

Vasopressin and the Transmission of Paternal Behavior Across Generations in Mated, Cross-Fostered *Peromyscus* Mice

Janet K. Bester-Meredith and Catherine A. Marler
University of Wisconsin—Madison

The role of arginine vasopressin (AVP) in the nongenomic transfer of paternal behavior from fathers to offspring was examined in *Peromyscus*. Male California mice (*P. californicus*) exposed to fewer retrievals by white-footed mouse (*P. leucopus*) foster parents displayed fewer retrievals of biological offspring. In contrast, white-footed mice were retrieved equally rarely by California mouse foster parents and by biological parents and displayed no changes in pup retrieval behavior. AVP-immunoreactive staining in the bed nucleus of the stria terminalis may predict paternal behavior because it correlated positively with retrievals and with a score consisting of huddling, grooming, and time inside the nest. The authors discuss AVP as a possible mechanism by which early experience shapes adult paternal behavior.

Plasticity in the brain during development allows environmental influences to shape the formation of the central nervous system. Rearing conditions can affect a variety of neural pathways ranging from the visual system (Hubel, Wiesel, & LeVay, 1977) to stress responsiveness (Liu et al., 1997). However, few researchers have examined interactions between the social environment during development and the formation of neural pathways, and how this interaction influences social behavior in adults.

One neural substrate that shows plasticity in response to social cues is the neuropeptide arginine vasopressin (AVP). Social subjugation by a larger animal during puberty altered adult aggressiveness in male golden hamsters and decreased the AVP content of the anterior hypothalamus, a brain area associated with aggression (Delville, Melloni, & Ferris, 1998). In male California mice (*Peromyscus californicus*), manipulating social cues much earlier in development by cross-fostering pups to a less parental and less aggressive species, the white-footed mouse (*P. leucopus*), caused a decrease in aggression and AVP-immunoreactive (AVP-ir) staining in the bed nucleus of the stria terminalis (BNST) and the supraoptic nucleus (SON; Bester-Meredith & Marler, 2001). In both of these studies, altering the social interactions that males

received during development influenced adult social behavior and AVP levels. Neither of these studies, however, identified which specific aggressive or parental behaviors correlated with variation in AVP levels, nor whether these differences in social behavior and AVP levels would persist after cross-fostered animals gained further experience as adults.

As well as influencing aggression, plasticity in the AVP system may influence other forms of social behavior, such as paternal behavior. AVP has been proposed to play a role in the control of paternal behavior in prairie voles (*Microtus ochrogaster*) because males showed decreased AVP-ir staining (Bamshad, Novak, & De Vries, 1993, 1994) and increased AVP messenger RNA (mRNA; Wang, Smith, Major, & De Vries, 1994; Wang, Liu, Young, & Insel, 2000) after cohabitation with females. These findings suggest that AVP may be important in paternal behavior because of increased production and release at a time when paternal behavior is initiated, although aggression also increases at this time. In addition, paternal meadow voles (*M. pennsylvanicus*) show less AVP receptor binding in the lateral septum than nonpaternal voles (Parker, Kinney, Phillips, & Lee, 2001). More direct evidence of a role for AVP in regulating paternal behavior is found in studies in which researchers examined the effects of intracerebroventricular injections of AVP and its antagonist. Injections of AVP increased paternal behavior in monogamous prairie voles (Wang, Ferris, & De Vries, 1994) and inhibited pup-directed aggression in addition to inducing paternal behavior in facultatively paternal meadow voles (Parker & Lee, 2001). In contrast, injections of an AVP antagonist decreased paternal behavior in prairie voles (Wang, Ferris, & De Vries, 1994) and meadow voles (Parker & Lee, 2001). When castration eliminated AVP-ir staining in the lateral septum of male prairie voles, it also decreased expression of paternal behavior (Wang & De Vries, 1993). Thus there are a number of lines of evidence that variation in AVP levels is associated with variation in paternal behavior and perhaps with the onset of paternal behavior.

In addition, by comparing the relationship between paternal care, AVP-ir staining, and AVP receptor distribution between species of *Peromyscus* mice, researchers found an opposite association between paternal behavior and these AVP measures than

Janet K. Bester-Meredith, Department of Psychology, University of Wisconsin—Madison; Catherine A. Marler, Department of Psychology and Department of Zoology, University of Wisconsin—Madison.

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Correspondence concerning this article should be addressed to either Janet K. Bester-Meredith or Catherine A. Marler, Department of Psychology, University of Wisconsin, 1202 West Johnson Street, Madison, Wisconsin 53706. E-mail: jkbester@students.wisc.edu or camarler@facstaff.wisc.edu

would be predicted by previous research in voles (Bester-Meredith, Young, & Marler, 1999). If anything, it appeared that aggression might better predict AVP-ir staining and receptor distribution than paternal care across a variety of rodent species. Therefore, it is possible that AVP contributes to the onset of parental care but may not be critical for the maintenance of paternal behavior.

To test the hypothesis that parental behavior and AVP are associated in mated *Peromyscus* mice in the current study, we cross-fostered mouse pups between two species with different social systems: (a) the California mouse, a monogamous species in which males provide extensive parental care toward offspring (Bester-Meredith & Marler, 2001; Bester-Meredith et al., 1999; Gubernick & Alberts, 1987, 1989; Trainor & Marler, 2001) and (b) the white-footed mouse, a promiscuous species in which males express little paternal behavior (Bester-Meredith & Marler, 2001; Bester-Meredith et al., 1999; Schug, Vessey, & Underwood, 1992). A similar methodology was used to increase paternal behavior in offspring by cross-fostering from the less parental meadow vole to the more parental prairie vole (McGuire, 1988). We worked with cross-fostered (mice fostered between species), in-fostered (mice fostered to parents of their biological species), and control animals (raised by their biological parents). All behavioral and AVP measurements described below were made on the pups raised by the foster parents (or controls) when they were tested as adults. This manipulation of the early postnatal environment allowed us to examine the relationship between paternal behavior of pups as adults and AVP-ir staining in the BNST, the medial amygdala (MA), and the SON. These are brain areas in which AVP has been associated with social behaviors including paternal care and aggression (e.g., Bester-Meredith & Marler, 2001; Bester-Meredith et al., 1999; Ferris et al., 1997; Wang, 1995). To provide a control, we also measured AVP-ir staining in the paraventricular nucleus of the hypothalamus (PVN), a brain area that is associated with stress and other nonreproductive functions (Ma, Lightman, & Aguilera, 1999; Wotjak et al., 1996).

Method

Subjects

Male white-footed and California mice were reared in a laboratory colony at the University of Wisconsin–Madison. The mice were housed under a 14:10-hr light–dark cycle with lights on at 0500 and received free access to Purina 5015 mouse chow and water. Room temperature was set at 25 °C. Animals were maintained in accordance with the recommendations of the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). The research presented here was described in Animal Research Protocol A-48-7400-L00221-3-01-98 and approved on February 26, 1998, by the University of Wisconsin Research Animal Resource Committee.

Cross-Fostering and In-Fostering

Methods have been described in detail in Bester-Meredith and Marler (2001). Briefly, within 36 hr of birth, entire litters from each species were separated from their parents, wiped clean with water-soaked cotton balls, and dipped in soiled bedding of their foster parents. Of these litters, 2 California mouse pups or 4 white-footed mouse pups from the same litter were exchanged to be raised by parents of the other species (cross-fostered) or to be raised by parents from their own species (in-fostered) that also

gave birth within the last 36 hours. In-fostering was used to control for any behavioral changes caused by being separated from biological parents and being raised by foster parents. Mice from the general colony provided additional controls because cross-fostering and in-fostering were not done simultaneously because of limitations in the number of individuals available for fostering at any one time. Colony control mice remained undisturbed with their birth parents and biological siblings and were housed in standard cages (white-footed mouse: 29.2 × 19.0 × 12.7 cm; California mouse: 48.3 × 26.7 × 15.6 cm) until separation from their parents. Fostered mouse pups were housed with their foster parents in clear Plexiglas observation cages until separation from their parents on Day 25 after birth. After weaning, cross-fostered, in-fostered, and control mice were housed in standard cages containing a total of 2 to 4 age-matched mice from the same species, sex, and treatment condition.

The numbers of siblings and the sex ratio in all litters were recorded at weaning for use in statistical analyses as covariates because control litters were not culled. For the males used in the study of AVP-ir staining, the number of siblings (excluding the male in the study) and the sex ratio of the litter (with an all-female litter having a sex ratio of 1 and an all-male litter having a sex ratio of 0) were as follows: control California mice: 1.75 ± 0.44 siblings, 0.31 ± 0.10 sex ratio; cross-fostered California mice: 0.86 ± 0.14 siblings, 0.14 ± 0.09 sex ratio; control white-footed mice: 2.29 ± 0.52 siblings, 0.25 ± 0.10 sex ratio; cross-fostered white-footed mice: 2.00 ± 0.37 siblings, 0.28 ± 0.09 sex ratio.

Paternal Behavior

Baseline levels of paternal behavior by fathers of cross-fostered offspring. We investigated whether paternal care varied between the two species when fathers were caring for biological or foster pups. Four different groups of mated pairs were used: 11 California mouse pairs from the general colony with their biological offspring, 17 California mouse pairs with cross-fostered white-footed mouse pups, 8 white-footed mouse pairs from the general colony with their biological white-footed mouse pups, and 19 white-footed mouse pairs with cross-fostered California mouse pups. Each pair was videotaped for 20 min at least 30 min after the onset of the dark cycle under illumination from a red light every other evening until weaning from Day 3 until Day 23 after the birth of pups. During each observation session, we recorded the amount of time that the male mouse in each pair spent huddling with pups, retrieving pups, grooming pups, and nest-building; we also recorded the amount of time the male spent inside of the nest during each observation session. *Huddling* was defined as the amount of time that the male spent in physical contact with a pup's body excluding the tail. *Retrievals* occurred when pups were carried in the father's mouth. *Grooming* was defined as when the father licked the body of the pup. *Nest-building* was defined as when the father manipulated nesting material by collecting shavings in his mouth and carrying them into the nest or when he used his nose to shape the shavings in the nest into a bowl-like shape (Xia & Millar, 1988). Because the boundaries of the nest were difficult to define, a father was recorded as being inside the nest when he was located in the small chamber or in the portion of the large chamber containing the bowl-like collection of shavings that constituted the nest.

Paternal behavior by cross-fostered offspring. We investigated the effects of cross-fostering on paternal behavior in a random subset of the cross-fostered pups described above as adult mice using males from nine litters each of white-footed mice raised by California mice, control white-footed mice, California mice raised by white-footed mice, control California mice, and in-fostered California mice. Only four litters of in-fostered white-footed mice were used because only 4 of 14 pairs of in-fostered white-footed mice bred successfully. Although this was a low rate of successful breeding in white-footed mice, it did not appear to affect the pattern of results, with all data being in the normal range for this species, and additional colony animals were used as controls (see Results). Paternal behavior data were obtained for 1 male per litter with the following

exceptions: An additional male sibling per litter was paired with a female, and the resulting data were averaged for all statistical analyses for two litters of cross-fostered white-footed mice, one litter of control white-footed mice, and one litter of in-fostered California mice, leading to the final litter sample sizes described above. The average ages of males at the birth of their first litter were not significantly different among species, among fostering groups (cross-fostered, in-fostered, or control), or among fostering groups when each species was treated independently (all p s > .23). On average, the first litter of pups was born when the male parent was 15 months of age, with a range of 11 to 22 months of age.

Within 36 hr after the birth of the first litter (Day 1), a pair of mice was placed into a clear Plexiglas observation cage as described above. As was also described above, each pair was videotaped for 20 min from Day 3 until Day 23, and paternal behaviors from each observation session were analyzed. For the present study, we sampled a subset of these taping sessions: Days 5, 9, 13, 17, and 21 after the birth of pups. We substituted the average of the measured behaviors of the male during the otherwise unused taping sessions that occurred 2 days prior to or after the intended taping session when the taping session was improperly recorded (13 out of 245 taping sessions). For example, if Day 13 was unusable, we substituted the average of Day 11 and Day 15.

Immunocytochemistry

We examined the effects of cross-fostering on AVP-ir staining in a subset of 32 sexually experienced males, obtained from the parental behavior study described above. Eight males that were on average 17 months old were taken from each of the following four groups: control white-footed mice, cross-fostered white-footed mice, control California mice, and cross-fostered California mice. Brains were assayed in eight sets of four (described below) such that each set included one brain from each of the four groups for both species. One animal from each group was eliminated from the image analysis of AVP-ir staining because of problems with the primary antibody during the first batch of immunocytochemistry. Tissue damage prevented measurement of the MA in 1 control California mouse and of the SON in 1 control California mouse and 1 cross-fostered white-footed mouse. Data for 2 cross-fostered white-footed mice and 2 control California mice were averaged because the animals were siblings, leading to a final sample size of 7 cross-fostered California mice, 6 control California mice (5 for measurements in the MA and SON), 7 control white-footed mice, and 6 cross-fostered white-footed mice (5 for measurements in the SON). Cross-fostered mice were compared with control mice instead of in-fostered mice because there was no significant difference in the time of year that the brains of cross-fostered and control mice were collected, $F(1, 24) = 0.38, p = .54$, whereas there was a difference between cross-fostered and in-fostered mice, $F(1, 23) = 9.96, p = .01$, and because parental behavior measurements revealed no behavioral differences between control and in-fostered mice (see Results). We collected behavioral data while each father was caring for a first litter of pups in order to correlate these behavioral data with AVP-ir staining measurements obtained after the birth of a second litter. These males had previously been exposed to aggression tests as sexually naive males at an average of 7 months of age (10 months prior to being killed) and as sexually experienced males at an average of 16 months of age (1 month prior to being killed). All males were decapitated between 1100 and 1300 on the 3rd day after the birth of their second litter of pups (Bamshad et al., 1994). To prevent disturbance of the animals, cages remained in the colony room until immediately prior to decapitation.

Brains were placed in 5% acrolein for 3.0 hr and fresh acrolein for an additional 2.5 hr. After being stored for 24 hr in a cryoprotectant solution (0.05 M phosphate buffer containing 0.9% NaCl, 30.0% sucrose, 30.0% ethylene glycol, and 1.0% polyvinylpyrrolidone), brains were frozen on dry ice and stored at 0 °C (Bester-Meredith & Marler, 2001; Bittman, Bartness, Goldman, & De Vries, 1991). Frozen brains were sectioned into 50- μ m

coronal sections with a vibratome set at a cutting angle of 17°. After sectioning, immunocytochemistry for AVP was performed on free-floating sections as described elsewhere (Bester-Meredith & Marler, 2001). Sections were stored overnight in Tris-NaCl and mounted onto slides, air dried, and coverslipped the following day. We previously tested the specificity of the antibody for AVP in the California mouse and white-footed mouse (Bester-Meredith et al., 1999). Using computerized image analysis software (NIH Image, Version 1.61; Rasband, 1996), we analyzed the density of AVP-ir staining in the BNST, MA, PVN, and SON (e.g., Bamshad et al., 1993, 1994; Wang, 1995; Wang & De Vries, 1993). Measurements of AVP-ir staining were not made in the lateral septum because coronal sections only revealed light AVP-ir staining (staining was less than 1%). 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, 1994; Bester-Meredith & Marler, 2001; Bester-Meredith et al., 1999). For each brain area, an observer who was unaware of the mouse's species and treatment condition identified the section with the highest amount of AVP-ir staining. The observer traced each brain area bilaterally on the section with the maximum percentage of staining and calculated the percentage of staining for each brain area. Additional measurements of cell size, cell optical density, and cell number were made on 10 cells on each side of the section with the maximum percentage of staining. If fewer than 10 cells were present, all were measured. Cell size was measured by tracing each cell and by calculating its surface area. Optical density was measured in these cells by comparing the optical density of each traced cell to a calibrated set of optical density standards. The total number of cells was also counted on the section with the maximum percentage of staining.

Statistical Analysis

All statistical analyses were conducted using the computer program Statistica (StatSoft, Tulsa, OK). For the study of species differences in paternal behavior, Mann-Whitney U tests were used to determine whether (a) the two species or (b) the parents of cross-fostered and colony control mice within each species differed in total time spent in each dependent variable (huddling with pups, retrieving pups, grooming pups, nest-building, and the amount of time spent outside of the nest). No composite scores were used in this analysis.

To reduce the number of statistical comparisons in the offspring of the parents described above, we formed a composite score of huddling, grooming pups, and the amount of time spent inside the nest because these variables were highly intercorrelated and occurred simultaneously (range of $r = .65$ to $.97$). This composite score was named the *HGI score* (an abbreviation for huddling, grooming, and inside the nest) and was calculated by averaging the Z scores of these intercorrelated variables. Averaging the Z scores to form the composite HGI scores allowed us to create an average of the variables in which relatively rare behaviors (such as grooming) and relatively common behaviors (such as huddling) both would contribute equally to the composite score. We tested whether offspring differed in the total duration of the three parental care dependent variables using a regression model with the month of pups' birth as a covariate that assumed a normal distribution for the HGI score and a Poisson distribution for retrievals and nest-building with corrections for overdispersion as needed.

We used analysis of covariance to examine whether the main effects of fostering group and species could predict the maximum percentage of AVP-ir staining, and we used multivariate analysis of covariance to analyze cell size, optical density, and cell number from the section with the maximum percentage of staining, with sibling sex ratio and assay date as covariates (Bester-Meredith & Marler, 2001). We examined the effects of cross-fostering within each species by using planned comparisons. We also tested whether the percentage of AVP-ir staining in any of the four brain areas correlated with any of the three measures of parental behavior using Pearson product-moment correlations. Data are presented as means plus or minus standard errors in the text and figures.

Results

Baseline Levels of Paternal Behavior by Fathers of Cross-Fostered Offspring

The two species showed different patterns of paternal care (see Figure 1). When data on parents of colony and cross-fostered mice were combined, male California mice spent significantly more time huddling ($U = 80.00, p < .0001$), grooming ($U = 2.00, p < .0001$), and retrieving pups ($U = 95.00, p < .0001$), as well as nest-building ($U = 120.50, p < .0001$), and they spent significantly less time out of the nest ($U = 93.00, p < .0001$) than white-footed mice ($n_1 = 28, n_2 = 27$). This pattern of paternal care, with California mice showing greater paternal care than white-footed mice, occurred even when mice were caring for unrelated foster offspring of the other species (all $ps > .17$) with one exception. When parenting cross-fostered mice, male Califor-

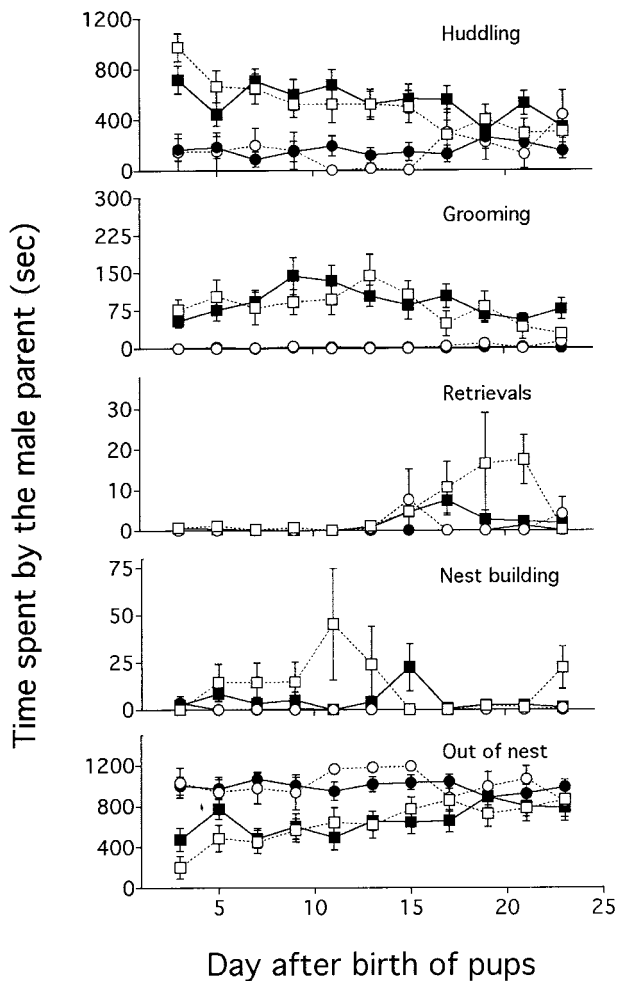


Figure 1. Paternal care differed between the colony white-footed mice (open circles, $n = 8$ pairs with their biological pups) and the colony California mice (open squares, $n = 13$ pairs with their biological pups). Caring for cross-fostered pups instead of biological pups altered the amount of time that male California mice (solid squares, $n = 20$) spent retrieving pups, but did not alter the behavior of male white-footed mice (solid circles, $n = 23$). Data are presented as means (\pm SE).

nia mice showed a marginally nonsignificant decrease in time spent retrieving pups (see Figure 2; $U = 52.50, p = .054$) that caused cross-fostered white-footed mouse pups to be retrieved at the same rate as control white-footed mouse pups.

Paternal Behavior by Cross-Fostered Offspring

Male California mice raised by the less parental white-footed mice displayed a decrease in one out of three measures of parental behavior, whereas male white-footed mice raised by the more parental California mice did not display any changes in parental behavior. Cross-fostered California mice showed a decrease in time spent retrieving pups (Wald statistic = 4.52, $p = .03$) and a nonsignificant trend toward a decrease in the number of pups retrieved (Wald statistic = 3.45, $p = .06$). Cross-fostering did not alter time spent retrieving pups or the number of pups retrieved in white-footed mice or in the control comparisons of in-fostered and control mice (all $ps > .9$). Therefore, being raised by white-footed mice caused California mice to decrease the amount of time spent retrieving their own pups, but being raised by California mice did not cause male white-footed mice to alter the degree to which they expressed this form of paternal behavior. There was no significant effect of cross-fostering or in-fostering on nest-building or the HGI score (see Table 1; all $ps > .10$). Because the smaller sample size of in-fostered white-footed mice may have reduced the power of our statistical analysis and there were no differences between in-fostered and control mice, we reanalyzed the data combining all in-fostered and control mice (increasing the sample size of the white-footed mouse control groups to 13). This additional analysis provided identical results in white-footed mice, with no effect of cross-fostering on time spent retrieving pups, number of pups retrieved, time spent nest-building, or the HGI score (all $ps > .4$).

Correlations Between AVP-ir Staining and Parental Behavior

Data on behavior and AVP-ir staining for the two species were combined because of the similarity in the patterns of changes and because the test of differences between correlations showed no statistically significant differences between species (all $ps > .33$), allowing us to use the larger sample size of the pooled sample (Cohen & Cohen, 1983). All four groups (control and cross-fostered animals of both species) were used in these analyses. The amount of parental behavior shown by males correlated significantly with the amount of AVP-ir staining in the BNST but not the other brain areas. The percentage of staining in the BNST correlated positively with the HGI score (see Figure 3), $r(24) = .58, p = .003$, and time spent retrieving pups, $r(24) = .41, p = .05$, but was not related to time spent nest-building, $r(24) = .17, p = .43$, suggesting that males that spent more time huddling, grooming, and in the nest (HGI score) or retrieving pups also showed the highest levels of AVP-ir staining. Because retrievals were relatively rare, we also analyzed whether males that retrieved pups differed in the percentage of staining in the BNST from males that did not retrieve pups. This analysis showed that males that retrieved pups showed higher levels of the percentage of staining in the BNST than males that did not retrieve pups (see Figure 4), $t(22) = -2.17, p = .04$. The maximum percentage of staining in the SON, PVN, and MA were unrelated to any of the parental

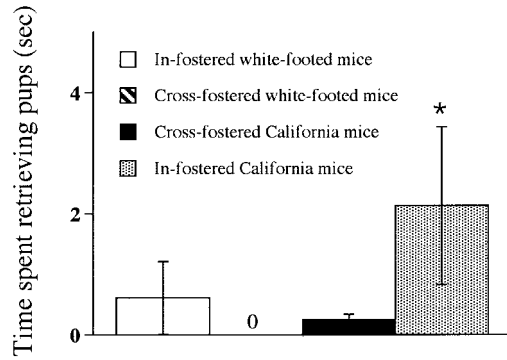


Figure 2. Average time spent retrieving pups by male in-fostered and cross-fostered white-footed and California mice. *n* = 9 per group for cross-fostered California mice, in-fostered California mice, and cross-fostered white-footed mice; *n* = 4 for in-fostered white-footed mice. Data are presented as means (\pm SE).

behavior measures (all *ps* > .11). To summarize, male mice that had high HGI scores or that had high retrieval durations also showed large amounts of AVP-ir staining in the BNST but not in the other brain areas that were examined. Nest-building behavior was not associated with any of the measures of AVP-ir staining.

Cross-Fostering and AVP-ir Staining

Despite the overall association between levels of paternal care and the amount of AVP-ir staining in the BNST and a change in retrievals in cross-fostered California mice, cross-fostering did not alter the density of AVP-ir staining in the section with the maximum percentage of staining or the cell measurements (cell number, cell area, cell optical density) in the BNST overall or in each species individually (see Figures 5 through 7; all *ps* > .10). Similarly, cross-fostering did not alter the density of AVP-ir staining in the section with the maximum percentage of staining or the cell measurements (cell number, cell area, cell optical density) in the SON, PVN, or MA overall or in each species individually (see Figures 6 and 7; all *ps* > .07).

Table 1
Paternal Behavior of Peromyscus Mice Reared by Biological (Control) or Foster Parents

Group	Retrieving pups	Nest-building	Components of the HGI score		
			Huddling	Grooming	In nest
White-footed mice					
Control	0.00 \pm 0.00	1.42 \pm 1.42	435.09 \pm 137.36	14.13 \pm 10.21	504.02 \pm 129.17
In-fostered	0.60 \pm 0.60	2.90 \pm 2.34	358.40 \pm 181.45	2.00 \pm 1.61	377.10 \pm 172.09
Cross-fostered	0.00 \pm 0.00	1.67 \pm 1.31	167.32 \pm 39.58	1.50 \pm 1.37	214.61 \pm 45.92
California mice					
Control	2.33 \pm 1.32	5.10 \pm 2.28	560.28 \pm 75.63	93.99 \pm 18.84	582.97 \pm 78.29
In-fostered	2.12 \pm 1.34*	5.79 \pm 3.15	503.89 \pm 86.06	78.20 \pm 14.75	604.07 \pm 110.08
Cross-fostered	0.27 \pm 0.15*	5.49 \pm 4.62	467.33 \pm 92.32	71.09 \pm 12.69	506.99 \pm 98.94

Note. All data are presented in seconds. The HGI score (the mean of Z scores of time spent huddling, grooming, and in the nest) is divided into its three component variables. Data are presented as raw means (\pm SE). Comparisons were made between cross-fostered and in-fostered mice and between in-fostered and control mice. Asterisks indicate values significantly different from each other at *p* < .05.

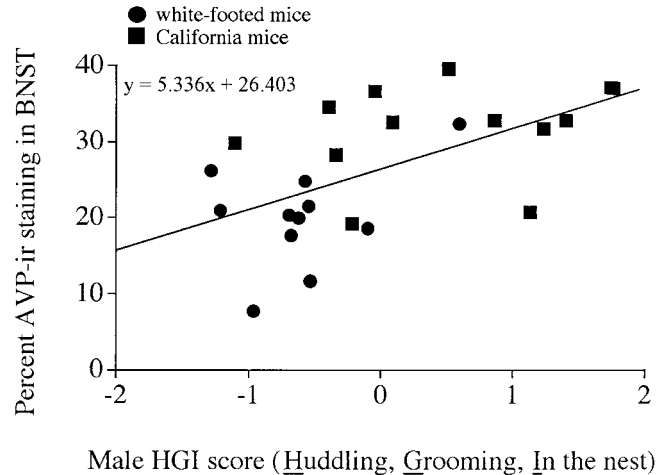


Figure 3. The statistically significant correlation between the maximum percentage of arginine vasopressin immunoreactive (AVP-ir) staining in the bed nucleus of the stria terminalis (BNST) and the HGI score (a composite score of the average time spent huddling, grooming, and in the nest) by in-fostered and cross-fostered white-footed and California mice. *n* = 24, *R*² = .34, *p* = .003.

Discussion

Paternal retrievals may play an important role in the cross-generational effects of paternal behavior on offspring social behavior. In the present study, we found that California mouse offspring exposed to fewer paternal retrievals (because they were raised by white-footed mouse parents) also displayed a decrease in pup retrievals when raising their own offspring. Therefore, changes in pup retrieval behavior in males may be transferred across generations. AVP may be a physiological mechanism important in this nongenomic transfer of paternal behavior in California mice because AVP-ir staining in the BNST was positively associated with retrievals, as well as with the HGI score consisting of huddling, grooming, and time spent inside the nest when the species and fostering groups were combined. Thus our current

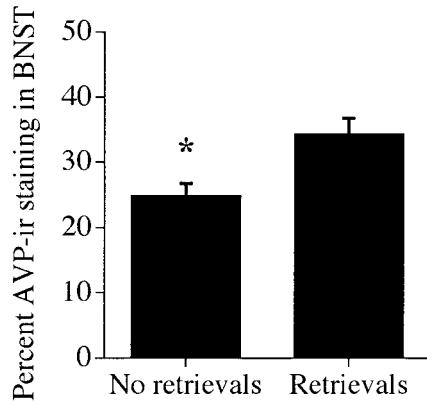


Figure 4. The maximum percentage of arginine vasopressin immunoreactive (AVP-ir) staining in the bed nucleus of the stria terminalis (BNST) of males that did not retrieve pups ($n = 20$) and males that did retrieve pups ($n = 4$). Data are presented as means (\pm SE). * $p < .05$.

study indicates a potential link between paternal behavior and AVP that may be transferred across generations.

AVP and Paternal Care

In the present study, AVP-ir staining in the BNST correlated with male retrievals and the HGI score when both species were combined. In addition, the two species did not differ statistically in the correlations between the behavioral and AVP measurements (Cohen & Cohen, 1983). These findings add to the body of evidence suggesting that AVP may facilitate paternal behavior in monogamous rodent species such as the prairie vole (Bamshad et al., 1993, 1994; Wang & De Vries, 1993; Wang et al., 2000; Wang, Ferris, & De Vries, 1994; Wang, Smith, et al., 1994; but see Lonstein & De Vries, 1999) and in facultatively paternal species such as the meadow vole (Parker et al., 2001; Parker & Lee, 2001). To our knowledge, this study is the first to demonstrate a correlation between specific paternal behaviors (the HGI score and paternal retrievals) and AVP in the BNST, a brain area associated with paternal behavior (e.g., Kirkpatrick, Kim, & Insel, 1994). Not

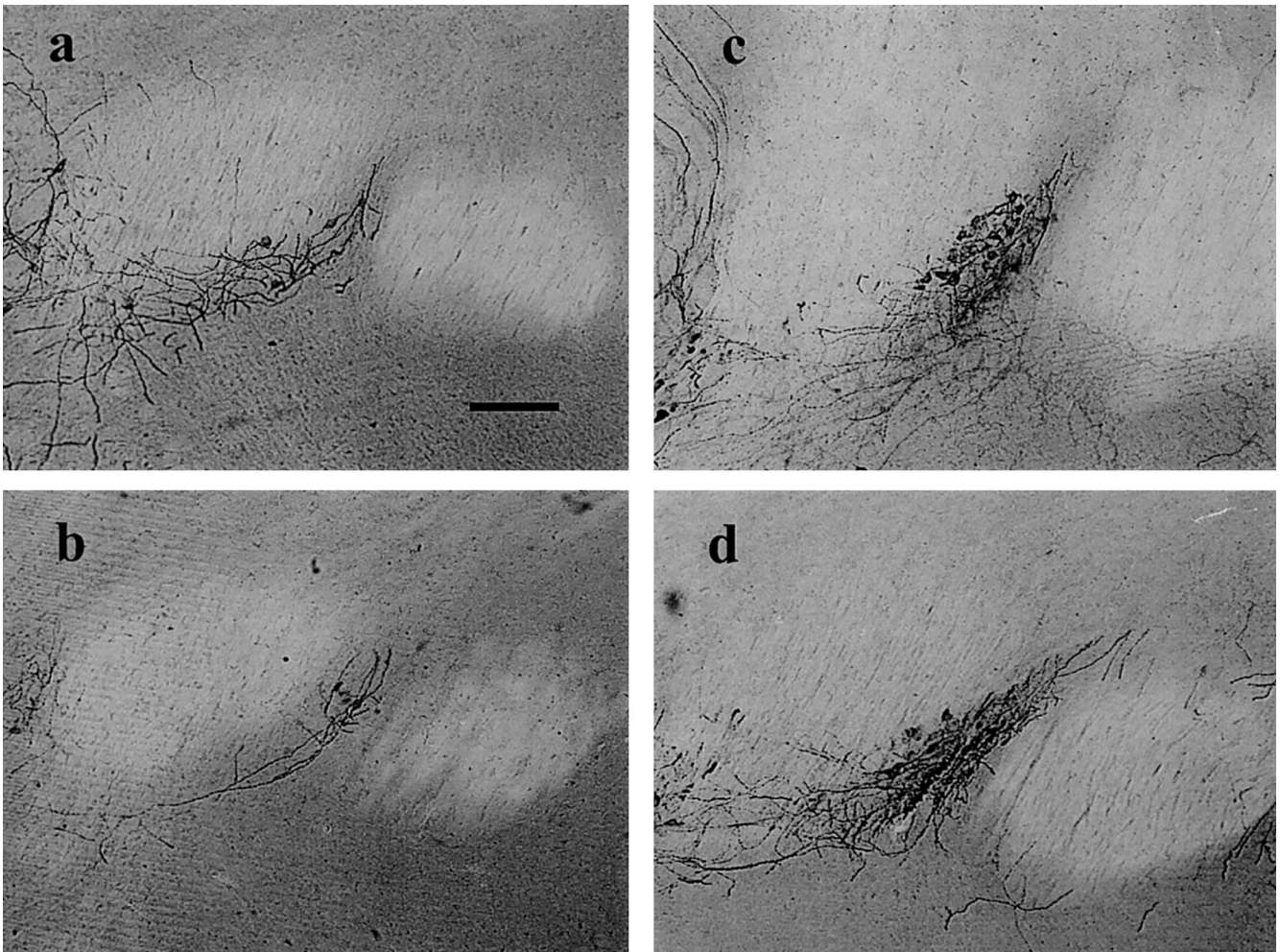


Figure 5. Representative photomicrographs of arginine vasopressin immunoreactive staining in the bed nucleus of the stria terminalis comparing sexually experienced male control white-footed mice (a), cross-fostered white-footed mice (b), control California mice (c), and cross-fostered California mice (d). Bar represents 100 μ m.

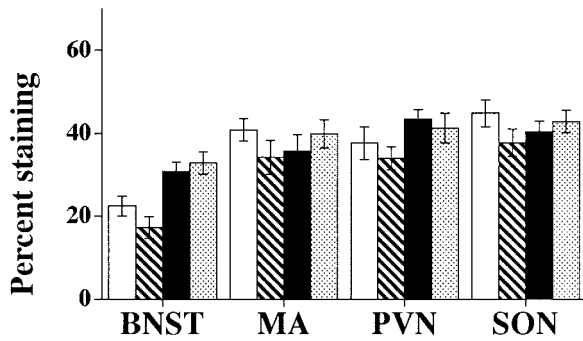


Figure 6. The maximum percentage of staining in the bed nucleus of the stria terminalis (BNST), medial amygdala (MA), paraventricular nucleus (PVN), and supraoptic nucleus (SON) of control and cross-fostered white-footed and California mice. $n = 7$ for cross-fostered California mice (solid bars) and control white-footed mice (open bars); $n = 6$ for control California mice (stippled bars) and cross-fostered white-footed mice (hatched bars). Data are presented as means ($\pm SE$). There were no statistically significant differences between groups.

only does this finding indicate that AVP may play an important role in the maintenance of paternal behavior in *Peromyscus* mice, it also suggests that the decreases in paternal behavior seen in California mice may be related to changes in the organization of AVP pathways. However, AVP manipulations will be necessary to verify this link between AVP and paternal behavior in *Peromyscus* mice.

Cross-Fostering and AVP

Although we found that the effect of cross-fostering on retrievals was sustained through the experience of mating, we did not find the same for AVP-ir staining in the BNST. We previously found lower AVP-ir staining in the BNST of cross-fostered male California mice compared with the more aggressive control California mice when tested while sexually naive (Bester-Meredith & Marler, 2001), but not in the comparisons of sexually experienced males in the present study. Two reasons may explain this finding. First, males in the present study were older (17 months) than those in the previous study (7 months) and had more social experience. In this study, males may have mated with their partners during the post-partum estrus, leading to changes in AVP levels (e.g., Bamshad et al., 1994). A second possible explanation for the lack of an effect of cross-fostering on AVP-ir staining is the use of only a subset of animals from the entire study in the AVP portion of the present study. Therefore, it is possible that an effect of cross-fostering on AVP-ir staining would have been found in these mice if they had been tested prior to mating or if a larger sample size had been used.

Cross-Generational Changes in Behavior and Neurochemistry

The finding that cross-fostering altered retrievals in California mice but not white-footed mice may be explained by three factors related to behavioral plasticity and cross-fostering. First, white-footed mice may never have been exposed to enough variation in paternal retrieval behavior. In the present study, despite species differences in paternal behavior, white-footed mice were retrieved

by males at the same rate whether they were raised by white-footed mice or California mice because male California mice decreased the time that they spent retrieving pups when raising white-footed mouse pups. A second possible explanation for the finding that cross-fostering altered retrievals in California mice but not white-footed mice is that California mice and white-footed mice may have differed in how strongly the patterns of paternal care were transmitted socially between generations from foster parents to their cross-fostered offspring. Cross-fostering did not alter paternal behavior in the white-footed mouse in our study or in a study of white-footed mice and the more parental deer mouse (*P. maniculatus*; Hawkins & Cranford, 1992). It is possible that California mice show more plasticity in the expression of paternal care, similar to polygamous meadow voles that spent more time huddling and in the nest after being raised by the more paternal prairie vole (McGuire & Novak, 1987). Finally, it is also possible that the quality of retrieval behavior may have been altered in a way that was not detectable using the methods of the current study. Regardless of the underlying cause of changes in retrieval behavior in California mice, the findings of the present study suggest the existence of a nongenetic behavioral mechanism that allows be-

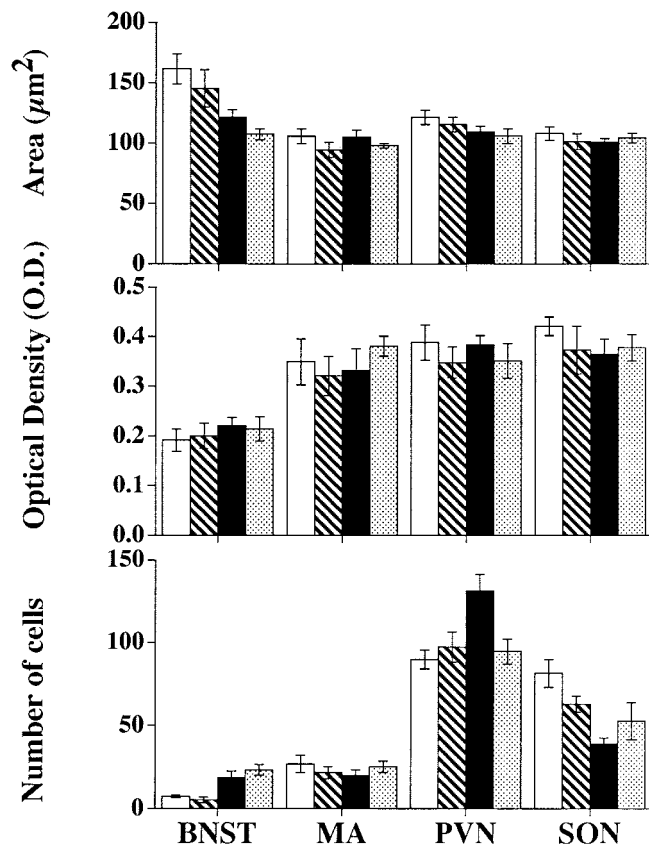


Figure 7. Average area, optical density, and number of neurons in the bed nucleus of the stria terminalis (BNST), medial amygdala (MA), paraventricular nucleus (PVN), and supraoptic nucleus (SON) of control and cross-fostered white-footed and California mice. $n = 7$ for cross-fostered California mice (solid bars) and control white-footed mice (open bars); $n = 6$ for control California mice (stippled bars) and cross-fostered white-footed mice (hatched bars). Data are presented as means ($\pm SE$).

havioral traits to be passed on to multiple future generations by altering an animal's early developmental experience.

The present study also adds to a potential mechanistic explanation for cross-generational changes in aggression. Using fostering paradigms in *Peromyscus*, we previously identified a positive association between paternal retrieval behavior and offspring resident-intruder aggression in unrelated individuals (Bester-Meredith & Marler, 2002) and found that a decrease in resident-intruder aggression was associated with a decrease in AVP-ir staining in the BNST (Bester-Meredith & Marler, 2001). In addition, we have unpublished data demonstrating a significant positive correlation between paternal retrievals and resident-intruder aggression in males and between maternal retrievals and resident-intruder aggression in females (Bester-Meredith & Marler, 1996, 2002). In the present study, we found a significant association between retrieval behavior and AVP-ir staining in the BNST. Thus, we hypothesize that lower AVP may be associated with lowered resident-intruder aggression and pup retrievals and that this decrease in pup retrievals may decrease resident-intruder aggression in future generations. Further studies may therefore reveal a suite of traits—retrievals, resident-intruder aggression, and AVP—that link generations through nongenomic mechanisms.

Researchers working with female rats have found suites of neurochemical and behavioral traits linking generations through maternal effects (e.g., Francis, Diorio, Liu, & Meaney, 1999; Gonzales, Lovic, Ward, Wainwright, & Fleming, 2001; Lovic, Gonzalez, & Fleming, 2001). These maternal deprivation and cross-fostering studies have identified licking and grooming behavior as key behaviors linking maternal behavior through generations. If mothers display a higher amount of licking and grooming behavior toward the pups, then the pups express more licking and grooming behavior once they became maternal as adults. In addition, individuals experiencing a higher level of maternal licking and grooming behavior also display less fearful behavior and show a weaker hypothalamic–pituitary–adrenal response to stress (Caldji et al., 1998; Liu et al., 1997). Our current results combined with our previous results showing an effect of fathers on offspring aggressiveness suggest that both fathers and mothers can potentially have significant effects on their offspring through nongenomic mechanisms.

In conclusion, the results of this study suggest that the rearing environment shapes aspects of adult paternal behavior in California mice. In combination with our previous work showing that cross-fostering decreased aggressiveness in California mice (Bester-Meredith & Marler, 2001) and that the amount of time that fathers spent retrieving pups predicted their future aggressiveness (Bester-Meredith & Marler, 2002), these results suggest that the social environment during development is critical for the formation of species-typical behavioral and neurochemical traits. This study also is among the first to demonstrate a role for early experience in shaping adult paternal behavior (see also McGuire & Novak, 1987) and identifies AVP as a possible underlying biological mechanism for early experience in shaping adult paternal behavior. Furthermore, because paternal retrievals of pups are associated with offspring aggressiveness (Bester-Meredith & Marler, 2002), the plasticity in pup retrievals demonstrated in this study may serve as a behavioral mechanism by which aggressive behavior is transferred between generations.

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