

Species Differences in Paternal Behavior and Aggression in *Peromyscus* and Their Associations with Vasopressin Immunoreactivity and Receptors

Janet K. Bester-Meredith,^{*,1} Larry J. Young,[†] and Catherine A. Marler^{*,1}

^{*}Department of Psychology, University of Wisconsin-Madison, Madison, Wisconsin 53706; and [†]Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, Georgia 30322

Received July 14, 1998; revised November 18, 1998; accepted January 2, 1999

Previous comparative studies have suggested that the distribution of arginine vasopressin (AVP) pathways within the brain is associated with species-typical patterns of social behavior. In the current study, male parental behavior and aggression were compared in two species of *Peromyscus*. As predicted based on other studies, male mice from a monogamous species, the California mouse *Peromyscus californicus*, spent more time providing parental care to offspring than males from a polygamous species, the white-footed mouse *Peromyscus leucopus*. Sexually naive male California mice also attacked opponents more rapidly than white-footed mice during resident-intruder and neutral aggression tests. Since AVP has been shown to modulate these behaviors, we compared the distribution of vasopressinergic neurons and receptors. We predicted that greater AVP-immunoreactive (AVP-ir) staining in the bed nucleus of the stria terminalis and AVP receptor density in the lateral septum would occur in the species with low levels of paternal care because this pattern was found in similar comparisons with sexually naive monogamous and polygamous voles. In contrast, in our study, monogamous male mice showed more AVP-ir staining in the bed nucleus of the stria terminalis than the polygamous species, as well as more AVP receptors in the lateral septum. Parental behavior therefore does not appear to predict differences in patterns of AVP-ir staining and receptor distribution across species or vice versa. We propose the hypothesis that aggression may be better

correlated with species patterns of AVP-ir staining density and receptor distribution. © 1999 Academic Press

Key Words: vasopressin; aggression; parental care; *Peromyscus*.

Members of the genus *Peromyscus* provide a valuable model for neurobiological studies of male parental care and aggression. Because different *Peromyscus* species show large differences in male parental care and male-male aggression, comparative studies can be used to examine associations between neuroanatomical markers and social behavior. For example, several recent studies have associated the neuropeptide arginine vasopressin (AVP)² with species-typical patterns of social behavior (i.e., Bamshad, Novak, and De Vries, 1993; Ferris, Delville, Grzonka, Lubernarod, and Insel, 1993). Within the pathway originating in the bed nucleus of the stria terminalis (BNST) and medial amygdala (MA) and projecting to the lateral septum (LS), AVP has been associated primarily with male parental or aggressive behavior, depending on the species which is examined.

Evidence for the involvement of this pathway in parental behavior has been provided by a series of

¹ To whom correspondence should be addressed. Fax: (608) 262-4029. E-mail: jkbest@students.wisc.edu or camarler@mac.wisc.edu.

² Abbreviations used: AVP, arginine vasopressin; AVP-ir, AVP-immunoreactive; BNST, bed nucleus of the stria terminalis; LH, lateral habenular nucleus; LS, lateral septum; MA, medial amygdala; PVT, paraventricular nucleus of the thalamus; PVN, paraventricular nucleus of the hypothalamus; SON, supraoptic nucleus.

comparisons of AVP-immunoreactive (AVP-ir) staining and AVP receptors in polygamous meadow voles (*Microtus pennsylvanicus*) and monogamous prairie voles (*Microtus ochrogaster*). Comparisons of sexually naive males have demonstrated that higher AVP-ir staining is observed in the BNST and MA of the species that shows less parental care, the meadow vole (Wang, 1995). In addition to this difference in presynaptic AVP, sexually naive meadow voles and montane voles possess more AVP receptors in the LS, the presumed target of the BNST and MA (Insel, Wang, and Ferris, 1994; Young, Winslow, Nilsen, and Insel, 1997). These findings suggest that higher levels of AVP in the BNST and MA and more AVP receptors in the LS of sexually naive males are associated with less potential for parental investment in voles by sexually experienced males. However, this hypothesis has yet to be tested in other rodent species.

Additional support for the correlation between AVP release and paternal behavior has been provided through studies of sexually experienced males. Although the absolute amount of AVP in the LS does not differ between sexually experienced males of monogamous and nonmonogamous vole species (Wang, Zhou, Hulihan, and Insel, 1996), monogamous males can show changes in AVP after becoming parental. In the prairie vole, an increase in paternal behavior after mating is accompanied by a decline in the amount of AVP-ir staining in the LS and lateral habenular nucleus (LH), two targets of the BNST and MA (Bamshad *et al.*, 1993; Bamshad, Novak, and DeVries, 1994). One interpretation of this observation is that the sudden decrease in AVP-ir staining in the BNST, MA, LS, and LH results from higher rates of AVP release and that this increase in neurotransmitter release may lead to more frequent paternal behavior. The existence of a positive relationship between AVP levels and paternal behavior has been supported by the results of AVP injections into the LS. While male prairie voles tend to spend more time in physical contact with pups after receiving injections of AVP into the LS, injections of an AVP antagonist into the LS produce the opposite effect: a decrease in paternal responsiveness (Wang, Ferris, and De Vries, 1994, but see Lonstein and De Vries, 1998). Together, these observations suggest that paternal behavior is associated with higher levels of AVP release from the BNST and MA, resulting in lower levels of AVP-ir staining in paternal animals.

Experimental evidence also suggests a relationship between central AVP and aggression. For example, after mating, male prairie voles not only become more

parental, but also become more likely to attack intruders to guard their mates (Winslow, Hastings, Carter, Harbaugh, and Insel, 1993). Intracerebroventricular injections of an AVP antagonist block this mating-induced increase in mate-guarding aggression. The association between AVP and aggression has been extended to other species as well. For example, AVP-ir staining is lowest in the LS and BNST of house mice that attack their opponents most rapidly during a resident-intruder test (Compaan, Koolhaas, Bujis, Pool, de Ruiter, and van Oortmerssen, 1992; Compaan, Bujis, Pool, de Ruiter, and Koolhaas, 1993). A similar effect has been observed in wild-type rats, with less aggressive rats differing from more aggressive rats in the amount of AVP-ir staining in the LS (Everts, de Ruiter, and Koolhaas, 1997).

Although these correlational studies do not test the direct relationship between AVP and aggression, experimental manipulations of AVP tend to alter resident-intruder aggression. Altering AVP levels with injections of AVP produces an increase in dominance in golden hamsters. In golden hamsters, the frequency of flank marking, a territorial behavior, increases after males receive AVP injections, while blocking AVP receptors reduces flank marking (Ferris and Potegal, 1988; Ferris, Meenan, Axelson, and Albers, 1986; Ferris *et al.*, 1993; Delville, Mansour, and Ferris, 1996). Together, these results suggest that AVP may play a key role in determining a male's aggressiveness and dominance.

No studies to date have attempted to determine whether species differences in parental behavior or in aggression are more closely associated with AVP pathways. It is possible that neither set of behaviors is correlated with AVP brain patterns across species. By examining associations between AVP and social behavior, it will be possible to determine whether AVP may serve a similar function in species that show large differences in their types of social behavior. This study was designed to examine the relationship between AVP-ir staining and receptors in the pathway originating in the MA and BNST and aggressive and parental behavior in two species with profiles of parental behavior similar to those of the prairie and meadow vole. First, we quantified differences in AVP-ir staining and AVP receptors in sexually naive and socially inexperienced males from two closely related species of mice, the monogamous California mouse (*Peromyscus californicus*) and the polygamous white-footed mouse (*Peromyscus leucopus*). Second, we measured the amount of parental and aggressive behavior

shown by males from each species. Our goal was to examine how AVP-ir staining and AVP receptor distribution is associated with the potential for an individual to show aggressive and parental behavior by measuring AVP-ir staining and AVP V_{1a} receptor distribution in sexually naive males without prior experience in staged aggressive encounters.

METHODS

Subjects

We used 42 male white-footed mice and 61 male California mice reared in a laboratory colony at the University of Wisconsin, Madison, in four studies. The animals were kept under a 13L:11D light cycle with lights on at 05:00, and received free access to Purina 5015 mouse chow and water. The first study, which compared parental behavior of the two species, involved 8 mated, male white-footed mice and 13 mated, male California mice. These animals were housed in male-female pairs with their offspring in standard cages until the beginning of the experiment. The second study, which compared aggression of sexually naive males of the two species in resident-intruder and neutral paradigms, used 22 male white-footed mice and 36 male California mice, which were housed in same-sex groups of 1–4 animals. The third study, which compared AVP-ir staining in sexually naive males, used 6 males from each species that ranged in age from 7 to 11 months and were housed in same-sex groups of 2 or 3 mice in standard cages. The fourth study, which compared species differences in the distribution of AVP receptors, also used 6 sexually naive males from each species. Animals were maintained in accordance with the recommendations of the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.

Male Parental Behavior

Within 36 h after the birth of a new litter of pups, animals were placed in clear Plexiglas observation chambers that were divided into three chambers by Plexiglas partitions. Two small chambers (22 × 30 × 14.5 cm each) were separated by a removable, clear Plexiglas partition, while a third, larger chamber (30 × 30 × 29 cm) could be entered from either small chamber and contained a running wheel, food, and a water bottle. Data collection began on the third day that a

pair was placed in the observation chamber and continued every other day until pups were weaned on the morning of day 25. All observations were conducted between 18:30 and 20:30. During each 20-min observation period, the duration of time spent huddling with infants, retrieving infants, licking infants, building a nest, and outside of the nest was recorded for the male parent.

Aggressive Behavior

Each mouse was tested three times in this experiment: as a resident, as an intruder, and in a neutral test. Half of the animals were residents first and intruders 1–2 weeks later, while the other half served as intruders first and residents 1–2 weeks later. Neutral tests were conducted 6 weeks after the completion of the resident-intruder tests. All tests were staged in the Plexiglas observation cages described above.

Each resident was removed from his home cage 48–60 h prior to the experiment and placed in a Plexiglas observation cage. At the beginning of each resident-intruder test, an intruder was placed into the smaller chamber of the observation cage. During the neutral aggression test, both animals were placed simultaneously in separate compartments of the observation cage. All tests were terminated as soon as an attack occurred to prevent injury to the animals. If no attack occurred, mice were separated after 10 min. All trials were videotaped for later measurement of attack latency.

Immunocytochemistry

Six individuals from each species were sacrificed by decapitation. Brains were placed in 5% acrolein for 3 h (twice) and stored overnight in a 30% sucrose buffer solution. Brains were sectioned into 50- μ m coronal sections with a vibratome at a cutting angle of 17°.

The free-floating sections were placed in 0.5% NaBH₄ for 10 min, a 20% goat serum solution in 0.05 M Tris-NaCl with 0.3% Triton (Tris 20%) (pH = 7.6) for 15 min, and then stored in 1:5000 solution of rabbit anti-vasopressin (ICN Immunobiologicals) in 2% goat serum on a refrigerated shaker. After 40 to 45 h, the sections were transferred into the following solutions: (1) Tris 2% goat for 15 min (twice); (2) 3% glutaraldehyde for 30 min; (3) 0.3% H₂O₂ in MeOH for 30 min; (4) Tris 2% goat for 15 min (twice); (5) goat anti-rabbit IgG 1:150 in Tris 2% for 45 min; (6) Tris 2% goat for 15 min; (7) rabbit PAP 1:300 in Tris 2% for 45 min; (8) Tris

2% goat for 15 min; (9) goat anti-rabbit IgG 1:150 in Tris 2% for 45 min; (10) Tris 2% goat for 15 min; (11) rabbit PAP 1:300 in Tris 2% for 45 min; (12) Tris 2% goat for 15 min; (13) Tris NaCl for 10 min (twice); (14) DAB (20 ml Tris NaCl, 0.010 g diaminobenzidinetetrahydrochloride, 20 μ l H₂O₂) for 10 min; and (15) Tris NaCl for 10 min. Sections were stored overnight in Tris-NaCl and mounted onto slides, air dried, and coverslipped the following day. Tissue sections were processed in four separate batches with individuals from each species balanced across each batch. Batch number was used as a covariate in the statistical analysis.

Specificity of the antibody for AVP was tested by pretreating antisera with 50 μ M oxytocin or 50 μ M vasopressin. Although AVP-ir staining was eliminated after pretreatment with vasopressin, pretreatment with oxytocin did not alter AVP-ir staining.

The density of AVP-ir staining was analyzed in the BNST, LS, PVN, and SON of the coronal sections using computerized image analysis software, NIH Image 1.44. Density of cells and fibers was expressed as the total area covered by AVP-ir cells and fibers within a sampling area (Bamshad *et al.*, 1993). All density measurements were taken by two independent observers. For each brain area, the section with the highest amount of AVP-ir staining was identified. Each observer, who was blind to species and the predicted outcome, traced all brain areas bilaterally and calculated the percentage staining for both bilateral measurements. These bilateral measurements were averaged for each brain area of each brain. Since two observers made independent measurements for each brain area, the average of the two scores for each brain area was used in the statistical analysis. The average interrater reliability was 0.80.

For the BNST, PVN, and SON, additional measurements of cell size, cell density, and cell number were made on approximately 10 sections that were evenly distributed throughout each brain area. Cell size was measured by tracing a maximum of 10 cells per section on 10 sections per brain and calculating the surface area of each cell with NIH Image 1.44. Cell density was measured by comparing the optical density of each traced cell to a calibrated set of optical density standards with NIH Image 1.44. On each section that cell size and density were measured, the number of cells was counted. For each brain section, the average cell size and cell density were calculated. These section averages were then averaged to determine the

average cell size, cell density, and cell number for each brain area for each subject.

Radioligand Receptor Autoradiography

A set of slide-mounted sections was processed for receptor autoradiography using ¹²⁵I-labeled linear vasopressin V_{1a} receptor antagonist (HO-Phenylacetyl¹-D-Tyr(Me)²-Phe³-Gln⁴-Asn⁵-Arg⁶-Pro⁷-Arg⁸-NH₂, NEN), as described elsewhere (Young *et al.*, 1997). Slides were prewashed in Tris-HCl (pH 7.4) and incubated for 60 min at room temperature in 50 pM ¹²⁵I antagonist in Tris with MgCl₂ (10 mM), BSA (0.1%), and bacitracin (0.05%). Nonspecific binding was determined in adjacent sections by adding 1 μ M of the selective V_{1a} ligand d(CH₂)₅(Tyr[Me]) AVP to the incubation buffer. After air drying, the slides were exposed to BioMax MR film (Kodak, Rochester, NY) for 48 h. Included in the cassette were ¹²⁵I autoradiographic standards (Amersham, Arlington Heights, IL) for quantification. Specific binding was quantified bilaterally on at least two sections per animal on the films using NIH image software as previously described (Young *et al.*, 1997).

Statistical Analysis

In the study of parental behavior, multiple regression was used to calculate the slope and intercept of each variable for each animal over the 3 weeks of observations. The duration of each behavior during each evening of observation, the slope, and intercept were compared between the two species using *t* tests. The *t* tests were also used to analyze species differences in attack latency and in receptor autoradiography. Results obtained in the study of AVP-ir staining were analyzed by calculating an average score for percentage staining, cell size, cell density, and cell number for each brain area. Percentage staining, cell size, cell density, and cell number were compared between the two species using a multivariate analysis of covariance (MANCOVA), with assay number as the covariate.

RESULTS

Parental Behavior

Overall, parents from the monogamous species, the California mouse, displayed more parental care than

their white-footed mouse counterparts (Fig. 1). Male California mice spent significantly more time huddling on Day 3 through Day 15 than white-footed mice (all values: $t(19) > 2.1$, $P < 0.05$). An overall species difference in huddling behavior also exists across the 3 weeks of observations. In addition to a significantly higher value for the intercept on the graph of huddling behavior for the California mouse ($t(19) = 6.5$, $P < 0.0001$), the slope of the two graphs also differs ($t(19) = -5.0$, $P < 0.0001$). While California mice tend to show less huddling behavior as their pups mature (slope = -29.8), white-footed mice tend to increase their contact with their pups (slope = 7.6). A similar pattern is observed for the amount of time spent outside the nest, with California mice spending significantly less time outside the nest than white-footed mice during Day 3 through Day 7 and Day 11 through Day 15 (all values: $t(19) > 2.2$, $P < 0.05$). A statistically significant difference also exists for the slopes and intercepts on the graph of time spent out of nest over the 3 weeks of observations (slope: $t(19) = 3.7$, $P = 0.001$; intercept: $t(19) = -5.8$, $P < 0.0001$).

A similar pattern of species differences in behavior also was observed with licking offspring, nest building, and retrieving offspring (Fig. 1). Again, California mice tended to spend more time licking their offspring during the first 2 weeks of observations (Days 3–5 and Days 9–15: $t(19) > 2.3$, $P < 0.05$). Not only did California mice spend more time licking their pups on Day 3 through Day 5 and Day 9 through Day 15, but the intercepts of the two graphs also were statistically different ($t(19) = 2.4$, $P < 0.05$). For nest building and retrievals of pups, we observed few statistically significant differences between the species. Although California mice tended to spend more time nest building, this difference was not statistically significant (all values: $t(19) > 1.6$, $P > 0.10$). California mice also tended to spend more time retrieving pups, but this difference was statistically significant only on Day 21 ($t(19) = 2.4$, $P < 0.05$).

Aggression

The aggression tests also show strong species differences (Fig. 2). Attack latency differs significantly between the species: sexually naive male California mice attack significantly more rapidly than sexually naive male white-footed mice in a resident–intruder test ($t(56) = 3.1$, $P < 0.01$). Although both species attacked their opponents much more slowly in the neutral aggression test, a similar pattern of species

differences in aggression was observed with male California mice attacking their opponents more rapidly than male white-footed mice ($t(52) = 3.1$, $P < 0.01$). There was no significant effect of test order on performance in the resident–intruder test ($F(5, 49) = 0.4$, $P = 0.8$) or in the neutral aggression test ($F(5, 44) = 0.9$, $P = 0.5$).

AVP-ir Staining

In both *P. californicus* and *P. leucopus*, we observed AVP-ir staining in the BNST, LS, MA, SON, and PVN. Both coronal and sagittal sections showed fiber tracts projecting from the ventrocaudal BNST toward the LS (Fig. 6). Measurements of AVP-ir staining were not made in the LS because coronal sections revealed only light AVP-ir staining (staining was less than 1%). In the area identified as the MA by Slotnick and Leonard (1975), we also identified AVP-ir cells and fibers that appear to project toward the BNST. However, no measurements are reported for the MA because the population of cells in the MA was located more medially than has been reported in voles. Further studies need to be performed to identify which cell populations project to the LS.

Species differences were identified in the AVP-ir pathway that originates in the BNST (Figs. 3, 4, and 5). In the BNST, AVP-ir fibers and cells cover more of the measured area in sexually naive male California mice than in sexually naive male white-footed mice ($F(1, 9) = 28.0$, $P < 0.001$). While 56% of the measured area of the ventrocaudal BNST is stained by AVP-ir cells and fibers in male California mice, only 33% of the measured area of the BNST is stained in sexually naive male white-footed mice. This difference in staining may result in part from a 14% difference in cell number ($F(1, 9) = 10.2$, $P = 0.01$), although there is also a nonsignificant trend toward a difference in the density of staining within cells ($F(1, 9) = 3.5$, $P = 0.09$). Despite this difference in staining in the BNST, no statistically significant differences were found in the LS (<1% staining, $F(1, 9) = 0.002$, $P = 0.97$) or LH (no staining), two presumed targets of this AVP pathway.

Outside of the AVP-ir pathway that originates in the BNST, no statistically significant differences in AVP-ir staining were observed in sexually naive males (Figs. 3, 4, and 5). Approximately 60% of the measured area of the PVN ($F(1, 9) = 0.001$, $P = 0.97$), 55% of the measured area of the SON ($F(1, 9) = 4.6$, $P = 0.06$), and 6% of the measured area of the PVT ($F(1, 9) = 0.5$, $P = 0.5$) are stained by AVP-ir fibers and cells in sexually

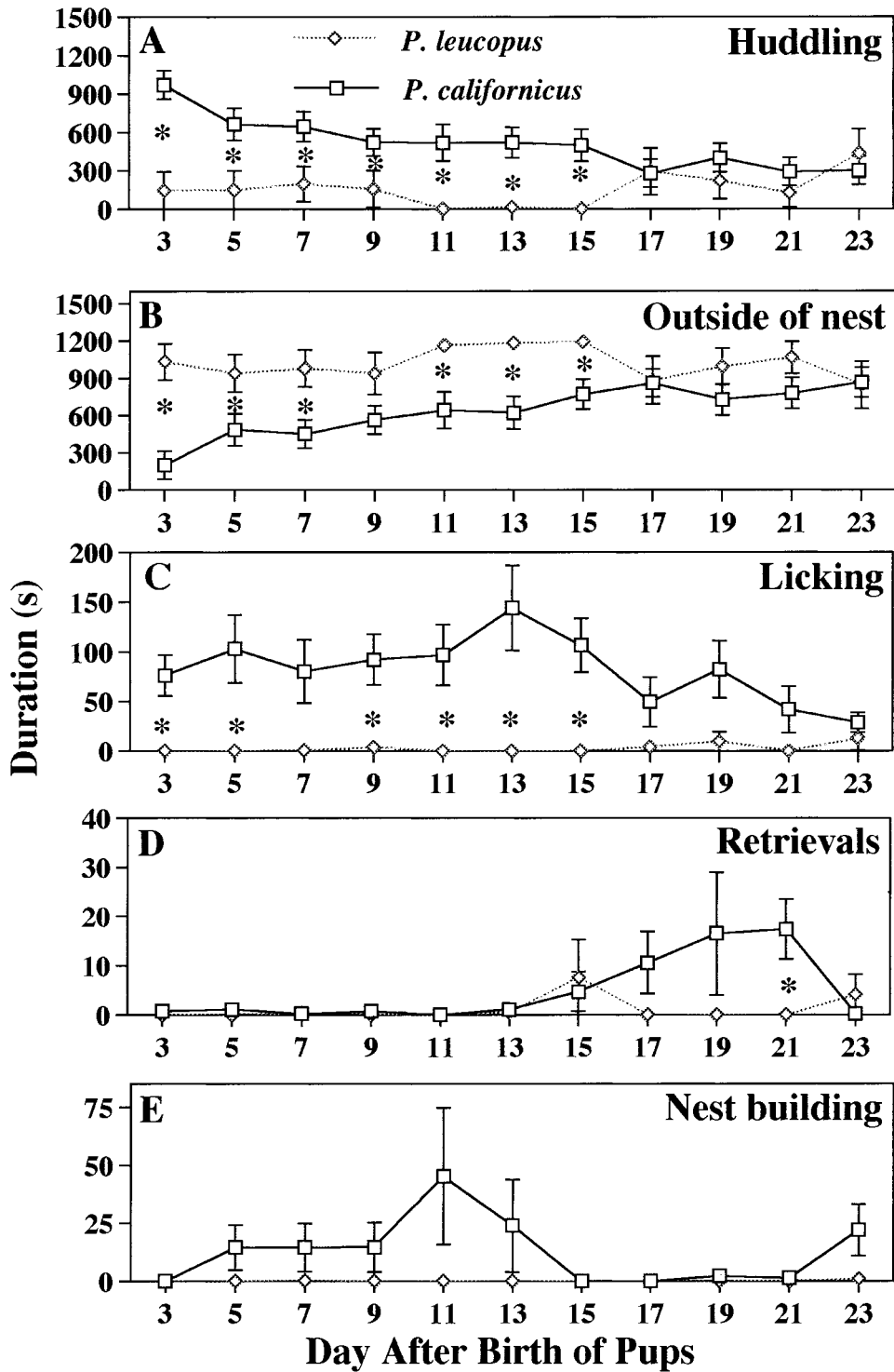


FIG. 1. Amount of time spent (A) huddling with pups, (B) outside of the nest, (C) licking pups, (D) retrieving pups, and (E) nest building by male white-footed mice *P. leucopus* and male California mice *P. californicus* during 1200-s observation periods beginning on Day 3 and ending on Day 23 after the birth of pups. Data are presented as means \pm standard errors. * $P < 0.05$.

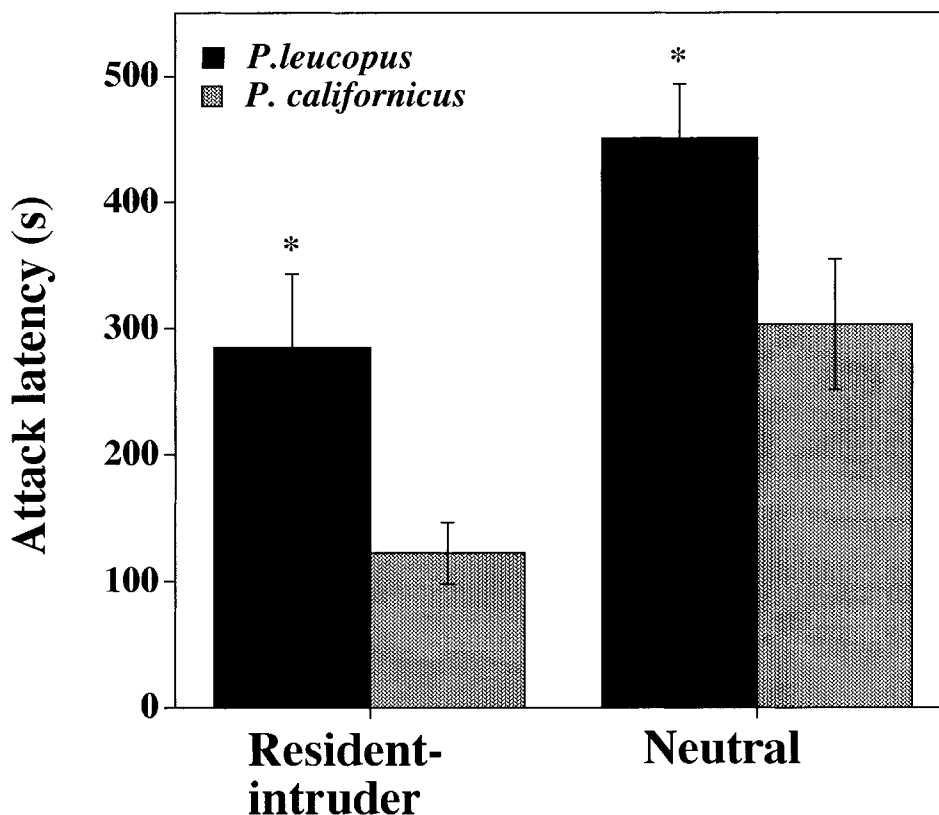


FIG. 2. Attack latency in resident-intruder and neutral aggression tests for male white-footed mice *P. leucopus* and male California mice *P. californicus*. Data are presented as means \pm standard errors. * $P < 0.01$.

naive male California and white-footed mice (Figs. 3 and 4). Within the PVN and SON, there also are no statistically significant differences in cell size (PVN: $F(1, 9) = 0.4$, $P = 0.54$; SON: $F(1, 9) = 2.1$, $P = 0.18$), number (PVN: $F(1, 9) = 0.8$, $P = 0.39$; SON: $F(1, 9) = 0.1$, $P = 0.75$), or density of staining within cells (PVN: $F(1, 9) = 0.2$, $P = 0.69$; SON: $F(1, 9) = 0.03$, $P = 0.87$).

AVP Receptor Autoradiography

Sexually naive male California mice have greater numbers of AVP V_{1a} receptors in the LS, interior layer of the cortex, and medial portion of the diagonal band than white-footed mice (areas identified by using Swanson, 1992). These differences were all statistically significant (septum: $t(10) = 2.5$, $P < 0.05$; cortex: $t(10) = 3.6$, $P < 0.01$; diagonal band: $t(10) = 2.5$, $P < 0.05$; Figs. 7 and 8).

DISCUSSION

It has been proposed that significant differences in social behavior may be associated with slight differ-

ences in the distribution of AVP-ir staining (i.e., Bamshad *et al.*, 1993) or AVP receptors (i.e., Young *et al.*, 1997). We were able to begin testing this association between AVP and social behavior in *Peromyscus* by comparing two species' differences in social behavior with differences in AVP-ir staining and receptor distribution. We examined how AVP receptor distribution and AVP-ir staining are associated with the potential for an individual to show aggressive and parental behavior by measuring AVP V_{1a} receptor distribution and AVP-ir staining in sexually naive males without prior experience in staged aggressive encounters.

Parental Behavior

While male California mice show high levels of parental care toward their own offspring, male white-footed mice spend most of their time outside of the nest and spend little time huddling with, retrieving, or licking their pups. The two species also show different temporal patterns of parental care. While California

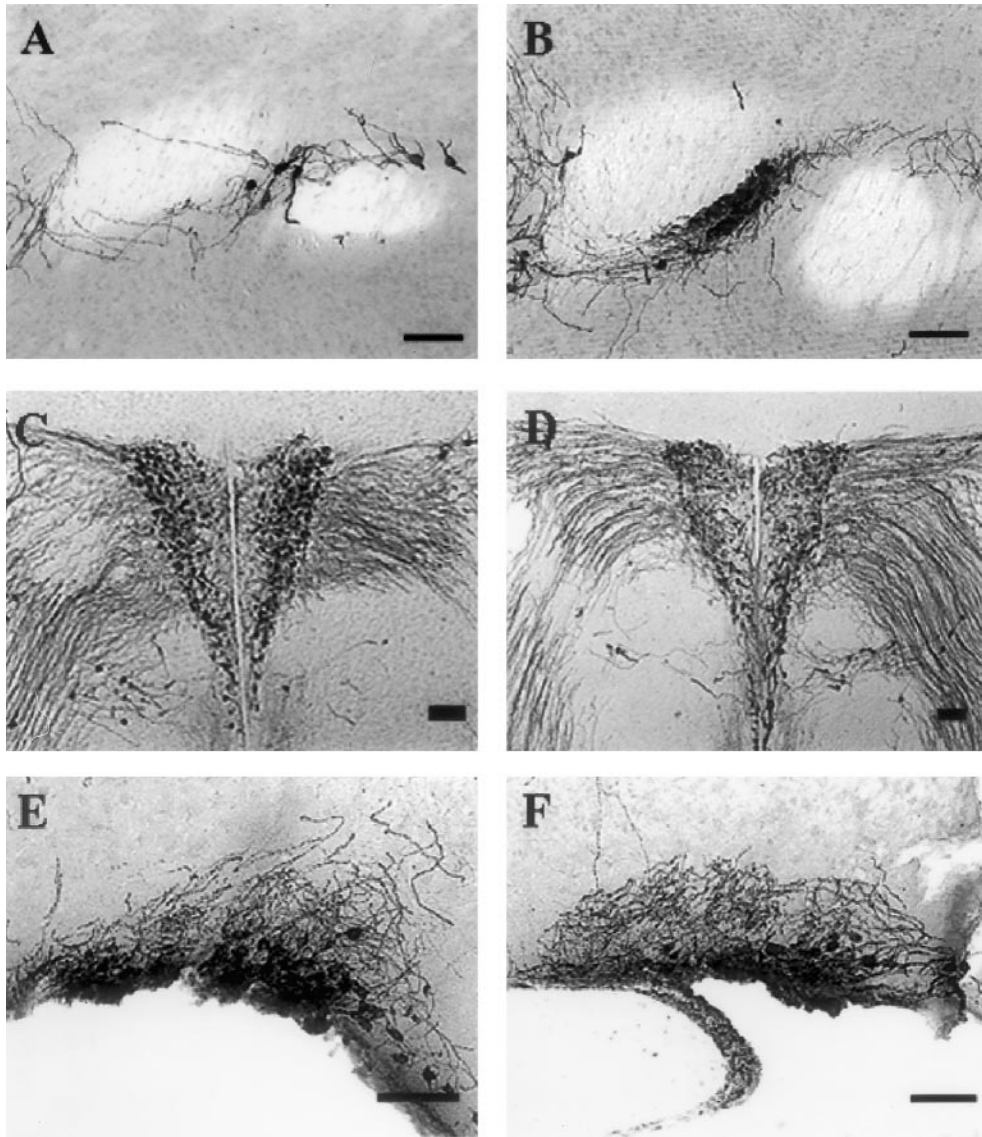


FIG. 3. Representative photomicrographs of AVP-ir staining in the bed nucleus of the stria terminalis (A and B), paraventricular nucleus (C and D), and supraoptic nucleus (E and F), comparing sexually naive, male white-footed mice *P. leucopus* (A, C, and E) and sexually naive male California mice *P. californicus* (B, D, and F). Bar represents 100 μm .

mice tend to spend less time in contact with pups as their offspring approach weaning age, white-footed mice show the opposite pattern. These data agree with field studies and studies of male parental care in large cages that suggest that male white-footed mice show little association with young until the pups reach weaning age (Schug, Vessey, and Underwood, 1992).

More importantly, this study is similar to previous comparisons of AVP and parental behavior in prairie and meadow voles because it shows large differences

in parental care between the monogamous and the polygamous species (McGuire, 1988). This study confirms that the polygamous white-footed mouse (Xia and Millar, 1991) resembles the polygamous meadow vole *M. pennsylvanicus* in that males show little parental care (Schug *et al.*, 1992; Wolff and Cicirello, 1990). In contrast, the monogamous California mouse (Ribble and Salvioni, 1990) shows high levels of biparental care similar to those of the monogamous prairie vole *M. ochrogaster* (McGuire, 1988; Gubernick and

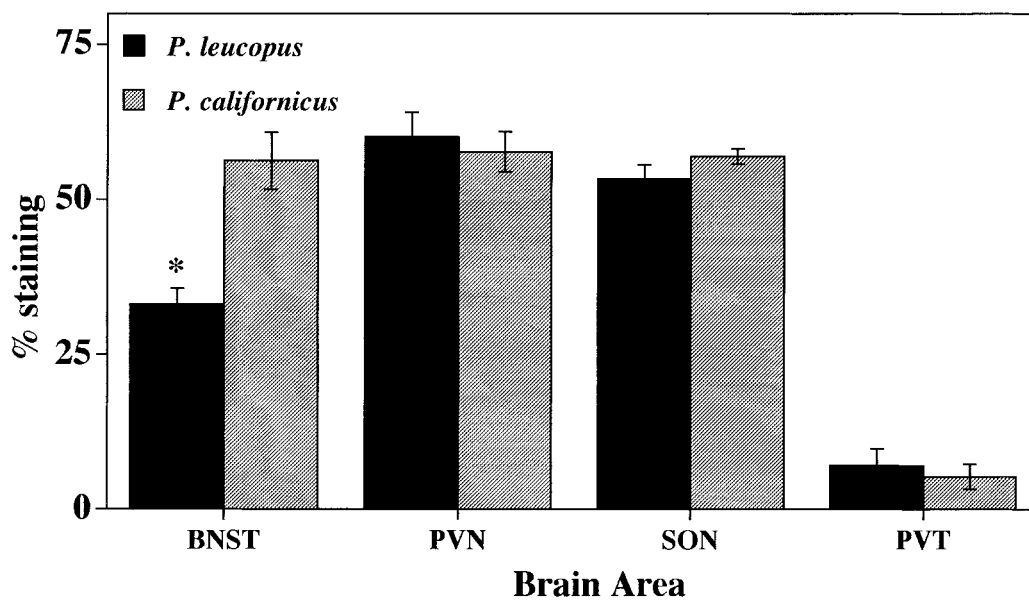


FIG. 4. Percentage staining as a measure of AVP-immunoreactive staining in the bed nucleus of the stria terminalis (BNST), paraventricular nucleus of the hypothalamus (PVN), supraoptic nucleus (SON), and paraventricular nucleus of the thalamus (PVT) in male white-footed mice *P. leucopus* and male California mice *P. californicus*. Data are presented as means \pm standard errors. * $P < 0.05$.

Alberts, 1987). These parallels in behavior are important because if parental care is an accurate predictor of AVP-ir staining and receptor patterns, parental male mice are predicted to have V_{1a} receptor distribution and low levels of AVP-ir staining similar to those of parental male voles.

V_{1a} Receptor Distribution

The two *Peromyscus* species differed in AVP-ir staining in the BNST, while they did not differ in brain areas that are not associated with this sexually dimorphic pathway. This result agrees with previous studies of voles, which found species differences in AVP-ir staining only in the pathway originating in the BNST and MA (Bamshad *et al.*, 1993, 1994; Wang, 1995; Wang *et al.*, 1996). In contrast to the voles, dramatic species differences in AVP receptor distribution occurred across a variety of brain areas not directly associated with this pathway, including the diagonal band and cortex. Since no studies have examined the behavioral consequences of AVP injections directly into the diagonal band or cortex, the function of species differences in AVP receptors in these areas remains unclear. However, it is possible that these areas are associated with other species-typical patterns of behavior or social memory (e.g., Young *et al.*, 1997).

Within the AVP pathway that has been associated with parental care and aggression, dramatic variation was observed in the distribution of AVP receptors and AVP-ir staining in white-footed mice and California mice. As in *Microtus*, AVP V_{1a} receptors are found in the LS, the target of the AVP pathway originating in the BNST. The two species of *Peromyscus* also show dramatic differences in receptor distribution that are opposite in direction to the differences in monogamous and polygamous voles. In contrast to previous studies in voles (Insel *et al.*, 1994; Young *et al.*, 1997), we observed a higher density of AVP V_{1a} receptors in the LS of the monogamous species, *P. californicus*, than in the polygamous species, *P. leucopus*. A previous comparison of AVP receptors in two species of *Peromyscus* also demonstrated that AVP receptors were more abundant in the LS of the monogamous species, *P. californicus*, in comparison to the polygamous deer mouse *P. maniculatus* (Insel, Gelhard, and Shapiro, 1991).

AVP-ir Staining

In addition to this difference in AVP receptor distribution between the California mouse and the white-footed mouse, the two species also differed in the amount of presynaptic AVP in the BNST in the

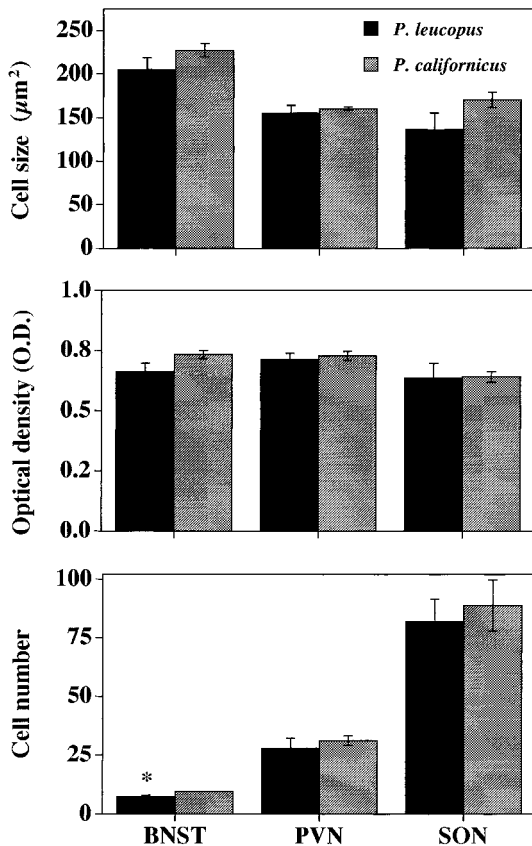


FIG. 5. Average cell size, optical density, and number of neurons per section of the bed nucleus of the stria terminalis (BNST), paraventricular nucleus (PVN), and supraoptic nucleus (SON) in male white-footed mice *P. leucopus* and male California mice *P. californicus*. Data are presented as means \pm standard errors. * $P < 0.01$.

same direction as did their receptors. Thus, in contrast to previous studies associating AVP and parental behavior in voles, more abundant AVP-ir staining was observed in the BNST of the monogamous species, the California mouse. These comparisons among monogamous and polygamous mice and voles suggest that patterns of both AVP receptor distribution and AVP-ir staining in this pathway may not be predicted by differences in mating systems or parental behavior (or vice versa). Although mating systems or parental behavior may be associated with differences in AVP receptor distribution between closely related species, neither monogamy nor a high level of parental care is universally associated with low AVP receptor density in the LS or low AVP-ir staining in the BNST in sexually naive males.

Species and Methodological Differences between Studies of AVP-ir Staining

As a caveat, differences in AVP-ir staining between voles and *Peromyscus* mice might also be influenced by general species differences between *Peromyscus* and *Microtus* and methodological differences between studies of AVP-ir staining. For example, in contrast to the abundant AVP-ir staining measured in the LS in *Microtus* (Bamshad *et al.*, 1993, 1994), very little AVP-ir staining in the LS (<1%) is observed in *Peromyscus*. In both species, the AVP-ir cells measured in the BNST appear to project toward the LS where V_{1a} receptors are located (Fig. 6). A direct connection between the BNST and the LS has been identified in rats using lesioning and track-tracing techniques (De Vries and Buijs, 1983), and the existence of a similar connection has been inferred for both domestic mice and *Microtus*. As a corollary, at least some of these fibers projecting from the BNST in *Peromyscus* probably reach the LS, although it is possible that these fibers are not homologous to the AVP-containing fibers in rats and *Microtus*. Similarly, it is possible that these BNST neurons in *Peromyscus* are homologous to neurosecretory magnocellular cells of other species because the cell sizes in the BNST population that we measured were larger than those found in domestic mice (Castel and Morris, 1988). However, since BNST neurons appear to be similar in size to those found in male *Microtus ochrogaster* (Wang, 1995), these cells could be homologous to those identified in male *M. ochrogaster* and therefore show direct connections to the LS.

One methodological difference from previous studies is that Wang (1995) measured cells in the most rostral portion of the BNST that are not visible without pretreatment with colchicine. These cells also tend to be located in a more lateral portion of the BNST than the cells that we measured. In our study, we avoided colchicine pretreatment in order to examine basal levels of AVP in the BNST and to avoid confounding stress effects (Ceccatelli, Villar, Goldstein, and Hökfelt, 1989; Romero, Levine, and Sapolsky, 1995). In addition, AVP-ir cell populations were clearly visible in the BNST with fibers apparently projecting in the direction of the LS, where the receptors are located, without the use of colchicine (Fig. 6). When colchicine treatment is used, it does not reveal any new populations of smaller cells in the area of the BNST in either *P. californicus* or *P. leucopus* (Bester-Meredith and Marler, unpublished data). The lack of an effect of col-

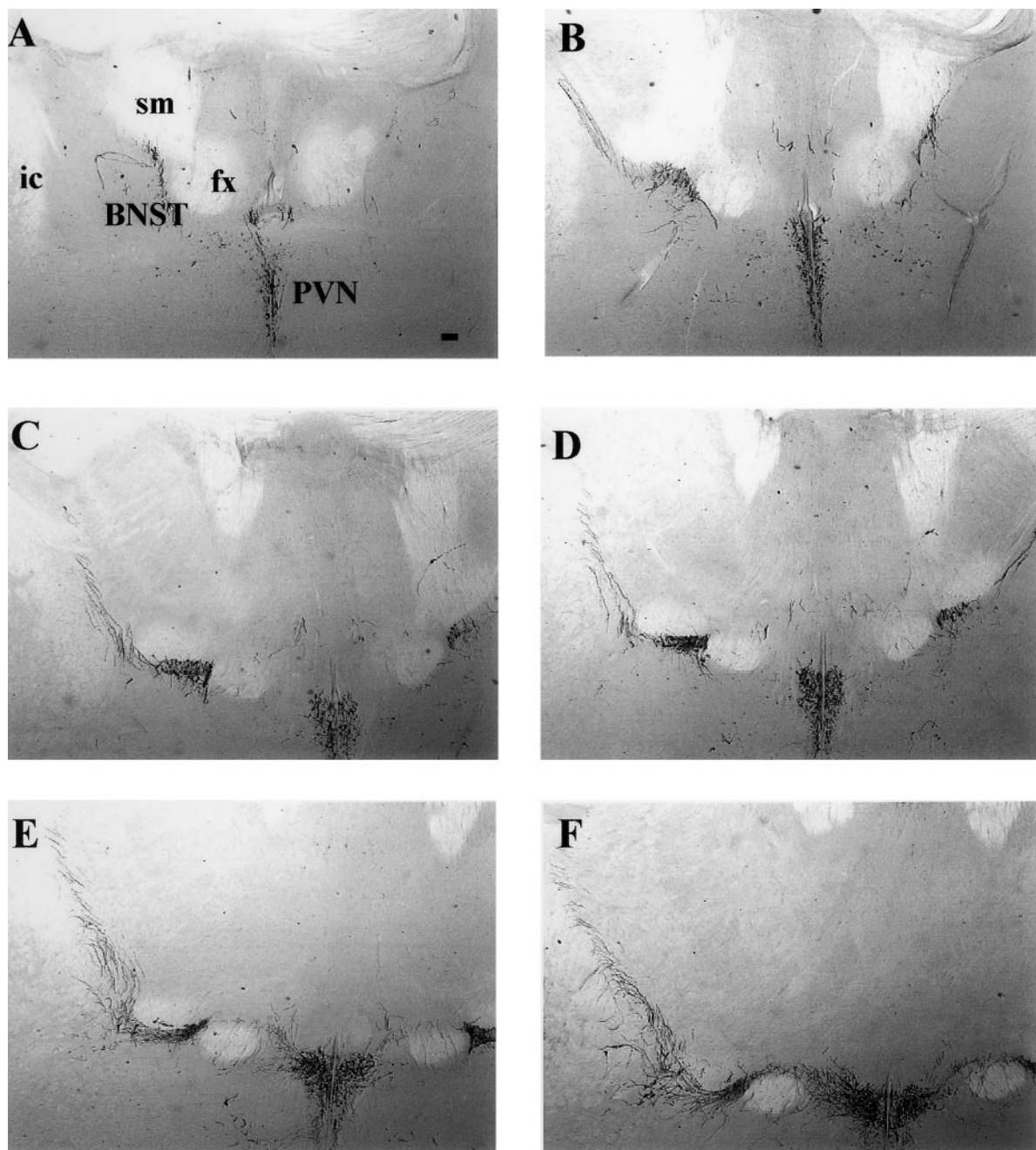


FIG. 6. Serial photomicrographs of coronal sections from a *P. californicus* brain showing fibers projecting upward toward the LS from the BNST. (fx = fornix; sm = stria medularis; ic = internal capsule). Bar represents 100 μ m.

chicine on neuronal visibility in the BNST may be related to species differences between *Peromyscus* and *Microtus* because the effects of colchicine appear to be quite variable across species. For example, in domestic mice, colchicine had no predictable effects on the visibility of neurons in the BNST or MA

(Castel and Morris, 1988). Despite the difference in colchicine treatment, studies in both *Peromyscus* and *Microtus* showed variation in AVP-ir staining between monogamous and polygamous species. In both *Peromyscus* and *Microtus*, these differences were observed in the BNST in populations of cells

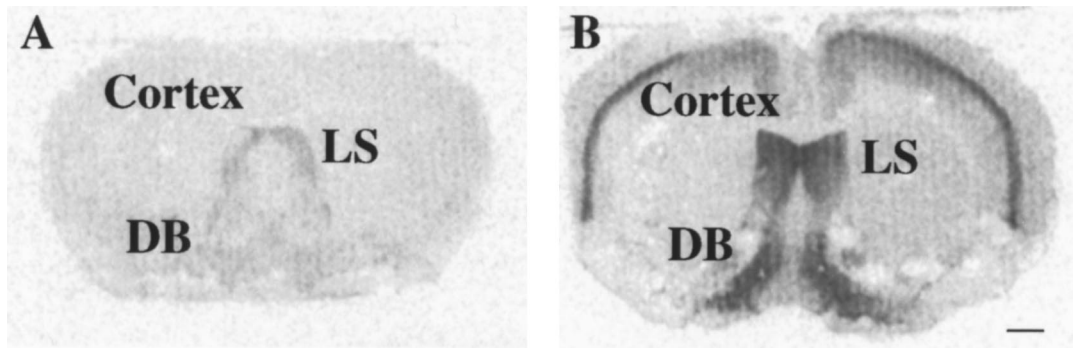


FIG. 7. Representative photomicrographs of AVP receptors in the lateral septum (LS), cortex, and diagonal band (DB) of (A) male white-footed mice *P. leucopus* and (B) male California mice *P. californicus*. Bar represents 500 μm .

that appear to project toward the LS. Therefore, we believe that regardless of the difference in the use of colchicine, these comparisons suggest that high AVP-ir staining in the BNST is not associated with monogamy or with high levels of parental care across distantly related species.

Aggression as a Possible Predictor of Patterns of AVP-ir Staining

Again, these comparisons among monogamous and polygamous mice and voles suggest that differences in mating systems or parental behavior may not be associated with predictable patterns of AVP-ir staining or AVP receptor distribution in this pathway. While closely related species may show correlations between parental behavior and AVP-ir staining or AVP receptor distribution, these patterns may not hold across more distantly related species. However, other

changes in the AVP pathway, including differences in AVP receptor binding affinity or rates of AVP production, release, or breakdown in the synaptic cleft, could also account for variation in parental behavior between species. In addition, it is likely that interactions among a variety of neurotransmitter systems are involved in the expression of complex social behaviors like parental behavior.

While it is possible that behaviors cannot be used to predict patterns of AVP-ir staining and receptor distribution, one plausible hypothesis that should be tested is whether patterns of aggression can explain the contradictions between this study and the findings of higher AVP-ir staining in the BNST of the polygamous meadow vole (Wang, 1995) and of more abundant AVP receptors in the LS of the polygamous montane vole (Insel *et al.*, 1994; Wang, Young, Liu, and Insel, 1997; Young *et al.*, 1997). While the mating system and parental behavior of voles parallel those of the *Peromyscus* used in this study, their patterns of aggression may differ. In this study, the monogamous California mouse shows a more rapid onset of aggression than the polygamous white-footed mouse. Sexually naive California mouse resident males attack intruders in less than half the time of sexually naive white-footed mouse resident males. A similar pattern was observed in a neutral aggression test. Although few studies of aggression in *Microtus* have been performed in the laboratory, a field study showed that the monogamous prairie vole is less aggressive than the polygamous male meadow vole across a variety of seasons and habitats using standard techniques for testing aggression (Hofmann, Getz, and Klatt, 1982). This conclusion is also supported by less detailed studies (Getz, 1962; Dewsbury, 1983; but see Colvin, 1973). These differences in aggression might explain

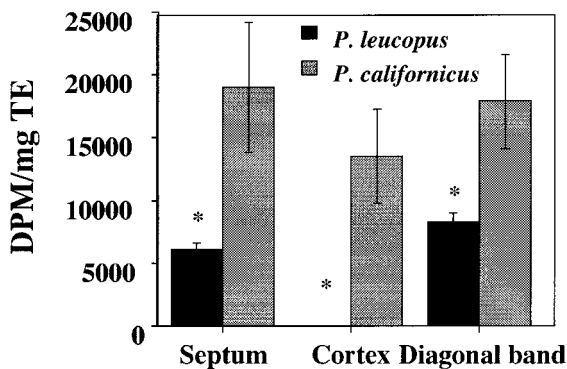


FIG. 8. The density of AVP receptors in the lateral septum, cortex, and diagonal band of male white-footed mice *P. leucopus* and male California mice *P. californicus*. Data are presented as means \pm standard errors. * $P < 0.05$.

why patterns of parental behavior do not accurately predict differences in AVP-ir staining and receptors. To begin testing this hypothesis more carefully, aggression in *Microtus* needs to be measured under more controlled laboratory conditions.

The results of this study and the vole studies described above suggest that high aggressiveness may be associated with high AVP levels in projections from the BNST and abundant AVP receptors in the target of this pathway, the LS. Higher levels of AVP-ir staining may indicate that more AVP can be released from the BNST and MA and bind to receptors in the LS during an aggressive encounter. Although this idea is supported by our study, the vole studies, and studies that have examined the effects of AVP injections on aggression as discussed earlier, there are studies on other species that appear to contradict this scenario. For example, house mice that rapidly attack their opponents tend to have less AVP in the BNST (Compaan *et al.*, 1993). These mice, however, were sacrificed after being housed with females and participating in staged aggressive encounters with unfamiliar males. In the current study, males were housed in same-sex groups and had not participated in staged aggressive encounters with unfamiliar conspecific males. It is therefore possible that social experience may alter presynaptic levels of AVP, as in Bamshad *et al.* (1993, 1994). Future comparisons of AVP-ir staining and AVP receptor distribution in experienced and inexperienced *P. californicus* and *P. leucopus* will add to our knowledge of how social and sexual experience alter AVP pathways.

When comparisons of AVP-ir staining are made in the LS, results are also variable. We found very low levels of AVP-ir staining in the LS (<1%) in *Peromyscus* in contrast to previous reports in voles (Bamshad *et al.*, 1993, 1994). The low levels of AVP-ir staining found in the LS are similar to findings in hamsters (Delville, Koh, and Ferris, 1994). Also in contrast to our findings in *Peromyscus*, more aggressive male rats have less AVP-ir staining in the LS than less aggressive rats, but here again they were housed with females and exposed to aggressive encounters 3 weeks earlier (Everts *et al.*, 1997). The variation among these within-species studies and the between-species studies may therefore be influenced by differences in social experience. Additional controlled studies of AVP and aggression across more species will be needed to verify if an association among AVP-ir staining, AVP receptor distribution, and aggression exists.

Our results clearly demonstrate that relative species differences in AVP-ir staining and receptor distribu-

tion cannot be predicted by parental care and that it is plausible that species differences in aggression can be used to predict variations in AVP-ir staining and receptor distribution more accurately. However, even if aggression is more closely associated with AVP-ir staining than parental behavior, this would not demonstrate that AVP is controlling one behavior and not the other. While several neurotransmitter systems have been associated with either aggression or parental care, in prairie voles, the results of AVP manipulations suggest that AVP is influencing both mate guarding aggression and parental behavior. These behaviors are temporally associated with pair bonding and natural selection may favor the linking of these two very disparate behaviors, perhaps in this case through vasopressin. How this control of dual behaviors is accomplished and how this ties in with the AVP-ir staining patterns still remains to be investigated in both voles and *Peromyscus*, but again the answer may still lie in plasticity in a variety of factors, such as rates of AVP production, AVP receptor distribution or sensitivity, or rates of AVP release. Recent reports have suggested that both AVP receptor distribution (Wang *et al.*, 1997) and absolute levels of AVP (Delville, Melloni, and Ferris, 1998) may be important in regulating social behavior in different species. By increasing our understanding of this neural pathway, we will gain a better awareness of the neural mechanisms that influence important social behaviors like aggression and male paternal care.

ACKNOWLEDGMENTS

The authors thank Zuoxin Wang, Brian Zahn, Patricia Martin, Melanee Clark, Taryn Haag, Charles Meredith, Kaarin Smyth, and Darren Holtzman for technical assistance. We also thank Craig Berridge and Mark Coleman for comments on the manuscript. This research was supported by Grants IBN-9703309 to C. A. Marler and MH56897 to L. J. Young.

REFERENCES

- Bamshad, M., Novak, M. A., and De Vries, G. J. (1993). Sex and species differences in the vasopressin innervation of sexually naive and parental prairie voles, *Microtus ochrogaster* and meadow voles, *Microtus pennsylvanicus*. *J. Neuroendocrinol.* **5**, 245–255.
- Bamshad, M., Novak, M. A., and De Vries, G. J. (1994). Cohabitation alters vasopressin innervation and paternal behavior in prairie voles (*Microtus ochrogaster*). *Physiol. Behav.* **56**, 751–758.
- Castel, M., and Morris, J. F. (1988). The neurophysin-containing

- innervation of the forebrain of the mouse. *Neuroscience* **24**, 937–966.
- Ceccatelli, S., Villar, M. J., Goldstein, M., and Hökfelt, T. (1989). Expression of c-Fos immunoreactivity in transmitter-characterized neurons after stress. *Proc. Natl. Acad. Sci. USA* **86**, 9569–9573.
- Colvin, D. V. (1973). Agonistic behaviour in males of five species of voles *Microtus*. *Anim. Behav.* **21**, 471–480.
- Compaan, J. C., Koolhaas, J. M., Buijs, R. M., Pool, C. W., de Ruiter, A. J. H., and van Oortmerssen, G. A. (1992). Vasopressin and the individual differentiation in aggression in male house mice. *Ann. N. Y. Acad. Sci.* **652**, 458–459.
- Compaan, J. C., Buijs, R. M., Pool, C. W., De Ruiter, A. J. H., and Koolhaas, J. M. (1993). Differential lateral septal vasopressin innervation in aggressive and nonaggressive male mice. *Brain Res. Bull.* **30**, 1–6.
- Delville, Y., Koh, E. T., and Ferris, C. F. (1994). Sexual differences in the magnocellular vasopressinergic system in golden hamsters. *Brain Res. Bull.* **33**, 535–540.
- Delville, Y., Mansour, K. M., and Ferris, C. F. (1996). Testosterone facilitates aggression by modulating vasopressin receptors in the hypothalamus. *Physiol. Behav.* **60**, 25–29.
- Delville, Y., Melloni, R. H., Jr., and Ferris, C. F. (1998). Behavioral and neurobiological consequences of social subjugation during puberty in golden hamsters. *J. Neurosci.* **18**, 2667–2672.
- De Vries, G. J., and Buijs, R. M. (1983). The origin of the vasopressinergic and oxytocinergic innervation of the rat brain with special reference to the lateral septum. *Brain Res.* **273**, 307–317.
- Dewsbury, D. A. (1983). A comparative study of rodent social behavior in a seminatural enclosure. *Aggress. Behav.* **9**, 207–215.
- Everts, H. G. J., de Ruiter, A. J. H., and Koolhaas, J. M. (1997). Differential lateral septal vasopressin in wild-type rats: Correlation with aggression. *Horm. Behav.* **31**, 136–144.
- Everts, H. G. J., and Koolhaas, J. M. (1997). Lateral septal vasopressin in rats: Role in social and object recognition? *Brain Res.* **760**, 1–7.
- Ferris, C. F., Delville, Y., Grzonka, Z., Luber-Narod, J., and Insel, T. R. (1993). An iodinated vasopressin (V_1) antagonist blocks flank marking and selectively labels neural binding sites in golden hamsters. *Physiol. Behav.* **54**, 737–747.
- Ferris, C. F., Delville, Y., Miller, M. A., Dorsa, D. M., and De Vries, G. J. (1995). Distribution of small vasopressinergic neurons in golden hamsters. *J. Comp. Neurol.* **360**, 589–598.
- Ferris, C. F., Meenan, D. M., Axelson, J. A., and Albers, H. E. (1986). A vasopressin antagonist can reverse dominant/subordinate behavior in hamsters. *Physiol. Behav.* **38**, 135–138.
- Ferris, C. F., and Potegal, M. (1988). Vasopressin receptor blockade in the anterior hypothalamus suppresses aggression in hamsters. *Physiol. Behav.* **44**, 235–239.
- Getz, L. L. (1962). Aggressive behavior of the meadow and prairie voles. *J. Mamm.* **43**, 351–358.
- Gubernick, D. J., and Alberts, J. R. (1987). The biparental care system of the California mouse, *Peromyscus californicus*. *J. Comp. Psychol.* **101**, 169–177.
- Hofmann, J. E., Getz, L. L., and Klatt, B. J. (1982). Levels of male aggressiveness in fluctuating populations of *Microtus ochrogaster* and *M. pennsylvanicus*. *Can. J. Zool.* **60**, 898–912.
- Insel, T. R., Gelhard, R., and Shapiro, L. E. (1991). The comparative distribution of forebrain receptors for neurohypophysial peptides in monogamous and polygamous mice. *Neurosci.* **43**, 623–630.
- Insel, T. R., Wang, Z.-X., and Ferris, C. F. (1994). Patterns of brain vasopressin receptor distribution associated with social organization in microtine rodents. *J. Neurosci.* **14**, 5381–5392.
- Lonstein, J. S., and De Vries, G. J. (1998). Sex differences in parental behavior of virgin prairie voles: Role of gonadal hormones and vasopressin. *Soc. Neurosci. Abstr.* **24**, 952.
- McGuire, B. (1988). Effects of cross-fostering on parental behavior of meadow voles (*Microtus pennsylvanicus*). *J. Mamm.* **69**, 332–341.
- Ribble, D. O., and Salvioni, M. (1990). Social organization and nest co-occupancy in *Peromyscus californicus*, a monogamous rodent. *Behav. Ecol. Sociobiol.* **26**, 9–15.
- Romero, L. M., Levine, S., and Sapolsky, R. M. (1995). Patterns of adrenocorticotropin secretagog release in response to social interactions and various degrees of novelty. *Psychoneuroendocrinology* **20**, 183–191.
- Schug, M. D., Vessey, S. H., and Underwood, E. M. (1992). Paternal behavior in a natural population of white-footed mice (*Peromyscus leucopus*). *Am. Midl. Nat.* **127**, 373–380.
- Slotnick, B. M., and Leonard, C. M. (1975). *A Stereotaxic Atlas of the Albino Mouse Forebrain*. U.S. Department of Health, Education, and Welfare, Rockville, Maryland.
- Swanson, L. J. (1992). *Structure of the Rat Brain*. Elsevier, Amsterdam.
- Wang, Z. (1995). Species differences in the vasopressin-immunoreactive pathways in the bed nucleus of the stria terminalis and the medial amygdaloid nucleus in prairie voles (*Microtus ochrogaster*) and meadow voles (*Microtus pennsylvanicus*). *Behav. Neurosci.* **109**, 305–311.
- Wang, Z., Ferris, C. F., and De Vries, G. J. (1994). Role of septal vasopressin innervation in parental behavior in prairie voles (*Microtus ochrogaster*). *Proc. Natl. Acad. Sci. USA* **91**, 400–404.
- Wang, Z., Young, L. J., Liu, Y., and Insel, T. R. (1997). Species differences in vasopressin receptor binding are evident in early development: Comparative anatomic studies in prairie and montane voles. *J. Comp. Neurol.* **378**, 535–546.
- Wang, Z., Zhou, L., Hulihan, T. J., and Insel, T. R. (1996). Immunoreactivity of central vasopressin and oxytocin pathways in microtine rodents: A quantitative comparative study. *J. Comp. Neurol.* **366**, 726–737.
- Winslow, J. T., Hastings, N., Carter, C. S., Harbaugh, C. R., and Insel, T. R. (1993). A role for central vasopressin in pair bonding in monogamous prairie voles. *Nature* **365**, 545–548.
- Wolff, J. O., and Cicirello, D. M. (1990). Comparative paternal and infanticidal behavior of sympatric white-footed mice (*Peromyscus leucopus noveboracensis*) and deermice (*P. maniculatus nubiterrae*). *Behav. Ecol.* **2**, 38–45.
- Xia, X., and Millar, J. S. (1991). Genetic evidence of promiscuity in *Peromyscus leucopus*. *Behav. Ecol. Sociobiol.* **28**, 171–178.
- Young, L. J., Winslow, J. T., Nilsen, R., and Insel, T. R. (1997). Species differences in V_{1a} receptor gene expression in monogamous and non-monogamous voles: Behavioral consequences. *Behav. Neurosci.* **111**, 599–605.