee, in press). Although the primary breeding season for meadow voles typically occurs during summer months, in autumn and winter of some years up to 50% of meadow vole females produce litters (Tamarin, 1977; Christian, 1980), and in unusually warm winters, 100% of females may continue breeding (Webster and Brooks, 1981). Under these socioecological conditions, the capacity to engage in facultative social behaviors (e.g., selective affiliation with a known breeding female, rejection of intruding conspecifics, and exhibition of paternal care) may act to confer a selective advantage by providing postpartum mating opportunities and increased offspring survivorship. Such facultative paternal behavior has been reported for other typically nonpaternal free-living rodents under unfavorable breeding conditions (e.g., *Marmota caligata,* Barash, 1975; *Peromyscus maniculatus,* Mihok, 1979; *Peromyscus leucopus,* Schug, Vessey, and Underwood, 1992; *Phodopus sungorus,* Wynne-Edwards, 1995). Although an adaptive argument can be posited for similar facultative changes in affiliative and parenting behaviors in meadow voles (Trivers, 1972; Emlen and Oring, 1977), whether or not these well-documented spatial

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variations reflect measurable changes in specific reproductive strategies has yet to be examined in free-living meadow vole populations. Nonetheless, because social systems are a composite of individual strategies, presumably of some biological function (Lott, 1984), a critical analysis of the capacity of a species to engage in facultative behavioral strategies should be considered.

The theory that meadow voles maintain the evolved capacity to display facultative behaviors is supported by laboratory research conducted on captive populations originating from habitats in which winter breeding is likely. Captive males and females from these populations display strong, selective partner preferences following 24 h of cohabitation with a mate (Parker, Phillips and Lee, in press), and these preferences are equivalent to those reported for monogamous prairie voles (Williams, Catania, and Carter, 1992; Insel, Preston, and Winslow, 1995). Similar to male prairie voles, meadow vole males also engage in stranger-directed aggression following cohabitation, and although mating is not necessary to induce aggression (as in prairie voles), mating does enhance the frequency of agonistic displays when compared with an unmated cohabitation condition (Parker *et al.*, in press). Meadow vole sires from these populations also share nests with a female mate rather than establishing separate nest sites (Storey, Bradbury, and Joyce, 1994), drive off intruding males (Storey, French, and Payne, 1995; Storey, 1996), demonstrate appreciable care for young (Hartung and Dewsbury, 1979; Dewsbury, 1982; Wilson, 1982; Storey and Snow, 1987; Storey *et al.*, 1994; Storey and Joyce, 1995), and even mate with an unfamiliar female without diminishing care for pups (Storey and Snow, 1987). Sexually and parentally inexperienced (hereafter naive) adult meadow voles also exhibit appreciable paternal care (Storey and Joyce, 1995; Parker and Lee, submitted for publication), and housing under winter, short day lengths (SD) (when males would be most likely to live with preweanling young in the field) enhances this effect (Parker and Lee, submitted for publication). Winter photoperiods also enable males to respond to social cues that have been previously associated with suppression of pup-directed aggression and paternal behavior onset in other rodent species (Huck, Soltis, and Coopersmith, 1982; Elwood, 1985). For example, 24 h of male–female unmated cohabitation induces paternal behavior in previously nonpaternal males, and 24 h of male–female mated cohabitation completely suppresses pup-directed aggression (Parker and Lee, in press). Finally, the presence of the male, in the absence of intruder males, results in more rapid weight gain by pups than when pups are reared by the dam alone (Storey and Snow, 1987). Collectively, these data suggest that nonmonogamous meadow voles from some geographic areas have evolved the ability to engage in facultative partner preferences and paternal care in the presence of specific social and environmental cues that contain reliable information about ecological conditions that favor biparental care.

In recent years, the examination of prairie vole affiliative and paternal behaviors has been extended to examine the neurobiology that regulates them (Winslow, Hastings, Carter, Harbaugh, and Insel, 1993; Wang, Ferris, and De Vries, 1994a). In male prairie voles, 24 h of copulation and cohabitation with a female mate is sufficient to induce selective partner preferences and rejection of intruding conspecifics (Insel, Preston, and Winslow, 1995). These behavioral changes are also associated with enhanced paternal responsiveness to neonates (Bamshad, Novak, and De Vries, 1994). Pharmacological experiments have implicated arginine-vasopressin (AVP), a 9-amino-acid peptide of hypothalamic origin with diverse neural forebrain projections, in the regulation of these behaviors in males. Central intracerebroventricular (ICV) administration of AVP induces selective partner preferences in the absence of mated cohabitation, and conversely, ICV administration of a selective, V_{1a} receptor antagonist (AVPA) blocks partner preference formation when delivered prior to mated cohabitation (Winslow *et al.*, 1993). Similar studies have also implicated a role for AVP in the regulation of paternal behavior. Administration of AVP by ICV or directly into the lateral septum enhances paternal behavior, and AVPA injections into the lateral septum inhibit paternal behavior expression (Wang *et al.*, 1994a).

Although others have examined the evolutionary origins and proximate mediation of facultative social behaviors associated with alternative social systems (Emlen and Oring, 1977; Lott, 1984), no research has yet examined the underlying neurobiology that mediates the facultative expression of paternal behaviors in a characteristically nonpaternal vole species. Consequently, it is not known whether the same or different neurobiological systems are involved in regulating paternal behavior in a consistently paternal, monogamous species (i.e., the prairie vole) and a nonmonogamous species (i.e., the meadow vole) when it behaves paternally. The following experiments were designed to investigate this question.

MATERIALS AND METHODS

Subjects, derived from wild-caught voles indigenous to northwestern Pennsylvania and southwestern New York, were born to breeding pairs in an established colony at the University of Michigan. Weanling meadow vole pups were removed from the dam and sire at 19 days of age and housed alone in winter, SD (10 h light/day) conditions. At weaning, all test subjects received a unique identification number (ID number), which subsequently permitted the experimenter to conduct behavior testing, injections, and behavior scoring blind to the subjects' baseline behavior scores. Subjects were housed in 26.67 × 21.59 × 13.97 cm polypropylene cages on pine shaving bedding with food (Purina mouse chow 5015) and water available *ad libitum*. Animal rooms were maintained at 21 ± 2°C with low ambient noise conditions. Subjects remained so housed until the beginning of the experimental procedure (11–13 weeks of age). All research was conducted according to the legal requirements of the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* (DHEW Publication 80-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD) and the University of Michigan's institutional guidelines.

Injection Site Validity and Reliability

Seven adult male voles from our colony were lightly anesthetized with Halothane and a clear plastic sleeve (with markings that delineated the midsagittal plane and ultimate injection site) was secured snugly over each subject's shaved head. (Removal of cranial fur served to increase successful injection rates.) Using a 10-μl Hamilton syringe attached to a 30-gauge needle and plastic stopper that reliably placed injections 1 mm below the lightly calcified skull (modified from Popick, 1976; see also Winslow *et al.*, 1993), the experimenter administered a 2-μl injection of cerebrospinal fluid (CSF) (Biofluids, Inc.) and 10% India ink solution percutaneously. Injections lasted approximately 1 min. Different injection sites were evaluated. After all injections, voles were euthanized and brains removed for gross coronal dissection. Brain sections were examined under a dissecting microscope to verify that the India ink, and therefore, the injections were into the lateral ventricle. After a suitable injection method was determined, injection reliability prior to beginning experiment 1 was 90% in 10 practice males. (Injection success rates were 90 and 94% for experiments 1 and 2, respectively.) It should be noted that neither abnormal behavior nor pain/suffering by subjects was observed following ICV injections.

Characterizing a Dose–Effect Relationship between AVP and Male Behavior

To establish whether central administration of AVP would effectively induce behavioral changes in male meadow voles, we conducted dosage trials using [Arg^8]-vasopressin (Peninsula Laboratories, Inc.). AVP doses and the experimental methods were similar to those used previously in prairie voles (Wang *et al.*, 1994a). Males (total $N = 28$) were naive adults from our laboratory colony that exhibited no interactions with pups (i.e., they exhibited no pup-aggressive or paternal behavior) during baseline paternal behavior testing (see below for detailed paternal behavior testing conditions). Each male received only one of the following doses of AVP by ICV in 2 μl of CSF: 0 ng ($N = 6$), 0.1 ng ($N = 5$), 1 ng ($N = 6$), 3 ng ($N = 6$), or 5 ng ($N = 5$). Males were allowed to recover for 15 min, and then every male was tested for post-drug administration paternal behavior at 15, 30, and 90 min following injections. Behaviors were scored categorically on an ordinal scale as unresponsive, aggressive, investigatory, or paternal. Directly following testing, subjects were euthanized and their brains were removed for gross coronal dissection. Brain sections were examined under a dissecting microscope to verify the injection site. Bilateral staining of ventricles I and II was required for inclusion in the study. Data were analyzed using cumulative logistic regression with proportional odds ratio.

Descriptive statistics showed that AVP doses below 1 ng (i.e., CSF, 0.1 ng AVP) were behaviorally ineffective, as all males in these two groups continued to behave unresponsively to pups during the three post-drug administration test periods. Animals in these two groups manifested no abnormal behavior following injections and as neither group responded to treatment, these groups were combined and served as the control group for subsequent inferential statistical analyses. Males that received 5 ng of AVP exhibited abnormal locomotion and excessive grooming during the three test times and remained far from pups during all behavioral tests. Logistical regression showed that injections of 1 and 3 ng per 2 μl of CSF most successfully altered male behavior ($\beta = -2.247$; $df = 1$; $\chi^2 = 10.493$; $P = 0.001$) and behavioral responses to the drug were observed only at test time 3 (i.e., 90 min after

drug treatment). Odds ratio analysis showed that males receiving 1 ng of AVP were 89% (odds ratio = 0.11) more likely to exhibit changes in social behavior than the control group, whereas males that received 3 ng of AVP were 99% (odds ratio = 0.01) more likely than the control group to exhibit changes in social behavior. Thus, we chose to examine whether central injections of 0, 1, or 3 ng of AVP altered male behavior 90 min after drug administration.

Paternal Behavior before and after Drug Administration

All test males were screened for baseline paternal behavior prior to receiving ICV injections. Each male was placed in a novel polypropylene 48.26 × 26.67 × 20.32 cm cage with fresh bedding. Males were allowed to become familiar with the new environment for 5 min, and then a 2- to 5-day-old pup was introduced to the opposite end of the cage from the male. Each test was carried out during the lighted phase of the light cycle and was videotaped for 10 min with a Panasonic camera and wide-angle lens on a time-lapse VCR. Based on the experimenter's rating, each male's behavior was scored categorically as aggressive behavior (rough handling/charging pup, resulting in pup vocalization and/or injury), unresponsive behavior (brief investigatory sniffing or no contacting/interacting with pup), or paternal behavior (grooming, huddling, and/or retrieving). As found previously (Parker and Lee, in press), male behavioral interactions with pups were distributed as follows: 15% were pup-aggressive, 35% were pup-unresponsive, and 50% were paternal. Sample sizes were matched for statistical/design reasons, and subjects were randomly assigned to a treatment condition [(i.e., experiment 1: 0, 1, or 3 ng of AVP; experiment 2: 0 or 5 ng [1-(β-mercapto-β,β-cyclo-pentamethylene propionic acid), 2-(*O*-methyl)tyrosine]-Arg^8-Vasopressin) (Peninsula Laboratories, Inc.)]. The experimenter was always present during the test. If any male was overtly aggressive to a pup (causing pup vocalization or injury), the pup was immediately removed from the cage and the test terminated. Attacked pups were rarely injured, and consequently, they were returned to the home cage and reared normally by the parents (personal observation). In the exceptional case of serious pup injury, the experimenter immediately euthanized the pup (<5% of pups were seriously injured during all phases of the study.)

Paternal Behavior Scoring

Detailed scoring of the videotaped behavior tests occurred 1 month after completion of the experiments. Because the videotapes contained only each subject's ID number and behavior test, the scorer was blind to drug treatment group (e.g., experiment 1: 0, 1, or 3 ng AVP; experiment 2: 0 or 5 ng AVPA), test time (i.e., whether the behavior test was before or after drug administration), and each subject's baseline behavior (e.g., pup-aggressive, pup-unresponsive, paternal) prior to random assignment to treatment group. ID numbers were decoded later, during statistical analysis. Based on videotaped tests, male behavior was scored categorically as described above: aggressive, unresponsive, or paternal. The number and duration of specific types of interactions were also scored (sniffing and contacting the pup, grooming the pup, huddling over the pup, time spent alone near the pup, time spent alone far from the pup, number of approaches, and number of retrievals). Finally, the latency to behave aggressively and the latency to behave paternally were calculated.

Experiment 1

The purpose of experiment 1 was to determine whether ICV administration of AVP, but not CSF, affected the suppression of pup-directed aggression and/or the onset of paternal behavior in naive adult male meadow voles. We also chose to examine whether differences in baseline behavior influenced the ability to respond to different drug treatment conditions, as prior research has shown that different mechanisms can be involved in pup-directed aggression suppression and the onset of paternal behavior, particularly when male baseline behavioral states vary (California mice, Gubernick, Schneider, and Jeannotte, 1994; meadow voles, Parker and Lee, in press).

The day after baseline paternal behavior testing, subjects (N = 60 for data analysis; pup-aggressive males: N = 19; pup-unresponsive males: N = 19; paternal males: N = 22) were lightly anesthetized with halothane and their heads shaved (see Table 1). Two days after baseline paternal behavior testing, each male was anesthetized with halothane and received an ICV injection of one of the following: 0, 1, or 3 ng AVP in a 2 μl CSF and 10% India ink solution. Once completed, each subject was placed in a neutral cage and closely monitored for 10 min until he fully recovered from the treatment. Males were assessed for paternal behavior 90 min after receiving ICV injections.

TABLE 1
Experimental Conditions for AVP Injections

Injection	Baseline behavior		
	Aggressive	None	Paternal
0 ng AVP/2 μl CSF + 10% India ink	$N = 6$	$N = 7$	$N = 6$
1 ng AVP/2 μl CSF + 10% India ink	$N = 6$	$N = 6$	$N = 6$
3 ng AVP/2 μl CSF + 10% India ink	$N = 7$	$N = 6$	$N = 10$

Note. Captive naive adult meadow vole males were tested for baseline paternal behavior and randomly assigned to a treatment group. Males were injected with central arginine-vasopressin (AVP) or control and tested for postinjection paternal behavior 1.5 h after drug administration. Following testing, voles were euthanized, brains were removed for gross coronal dissection, and injection sites were verified.

Experiment 2

Experiment 2 was designed to test the pharmacological specificity of AVP. Previous studies in our laboratory have found that 24 h of unmated cohabitation with a female reliably induces paternal behavior in short photoperiod housed males previously unresponsive to pups (Parker and Lee, in press). Thus, experiment 2 examined whether ICV injections of a long-acting, selective vasopressin V_{1a} antagonist, but not CSF, administered prior to 24 h of unmated cohabitation with a female would block the development of paternal behavior in naive, nonpaternal meadow voles.

Thirty-two naive adult meadow voles ($N = 16$ males and $N = 16$ females) served as subjects in experiment 2. Test males were approximately 11 weeks of age and females were roughly age matched and ranged from 11 to 20 weeks of age. Because SD females rarely mate and nearly 100% of long day (LD; 14 h light/day) females mate within 48 h of pairing (Meek and Lee, 1993), we paired SD males with LD females to ensure that failure to mate could be more accurately attributed to male, rather than female, choice. At 11 weeks of age, naive male meadow voles were assessed for baseline paternal behavior. Only pup-unresponsive males (i.e., no pup-aggressive or paternal males) were used. Following testing, subjects were randomly assigned to a treatment group (e.g., 0 or 5 ng of AVPA).

The same injection procedure and experimental criteria were used as in experiment 1. Winslow *et al.* (1993) reported that 5-ng ICV injections blocked AVP V_{1a} receptors for at least 18 h. Following preliminary testing to ensure that no adverse effects occurred with 5-ng AVPA injections, experimental males were injected with either 5 ng AVPA in 2 μl CSF + 10% India ink solution or 2 μl of CSF + 10% India ink solution. As before, males were closely monitored for 10 min following injections until they fully recovered.

Approximately 2 h after ICV drug administration, each male was paired with an unfamiliar female in a 10-gallon aquarium. The floor of each aquarium was lightly covered with pine shavings and food and water were available *ad libitum.* Pairs were videotaped overnight (using 25-W red lighting) to determine whether mating did or did not occur. (Only 5% of males mated.) The males that did not mate were tested for paternal behavior after 24 h of unmated cohabitation with a female.

Statistical Analyses

For experiment 1, the effect of drug treatment on post-drug interactions with pups was analyzed separately for each baseline behavior group. Using repeated-measures ANOVA, with drug dosage as the primary factor, pup-directed aggression suppression was assessed in males that were aggressive during baseline testing. Also using repeated-measures ANOVA with drug dosage as the primary factor, males in each of the three baseline groups were assessed for duration of two aggregate measures of paternal behavior (e.g., investigatory and paternal), retrieval counts, and time spent far from the pup. Principal components analysis using varimax rotation was used to simplify data analysis of highly related dependent variables (Morrison, 1983). Two principal component loadings emerged from our analysis: investigatory behaviors (e.g., time spent sniffing and approaching) and paternal behaviors (e.g., time spent grooming, huddling, and contacting). For survival analysis (see below), when post hoc pairwise comparisons indicated no significant differences between conditions, we collapsed across drug treatment groups to simplify subsequent data analyses. Finally, the latency to engage in any pup-directed aggressive behavior and latency to behave in any paternal behavior were compared between different drug treatments (within baseline behavior groups) using survival analysis, a statistical test that accounts for the probability of a behavioral event occurring during a fixed elapsed time period (Systat 7.0, Inc.).

For experiment 2, duration measures for postinjection investigatory behaviors, paternal behaviors, soli-

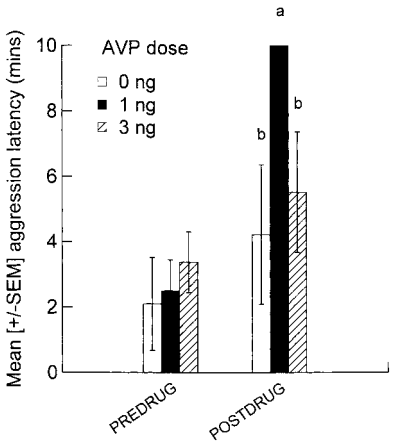

FIG. 1. Preinjection and postinjection mean (±SEM) pup-directed aggression latencies during 10-min paternal behavior tests (N = 19). "a" indicates a significant difference ($P < 0.05$) between groups with "b" where those with "b" do not differ.

tary behavior, and number of pup retrievals were compared between the two drug treatment groups using two-sample t tests (with Bonferroni corrections to protect against multiple comparisons). Finally, the latency to engage in any pup-aggressive behavior and latency to behave in any paternal behavior were compared between drug treatment using survival analysis.

RESULTS

Experiment 1

Baseline pup-aggressive males. ICV administration of AVP significantly suppressed pup-directed aggression in adult SD meadow voles ($F_2 = 4.158$; $P = 0.035$). Specifically, males that received 1 ng AVP/2 μl CSF completely suppressed pup-directed aggression in post-drug administration behavior tests and differed significantly from males that received either 2 μl CSF ($P = 0.018$) or 3 ng AVP/2 μl CSF ($P = 0.032$) (see Fig. 1). Males in the other two groups did not differ from one another ($P = 0.700$). A similar dose effect was found for longer pup-directed aggression latency (log survival test statistic$_1$ = −2.126; P = 0.034); males that received 1 ng of AVP, but not 3 ng AVP or CSF, failed to behave aggressively toward pups during the 10-min test, whereas the other two groups still engaged in pup-aggressive behavior during testing. No significant differences between drug conditions were found for the aggregate measures of investigatory or paternal behaviors, paternal latency, retrieval counts, or time spent far from the pup, as males that suppressed pup-directed aggression during the second behavior test exhibited unresponsive, rather than paternal, behavior.

Baseline pup-unresponsive males. ICV administration of AVP significantly increased paternal behavior (indexed by an aggregate measure of grooming, huddling, and time spent in contact with a pup) in previously pup-unresponsive males ($F_2 = 5.944$; $P = 0.014$) (see Fig. 2). Specifically, males that received ICV administration of 3 ng AVP/2 μl CSF increased paternal behavior after drug treatment and differed significantly from males that received either 0 ng AVP/2 μl CSF ($P = 0.010$) or 1 ng AVP/2 μl CSF ($P = 0.008$). Males in the other two groups did not differ from one another ($P = 0.756$). Injection of 3 ng AVP/2 μl CSF also decreased paternal behavior latency when compared with the other two treatment conditions (log survival test statistic$_1$ = 2.764; $P = 0.006$). AVP also decreased the amount of time males spent alone far from the pup ($F_2 = 6.174$; $P = 0.012$); males that received 3 ng AVP/2 μl CSF spent significantly more time near the pup than males that received either 2 μl CSF ($P = 0.009$) or 1 ng AVP/2 μl CSF ($P = 0.007$), and these males did not differ from each other ($P = 0.700$). AVP-treated males did increase investigatory behavior, but this effect was not significant ($F_2 = 2.046$; $P = 0.166$). No significant differences were found for pup-directed aggression latency (no males in this group became pup-aggressive after drug treatment) or retrieval counts.

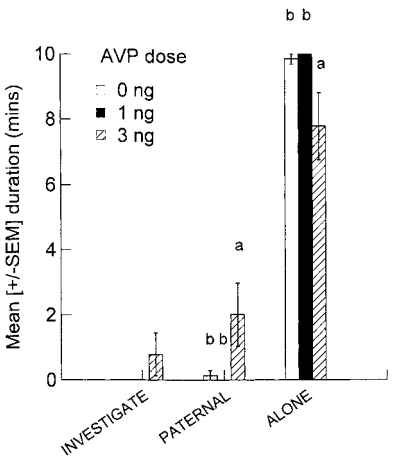

FIG. 2. Postinjection mean (±SEM) investigatory behaviors (e.g., sniffing and approaching the pup), paternal behaviors (e.g., grooming, huddling, and contacting the pup), and time spent alone far from the pup during 10-min paternal behavior tests (N = 19). For an explanation of "a" and "b" see Fig. 1.

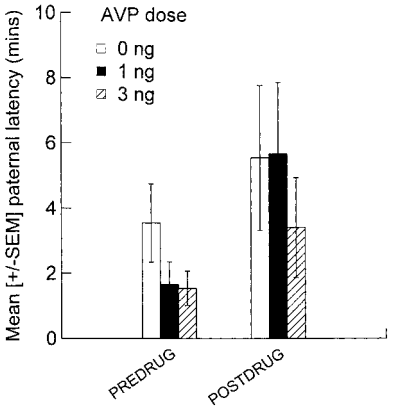

FIG. 3. Preinjection and postinjection mean (±SEM) paternal latencies during 10-min paternal behavior tests ($N = 22$). No significant differences were found.

Baseline paternal males. AVP did not alter paternal behavior in already paternal males. Thus, no significant differences were found for pup-directed aggression latency, paternal latency, retrieval counts, time spent far from the pup, or the aggregate investigatory or paternal behavior measures (see Fig. 3).

Experiment 2

Following 24 h of unmated cohabitation with a female, previously pup-unresponsive males that received 0-ng AVPA injections engaged in more investigatory behaviors (e.g., sniffing and approaching; $t_{14} = 2.817$; $P = 0.014$), paternal behaviors (e.g., grooming, huddling, and contacting; $t_{14} = 4.915$; $P < 0.0001$), and less time alone ($t_{14} = -4.915$; $P < 0.0001$) than males that were treated with 5 ng of AVPA (see Fig. 4). Similarly, following 24 h of unmated cohabitation with a female, previously pup-unresponsive males that received 0-ng AVPA injections engaged in paternal behavior faster than males that received 5 ng of AVPA (survival analysis test statistic$_1 = -2.366$; P = 0.018), but males did not differ on pup-directed aggression latency or number of retrievals. Thus, ICV administration of AVPA completely blocked paternal behavior onset in males paired with a female for 24 h of unmated cohabitation.

DISCUSSION

Data from experiment 1 indicate that administration of 1 ng of AVP (but not 0 or 3 ng) inhibited pup-directed aggression in previously aggressive males (Fig. 1). AVP also affected paternal attendance; ICV administration of 3 ng of AVP (but not 0 or 1 ng) induced paternal behaviors in previously pup-unresponsive males (Fig. 2). However, injections of AVP did not enhance paternal behavior in already paternal males (Fig. 3). Data from experiment 2 further support a role for AVP in the regulation of paternal behavior, as ICV administration of 5 ng of AVPA (but not 0 ng) completely inhibited the onset of paternal behavior (Fig. 4). This finding is particularly compelling, as SD male meadow voles reliably develop paternal behavior following 24 h of unmated cohabitation with a female (Parker and Lee, in press).

Neural and Cellular Regulation of Rodent Paternal Behavior

Although these experiments suggest a role for central AVP in both suppressing pup-directed aggression and promoting the onset of paternal behavior, administration of AVP into the ventricular system does not afford a clear neuroanatomical understanding of where (or how) AVP exerts its target effects. However, a series of lesion, early gene (*c-fos*) expression [indexed by immunoreactivity (Fos-ir)], AVP-ir fiber density, and AVP mRNA peptide expression studies indicates a possible role for specific neural pathways in the regulation of these behaviors in other rodent species. (Experimental subjects were prairie voles unless otherwise stated.) Bilateral bulbectomy reduces paternal behavior (Kirkpatrick, Williams, Slotnick, and Carter,

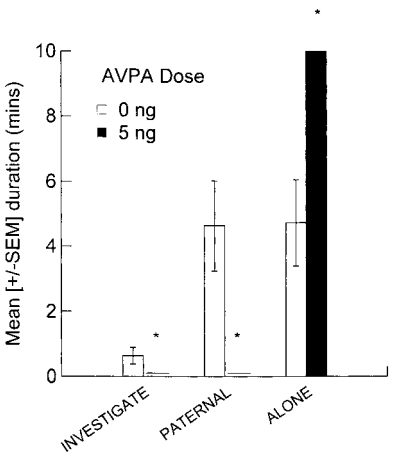

FIG. 4. Postinjection mean (±SEM) investigatory behaviors (e.g., sniffing and approaching the pup), paternal behaviors (e.g., grooming, huddling, and contacting the pup), and time spent alone far from the pup during 10-min paternal behavior tests ($N = 16$). *Indicates a significant difference ($P < 0.05$) between groups.

1994), as do lesions of the corticomedial and medial nuclei of the amygdala (MeAmyg) (Kirkpatrick, Carter, Newman, and Insel, 1994a), and the medial preoptic area (MPOA) of the hypothalamus (rats, Rosenblatt, Hazelwood, and Poole, 1996). The importance of these neuroanatomical areas is also supported by an increase in Fos-ir in both the MeAmyg (Kirkpatrick, Kim, and Insel, 1994b) and the MPOA (Wang, Hulihan, and Insel, 1997a) following male interactions with pups. After mating and cohabiting with a female, males show increased AVP mRNA expression in bed nucleus of the stria terminalis (BNST) cell bodies and a decrease in AVP-ir fibers in the lateral septum (Bamshad *et al.*, 1994; Wang, Smith, Major, and De Vries, 1994b). Because AVP cells in the BNST project to the lateral septum, the increase in AVP synthesis in cell bodies and the decrease in AVP staining in the terminals suggest that mating induced septal AVP release (Wang, Young, De Vries, and Insel, 1998). Moreover, reduced AVP-ir staining in the terminals of both the lateral septum and the lateral habenular nucleus occurs following mating and the birth of pups, each of which is associated with increased paternal responsiveness (Bamshad *et al.*, 1994). These brain areas are part of the extended amygdala neural pathway [accessory olfactory nucleus (AON)–amygdala–BNST–septum–MPOA] (hamsters; for a review see Newman, 1999), which expresses Fos-ir after social / sexual / paternal interactions (Kirkpatrick *et al.*, 1994b; Wang *et al.*, 1997a). Many of these areas also contain AVP receptors (AON, amygdala, BNST, septum) (prairie, pine, montane, and meadow voles; Insel, Wang, and Ferris, 1994; Wang, Young, Liu, and Insel, 1997b; Wang, Liu, Young, and Insel, 2000). Thus, these findings suggest that, in some species, paternal behavior is generated from the extended amygdala neural circuit that releases and / or binds AVP. (For exceptions, see Bamshad, Novak, and De Vries, 1993; Bester-Meredith, Young, and Marler, 1999; Lonstein and De Vries, 1999.)

Although these studies clearly delineate specific neural pathways involved in the regulation of paternal behavior, exactly how AVP acts to regulate paternal behavior may vary by whether a species displays paternal behavior consistently (i.e., the species is characteristically monogamous) or facultatively (i.e., the species is characteristically nonmonogamous). For instance, monogamous prairie voles show alterations in AVP pathways following cohabitation with a female (Wang *et al.*, 1994b) and following delivery of the litter (Bamshad *et al.*, 1993), whereas nonmonogamous long day length housed meadow voles do not. In prairie voles, central AVP receptor-binding studies have found no detectable receptor differences (Wang *et al.*, 2000), indicating that paternal behavior in prairie voles does not require postsynaptic changes in AVP receptors. This combined evidence suggests that prairie voles, a characteristically pair-bonding and paternal species, may be postsynaptically primed to consistently generate rapid social preferences, stranger-directed aggression, and enhanced paternal behavior when given the proper environmental or social stimulus to induce presynaptic AVP release. Whether or not nonmonogamous, SD housed meadow vole paternal behavior is regulated in a similar or different manner has yet to be determined. However, because meadow voles do not consistently display paternal care, it is likely that the facultative initiation of pup-directed aggression suppression and paternal behavior onset is dependent on a variety of social and environmental stimuli to alter activity in the relevant neural pathways.

Regulation of Meadow Vole Paternal Behavior

Data from our pharmacological experiments suggest that AVP is required to suppress pup-directed aggression in previously aggressive males and promote paternal behavior in previously pup-unresponsive males. However, exactly why individual variation in baseline behavior (e.g., aggressive, unresponsive, or paternal) is an important factor in determining whether or not meadow vole males suppress pup-directed aggression or display paternal behavior in response to central administration of different AVP doses remains unclear. Nevertheless, a series of behavioral experiments in our laboratory suggests possible areas for future investigation. In these experiments, we found that copulation and cohabitation suppressed pup-directed aggression in previously aggressive males, but these males (that cohabited with their mates throughout pregnancy and parturition) exhibited paternal behavior only following 24 of postpartum exposure to pups (Parker and Lee, in press). Similarly, following copulation and cohabitation, baseline pup-unresponsive males and already paternal males did not display paternal behavior or increase paternal behavior (respectively) until after parturition and pup exposure. These behavioral data suggest that copulation and cohabitation with a female are sufficient to suppress pup-directed aggression in previously aggressive males, but these social stimuli are ineffective regulators of paternal behavior onset (in baseline pup-aggressive and pup-unrespon-

sive males) and paternal behavior enhancement (in baseline paternal males).

Findings from these behavioral and pharmacological experiments show a similar differentiation in male responsiveness to AVP prior to cohabitation, which suggests that the receptor systems of the different baseline behavioral groups of naive males are not identical. One possible explanation for these findings is that baseline pup-aggressive males (compared with baseline pup-unresponsive males) may be unable to demonstrate paternal behavior following central administration of 3 ng of AVP because they lack sufficient receptors in the necessary brain areas. If this hypothesis is correct, social interactions may alter AVP receptor patterns through either hormonal or neural changes, similar to those described in female mammals (which exhibit increased oxytocin receptors in the extended amygdala late in pregnancy and which covary with maternal behavior onset) (Insel, 1986; Insel and Shapiro, 1992). These possible receptor variations might also explain why pup-aggressive males given 3 ng of AVP are more aggressive than those treated with 1 ng of AVP. Because AVP also stimulates adult-directed conspecific aggression in males of other species (hamsters, Ferris, 1992; prairie voles, Winslow *et al.*, 1993), the higher, but not lower, dose of AVP may act to stimulate additional receptors in other neuroanatomical areas, producing generalized aggressive behavior. Taken together, these data suggest that central AVP may act in various target sites to generate context and species-specific patterns of paternal and aggressive behaviors in response to different social stimuli.

In conclusion, data from these experiments support the hypothesis that the same neurobiological system regulates the expression of behavior in both consistently paternal (prairie) and facultatively paternal (meadow) vole species. In light of behavioral and pharmacological findings that (1) meadow vole paternal behavior is most fully expressed following mated cohabitation and postpartum pup exposure, (2) incremental increases of central AVP do not induce paternal behavior in pup-aggressive males, and (3) unlike prairie voles, central AVP administration does not increase paternal behavior in already paternal meadow vole males, it is highly likely that postsynaptic changes in neural sensitivity are also required to regulate paternal behavior expression in nonmonogamous meadow voles when they behave paternally. Experiments are in progress to examine whether paternal state covaries with central AVP receptor binding and distribution.

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