

Vasopressin and Aggression in Cross-Fostered California Mice (*Peromyscus californicus*) and White-Footed Mice (*Peromyscus leucopus*)

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To examine how developmental experiences alter neural pathways associated with adult social behavior, we cross-fostered pups between the more aggressive and monogamous California mouse (*Peromyscus californicus*) and the less aggressive and polygamous white-footed mouse (*P. leucopus*). Cross-fostered males became more like their foster parents when tested as adults. Male white-footed mice became more aggressive only in an aggression test in a neutral arena, whereas the territorial California mice became less aggressive in resident-intruder aggression test, as measured by attack latency. Only the species that displayed a change in resident-intruder aggression showed a change in arginine vasopressin (AVP) levels: cross-fostered California mice had significantly lower levels of AVP-immunoreactive (AVP-ir) staining than controls in the bed nucleus of the stria terminalis (BNST) and the supraoptic nucleus (SON) and a nonsignificant trend toward lower levels in the medial amygdala (MA). Neither species showed changes in AVP-ir staining in a control area, the paraventricular nucleus (PVN). The changes in AVP-ir staining in the BNST and SON may not be caused by stress because cross-fostering was not associated with changes in adult plasma concentrations of two steroid hormones, corticosterone and testosterone, that have been associated with stress-related alterations in AVP pathways. These results suggest that manipulating the early parental environment can directly alter both a neurotransmitter system and species-typical patterns of social behavior, but that these effects may vary between species and under different social contexts. © 2001 Academic Press

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A large body of work indicates a critical role of environmental conditions and experience in the development of the central nervous system and various behavioral processes. Lacking in this work has been

the systematic examination of the impact of the postnatal environment on neural pathways associated with adult social behaviors such as aggression. Although adult stress responsiveness is clearly affected by maternal behaviors like pup grooming (Liu, Diorio, Tannenbaum, Caldji, Francis, Freedman, Sharma, Pearson, Plotsky, and Meaney, 1997), it is unknown how the postnatal environment alters the development of these brain pathways more directly associated with social behavior than stress responsiveness. While recent attention has focused on the association between neurotransmitters like vasopressin (AVP) and adult male social behavior (reviewed in De Vries and Villalba, 1997; De Vries and Miller, 1998), the role of the early rearing environment in shaping this association in adults has received less consideration.

For example, studies involving administration of AVP and antagonists demonstrate a positive relationship between neurotransmission and aggression and/or dominance behaviors in prairie voles (Winslow, Hastings, Carter, Harbaugh, and Insel, 1993), golden hamsters (Ferris, Melloni, Koppel, Perry, Fuller, and Delville, 1997), and Syrian hamsters (Bamshad and Albers, 1996). In humans, aggressiveness correlates with levels of AVP in cerebrospinal fluid (Coccaro, Kavoussi, Hauger, Cooper, and Ferris, 1998). Differences in AVP-immunoreactive (AVP-ir) staining also are associated with differences in attack latency between species of *Peromyscus* mice (Bester-Meredith, Young, and Marler, 1999) and within different strains of house mice (Compaan, Koolhaas, Bujis, Pool, de Ruiter, and van Oortmerssen, 1992; Compaan, Bujis, Pool, De Ruiter, and Koolhaas, 1993).

Despite this association between AVP and adult attack latency, it remains unclear how the early envi-

ronment can alter both social behavior and this neuropeptide system. Limited evidence suggests that the social environment might influence both aggression and neural function, including AVP. For example, in golden hamsters social subjugation during puberty has been linked to short-term changes in aggression, AVP levels, and serotonin-immunoreactive fiber density (Delville, Melloni, and Ferris, 1998). However, these observations do not address the extent to which the early postnatal environment produces long-term effects that shape adult social behavior and AVP levels or the degree to which AVP participates in early experience-induced changes in social behavior.

To better test the hypothesis that developmental experiences alter neural pathways associated with adult social behavior, we cross-fostered mouse pups between two species with different social systems: (1) the California mouse (*Peromyscus californicus*), a species in which males are highly aggressive and provide extensive parental care toward offspring (Gubernick and Alberts, 1987; Bester-Meredith *et al.*, 1999; Trainor and Marler, in press; Oyegbile and Marler, unpublished data; Burg, Martin, and Marler, unpublished data), and (2) the male white-footed mouse (*P. leucopus*), a species in which males show less aggression and parental care (Schug, Vessey, and Underwood, 1992; Bester-Meredith *et al.*, 1999; Oyegbile and Marler, unpublished data; Burg, Martin, and Marler, unpublished data). Recent studies have demonstrated influences of cross-fostering on dominance and aggression (Westman, 1990; Carrier, Roubertoux, and Pastoret, 1991; Drummond and Canales, 1998; but see Hood and Cairns, 1989; Benus and Röndigs, 1997). Cross-fostering between these two *Peromyscus* species allowed us to examine influences of the early postnatal environment on adult attack latency and AVP-ir staining in the bed nucleus of the stria terminalis (BNST) and medial amygdala (MA), two brain areas in which AVP has been associated with social behavior (i.e., Wang, 1995). This study also examined the extent to which changes in attack latency are correlated with changes in AVP-ir staining in these cross-fostered mice and determined whether cross-fostering altered corticosterone (B) and testosterone (T) levels, because these hormones have been linked with this AVP pathway (van Leeuwen, Caffé, and De Vries, 1985; Watters, Wilkinson, and Dorsa, 1996). Together, the results of this study indicate a prominent influence of the parental environment on adult social behavior and on central and peripheral mechanisms.

MATERIALS AND METHODS

Subjects

We used 92 male white-footed mice and 92 male California mice reared in a laboratory colony at the University of Wisconsin, Madison, in two studies. The animals were kept under a 14L:10D light cycle with lights on at 05:00 and received free access to Purina 5015 mouse chow and water. Room temperature was set at 25°C. After weaning, animals were housed in standard cages (white-footed mouse, 29.2 × 19.0 × 12.7 cm; California mouse, 48.3 × 26.7 × 15.6 cm). During the observation period from birth until weaning, animals were housed in clear Plexiglas observation chambers that were divided into three chambers by Plexiglas partitions. Two small chambers (22 × 14.5 × 30 cm each) were separated by a removable, clear Plexiglas partition, while a third, larger chamber (30 × 29 × 30 cm) could be entered from either small chamber and contained a running wheel, food, and a water bottle. Animals were maintained in accordance with the recommendations of the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.

Cross-Fostering and In-Fostering

Within 36 h of birth, entire litters of pups from each species were separated from their parents, wiped clean with water-soaked cotton balls, and dipped in litter containing urine and feces of their foster parents. Two California mouse pups or four white-footed mouse pups from the same litter were then 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 had given birth within the last 36 h. In-fostering was used to control for any changes in behavior due to separation from biological parents and being raised by foster parents. Litter size was standardized for the two fostered groups in each species to minimize potential variance in attack latency due to the effects of siblings. Because of the difference in litter size and body size, when standardizing litter sizes to minimize variation within each species, California mouse litters were culled to two pups and white-footed mouse litters were culled to four pups before fostering. Fostered mouse pups were housed with their foster parents in the clear Plexiglas observation cages until separation from their parents on day 25 after birth. Because cross-fostering and in-fostering were not done simultaneously due to limitations in the number of individuals available for fos-

tering at any one time, control animals from the general colony were used in all comparisons. Control animals remained undisturbed with their birth parents and biological siblings and were housed in standard cages until separation from their parents. Similar to the cages of cross-fostered and in-fostered animals, the cages of control animals were changed weekly. Otherwise, control animals were left undisturbed until weaning. The numbers of siblings and the sex ratio in all litters were recorded at weaning for use in all later statistical analyses because control litters were not culled. After weaning, cross-fostered, in-fostered, and control animals from both species were housed in standard cages containing groups of two to four animals from the same species and treatment condition. Therefore, the only difference within each species was the fostering manipulation.

The first study investigated the effects of cross-fostering on adult male–male attack latency and involved 24 cross-fostered white-footed mice from 14 litters, 31 control white-footed mice from 13 litters, 14 cross-fostered California mice from 12 litters, and 39 control California mice from 25 litters. To control for the process of fostering, 8 in-fostered white-footed mice from 7 litters, 13 control white-footed mice from 10 litters, 13 in-fostered California mice from 11 litters, and 16 control California mice from 12 litters also were tested to determine the effects of in-fostering on adult male–male attack latency. On average, animals were tested in aggression tests at 7 months of age. In-fostered and cross-fostered animals were not compared directly because most of the tests on these two groups were not conducted on the same testing dates and these species show seasonal variation in attack latency in the laboratory (Bester-Meredith and Marler, unpublished data). Instead, cross-fostered and in-fostered animals were compared to control animals tested on the same testing dates. Because of overlap between the test dates of the cross-fostered and in-fostered mice and our efforts to minimize the number of animals tested, data from 6 California mice from 4 litters are reported as controls for both cross-fostered and in-fostered mice. The average weights of males that were tested in aggression tests were the following (siblings are averaged together): cross-fostered California mice, 41.99 g; control California mice, 46.26 g; in-fostered California mice, 41.49 g; cross-fostered white-footed mice, 22.46 g; control white-footed mice, 25.09 g; and in-fostered white-footed mice, 22.11 g.

The second study, which examined the effects of cross-fostering on AVP-ir staining and corticosterone and testosterone concentrations in sexually naive

males, used eight males from each group (control white-footed mice, cross-fostered white-footed mice, control California mice, cross-fostered California mice) that were an average of 7 months old. One animal from each group was eliminated from the analysis of AVP-ir staining and hormone concentrations due to technical problems with the third batch of immunocytochemistry. Data for two control white-footed mice were averaged because the animals were siblings.

Aggressive Behavior

Each mouse was tested as a resident in a resident–intruder test, as an intruder in a resident–intruder test, and as a participant in a test of aggression in a neutral arena. The order of these three tests was randomized to allow all possible combinations of test orders. A lack of order effects on attack latency is discussed under Results. All opponents were unfamiliar, male nonsiblings from the same species and treatment group (cross-fostered, in-fostered, or control). Only one strain of mice was used in each species. The attack latencies for each male when tested in a neutral arena and as a resident in the resident–intruder test were used in the statistical analyses. All tests were conducted in the clear Plexiglas observation chambers described above. The removable partition was not used during the resident–intruder test. For the resident–intruder test, the resident was placed in the Plexiglas observation cage 48–60 h prior to the experiment. At the beginning of each resident–intruder test, an intruder was placed into the smaller chamber of the observation cage. During the test of aggression in a neutral arena, both animals were placed simultaneously in separate, small compartments of an unfamiliar observation cage immediately prior to the test.

All tests were terminated as soon as an attack occurred to prevent further injury to the animals. Attack latency was defined as the time from the onset of the aggression test until the focal male lunged and bit its opponent or until the two males wrestled by locking together in ventral–ventral contact and rolling, biting, and kicking each other. If no attack occurred, mice were separated after 10 min. All trials were videotaped for later measurement of attack latency. Attack latency is negatively correlated with other measures of aggression in *Peromyscus* mice, including the probability of winning the encounter, the frequency of biting behavior, and the frequency of chasing behavior (Oyegbile and Marler, unpublished data), and has been used as the main measure of aggression in stud-

ies examining the association between aggression and vasopressin in other species of mice (i.e., Compaan *et al.*, 1992, 1993). The age, weight, anogenital distance, number of sisters, number of brothers, and number of cage mates also were recorded for each individual tested.

Immunocytochemistry

Brains were assayed in eight sets of four such that each set included one sexually naïve male that had not been tested in aggression or other behavioral tests from each of the following groups: cross-fostered California mice, control California mice, cross-fostered white-footed mice, and control white-footed mice. Brains from each set of four animals were collected so that the two brains from each species were collected on the same day, with the exception of one set of animals that were collected within 19 days of one another. To minimize the number of animals used in this study and because aggression testing revealed no behavioral differences, we did not compare AVP-ir staining between in-fostered mice and control mice. Cross-fostered and control animals were sacrificed between 11:00 AM and 1:00 PM by decapitation to harvest the brains and trunk blood was immediately collected for hormone assays (described below). To prevent disturbance of the animals, cages were left in the colony room until immediately prior to sacrifice and only one animal was removed per cage. Less than 30 s typically elapsed between opening of the cage and decapitation. Trunk blood was collected immediately after decapitation from the body cavity. Tubes were spun in a centrifuge for 10 min before plasma was removed from the tube using a Hamilton syringe, placed into a clean, labeled centrifuge tube, and frozen. Plasma samples were stored at -80°C before analysis of steroid hormones.

Brains were placed in 5% acrolein for 3 h and fresh acrolein for an additional 2.5 h. After being stored for 24 h in a cryoprotectant solution (0.05 M phosphate buffer containing 0.9% NaCl, 30% sucrose, 30% ethylene glycol, and 1% polyvinylpyrrolidone), brains were frozen on dry ice and stored at 0°C (Bittman, Bartness, Goldman, and De Vries, 1991).

Frozen brains were sectioned into 50- μm coronal sections with a vibratome set at a cutting angle of 17° . After sectioning, free floating sections were placed in 0.5% NaBH_4 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 overnight in 2% goat serum. After 12–18 h, sections were transferred into

the following solutions: (1) Tris 2% goat for 15 min; (2) 3% glutaraldehyde in phosphate buffer for 30 min; (3) 0.3% H_2O_2 in MeOH for 30 min; (4) Tris 2% goat for 15 min (twice); a 1:5000 solution of rabbit anti-vasopressin (ICN Immunobiologicals) in 2% goat serum at 37°C for 90 min; (5) Tris 2% goat for 15 min; (6) goat anti-rabbit IgG 1:150 in Tris 2% at 37°C for 45 min; (7) Tris 2% goat for 15 min; (8) rabbit PAP 1:300 in Tris 2% for 45 min; (9) Tris 2% goat for 15 min; (10) goat anti-rabbit IgG 1:150 in Tris 2% at 37°C for 45 min; (11) Tris 2% goat for 15 min; (12) rabbit PAP 1:300 in Tris 2% for 45 min; (13) Tris 2% goat for 15 min; (14) Tris-NaCl for 10 min; (15) DAB (20 ml Tris-NaCl, 0.01 g of diaminobenzidinetetrahydrochloride, 20 μl of H_2O_2 for 7 min; and (16) Tris-NaCl for 10 min (twice) (modified from Bester-Meredith *et al.*, 1999, to allow incubations at 37°C , similar to Delville *et al.*, 1998). 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 the white-footed mouse (Bester-Meredith *et al.*, 1999).

The density of AVP-ir staining in the BNST, MA, PVN, and SON was analyzed using computerized image analysis software, NIH Image 1.61. The density of cells and fibers was expressed as the total area covered by AVP-ir cells and fibers within a sampling area (Bamshad, Novak, and De Vries, 1993, 1994; Bester-Meredith *et al.*, 1999). For each brain area an observer who was blind to the subject's species and treatment condition identified the section with the highest amount of AVP-ir staining. The observer traced each brain area 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 the section with the maximum percentage of staining for the three brain areas where we measured species or cross-fostering differences in AVP-ir staining. Cell size was measured by tracing cells and calculating the surface area of each cell with NIH Image 1.61. Optical density was measured in these cells by comparing the optical density of each traced cell to a calibrated set of optical density standards in NIH Image 1.61. The total number of cells also was counted on the section with the maximum percentage of staining. A maximum of 10 cells per brain was measured on each MA and SON section. Because fewer cells were present in the BNST, 5 cells per brain were measured.

Testosterone and Corticosterone Measurements

Hormone assays were completed at the Wisconsin Regional Primate Research Center Assay Laboratory. As described above, plasma samples were collected from animals that had not been tested previously in any behavioral tests when they were sacrificed for immunocytochemical analysis of AVP-ir staining. Plasma samples were extracted in ethyl ether and steroid hormones were separated using celite chromatography using the System I technique (Abraham, Buster, Lucas, Corrales, and Teller, 1972). T was eluted with 4.0 ml of 20% ethyl acetate in isoctane (EA/ISO), whereas B was eluted with 4.0 ml of 50% EA/ISO. To estimate procedural loss, external recoveries of tritiated testosterone (T) (2500 cpm $^3\text{H-T}$) were run in triplicate and internal recoveries of corticosterone (B) (2500 cpm $^3\text{H-B}$) were used.

T was measured using an enzyme immunoassay (EIA) procedure modified by Ginther, Ziegler, and Snowdon (in press) from Munro and Stabenfeldt (1984). Briefly, microtiter plates (Nunc-Immuno Plate Maxisorb F96 certified, VWR Scientific, Chicago, IL) were coated with T antibody (R156, University of California-Davis diluted to 1:35,000) and with coating buffer. Column separated T fractions and standards (0.5–100 pg, $n = 8$, Sigma Diagnostics, Inc., St. Louis, MO) were assayed on microtiter plates. Absorbance was read at 420 nm. Data reductions (log-logit transformation) were analyzed by weighted least-squares regression analysis and reported as ng/ml of plasma. When assay concentrations for serial dilutions of T-spiked California mouse plasma pool (100–0.8 μl , $n = 8$) and the T-spiked white-footed mouse plasma pool (100–0.8 μl , $n = 8$) were compared with T standards, computed regression lines did not differ in slope ($P > 0.05$). Accuracy measured at each standard curve point (0.5–100 pg, 60 μl plasma, $n = 8$) was $97.12 \pm 1.61\%$ for the California mouse and $102.94 \pm 1.24\%$ for the white-footed mouse. All samples were measured in one assay. Quality control pools were assayed in duplicate on each microtiter plate. High (1:4) and low (1:29) concentration quality control pools were assayed on each plate and the intra-assay coefficients of variation were 7.6% (high) and 5.4% (low).

B was measured using a radioimmunoassay described by Moore (1986) and Marler and Ryan (1996) using a B antibody (3-Oxime-BSA, Endocrine Sciences Products, Calabasas Hills, CA, final dilution 1:50,000). After an overnight incubation at 20°C, dextran-coated charcoal was used to separate bound and free counts and the supernatant was counted on a scintillation

counter. When assay concentrations for serial dilutions of the B-spiked California mouse plasma pool (1000–7.8 pg, $n = 8$) and the B-spiked white-footed mouse plasma pool (1000–7.8 pg, $n = 8$) were compared with B standards, computed regression lines did not differ in slope ($P > 0.05$). Accuracy measured at each standard curve point (5–2000 pg, 20 μl plasma, $n = 10$) was $108.31 \pm 4.98\%$ for the California mouse and $97.73 \pm 2.49\%$ for the white-footed mouse. All samples were measured in one assay along with high (1:4) and low (1:29) concentration quality control pools. Quality control pools were assayed in duplicate. The intra-assay coefficient of variation was 2.8% for the low concentration plasma pool and 12.4% for the high concentration plasma pool.

Statistical Analysis

In both studies, we averaged data from individuals from the same biological parents to minimize the effects of parentage on the three dependent measures: attack latency, percentage of AVP-ir staining, and hormone concentrations. We used analysis of variance (ANOVA) to examine whether the main effects of fostering group and species and/or the interactions between the effects of species and group could predict each dependent measure and used multivariate analysis of variance (MANOVA) to analyze cell size, optical density, and cell number from the section with the maximum percentage of staining. Within each species, we used planned comparisons to examine the effects of cross-fostering on each dependent measure.

Using Pearson product-moment correlations, we correlated hormone concentrations with the maximum percentage of staining in the BNST, MA, SON, and PVN for each species. Multiple regression was used to determine whether hormone concentrations correlated with the multiple cell measures from the section with the maximum percentage of staining described above. We also correlated the variables recorded during the course of the experiment with the dependent measures and ran ANCOVA using the significant variables as a covariate. In the study of AVP-ir staining, sibling sex ratio and assay date were used as covariates. Assay date was used to control for variation between assays. Sex ratio of the litter in which the animal was raised was used as a covariate because sibling sex ratio has been shown to be an important predictor of AVP-ir staining (Smythe and Marler, unpublished data) and sex ratios could not be standardized because the sex of pups could not be determined at birth.

Data are presented as means \pm standard errors in the text and figures. However, when a covariate was used, the adjusted means \pm standard errors for each group are presented. For the study of hormone concentrations, T and B concentrations were log-transformed prior to use in the statistical analyses because they were not normally distributed and are presented in the text as back-transformed means and in the graphs as log-transformed means.

In the study of aggressive behavior, only the controls tested at the same time as the experimental animals were included in the same ANOVA. Because animals were not weighed prior to the aggression tests, attack latencies from aggression tests were excluded when the differences in the weights of the opponents were greater than 20 g. In the first set of tests, by excluding data from trials where males fought an opponent that weighed 20 g more or less than the focal animal, we eliminated weight differences that were more than 2 SD from the mean.

RESULTS

Attack Latency

Cross-fostering produced individuals that more closely resembled their foster parents in aggression test attack latencies. However, the two species showed different responses to cross-fostering depending on the testing paradigm. In the resident-intruder test, there was a significant interaction between species and cross-fostering ($F(1, 56) = 7.21, P = 0.01$) that was driven by a decrease in aggression in the California mouse as measured by longer attack latencies (Fig. 1). Planned comparisons showed that cross-fostering significantly reduced resident-intruder aggression as measured by longer attack latencies only in the California mouse ($F(1, 56) = 5.87, P = 0.02$). Cross-fostering did not significantly influence resident-intruder attack latency in the white-footed mouse. The opposite pattern was observed in the test of aggression in a neutral arena, with a change in attack latency observed only in the white-footed mouse, not the California mouse (Fig. 1). As in the resident-intruder test, a significant interaction existed between species and group ($F(1, 54) = 5.38, P = 0.02$) in the test of aggression in a neutral arena. Planned comparisons showed an increase in aggression in a neutral arena as measured by shortened attack latency in cross-fostered white-footed mice ($F(1, 54) = 6.64, P = 0.01$) and no change in cross-fostered California mice.

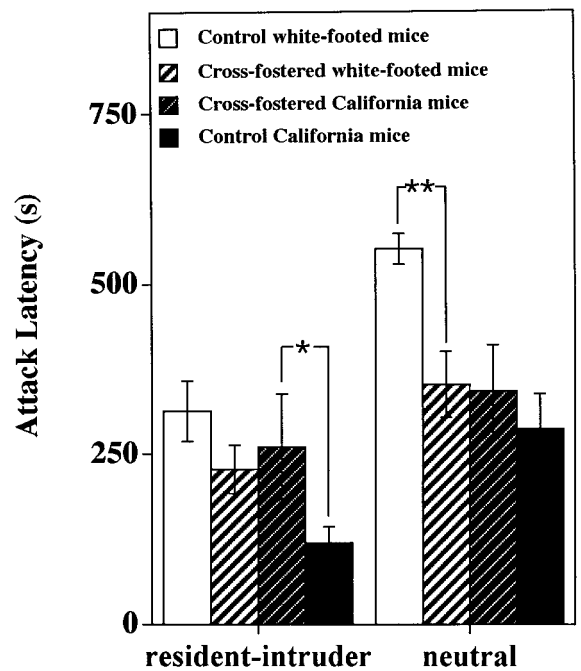


FIG. 1. Attack latency in the resident-intruder test and test of aggression in a neutral arena for control white-footed mice (resident-intruder, $n = 12$; neutral arena, $n = 13$), cross-fostered white-footed mice (resident-intruder, $n = 14$; neutral, $n = 13$), cross-fostered California mice (resident-intruder, $n = 10$; neutral, $n = 9$), and control California mice (resident-intruder, $n = 24$; neutral, $n = 23$). Data are presented as means \pm standard errors. * $P < 0.05$. ** $P = 0.01$.

As predicted, in-fostering did not alter attack latency in either testing paradigm. In the comparisons of in-fostered and control mice in the resident-intruder test, there was no interaction between species and in-fostering. Planned comparisons showed that there was no effect of in-fostering in the white-footed mouse alone (control, 363.56 ± 79.09 s; in-fostered, 368.57 ± 101.78 s) or in the California mouse alone (control, 292.10 ± 74.72 s; in-fostered, 338.38 ± 84.81 s). A similar pattern was observed in the test of aggression in a neutral arena, with no interaction between species and in-fostering, no effect of in-fostering in the white-footed mouse alone (control, 335.40 ± 61.29 s; in-fostered, 332.57 ± 80.88 s), and no effect of in-fostering in the California mouse alone (control, 524.40 ± 57.74 s; in-fostered, 491.89 ± 58.27 s). The slower attack latencies of these in-fostered and control animals in comparison to the cross-fostered and control animals described above may relate to a seasonal difference in aggression. There is a significant difference in time of year that tests occurred in the two data sets, with in-fostered mice more likely to be tested in the winter

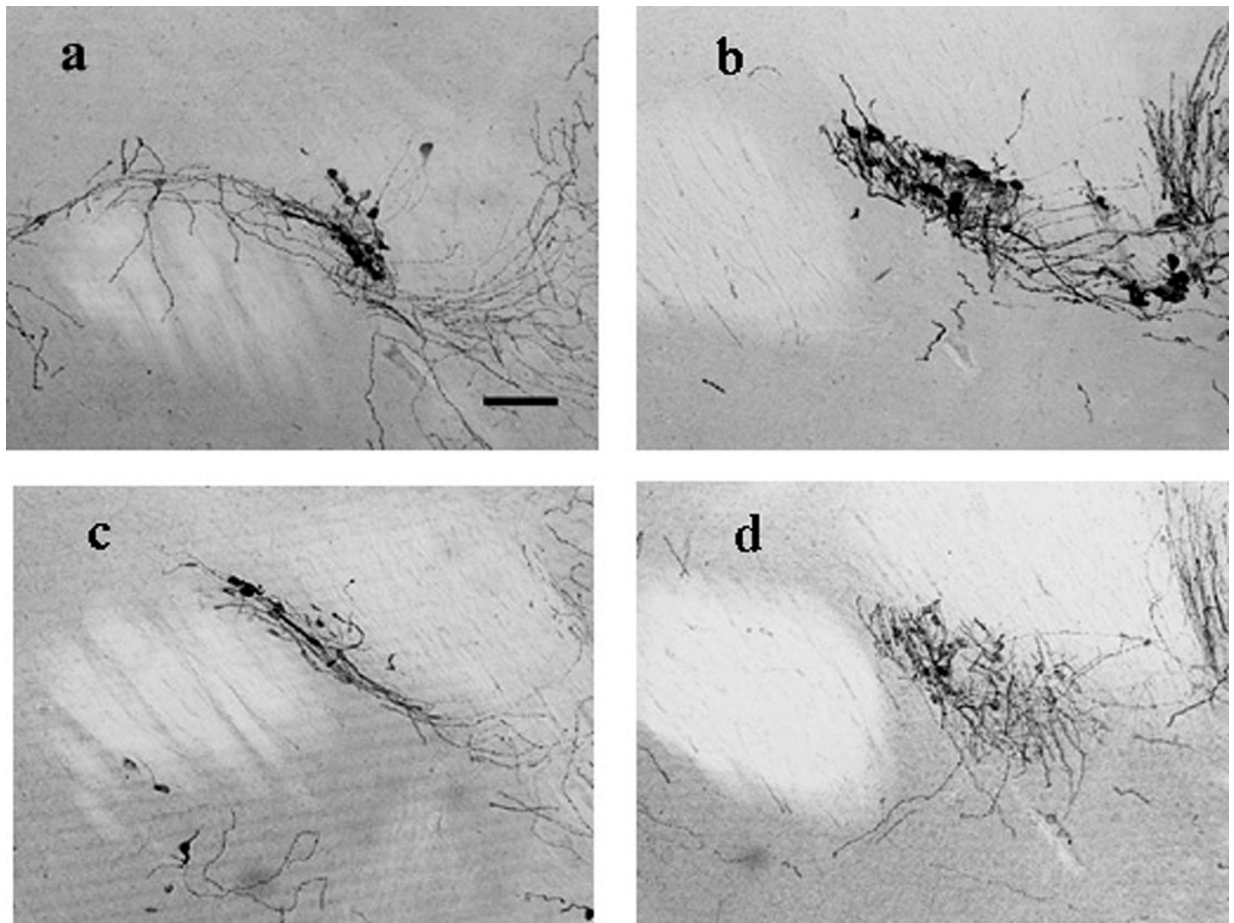


FIG. 2. Representative photomicrographs of AVP-ir staining in the bed nucleus of the stria terminalis (BNST) comparing sexually naïve, male (a) control white-footed mice, (b) control California mice, (c) cross-fostered white-footed mice, and (d) cross-fostered California mice. Bar represents 100 μm .

(resident–intruder test, $t(92) = -3.22$, $P = 0.002$; aggression test in neutral arena, $t(90) = -5.08$, $P < 0.0001$).

Attack latency did not correlate significantly with number of brothers, number of sisters, sex ratio of the litter in which a male was raised, number of cage mates, test date, age on the day of the test, anogenital distance, weight, intruder weight, weight difference between the two animals in each test, or test order in either the resident–intruder test or test of aggression in a neutral arena for either species. Neither resident–intruder attack latency nor attack latency in the test of aggression in a neutral arena in this study were associated with test order among the animals tested in the aggression trials of control and cross-fostered animals or the aggression trials of control and in-fostered animals for all animals, California mice only, or white-footed mice only. However, resident–intruder attack

latency did correlate with date of birth for in-fostered and control white-footed mice, but using date of birth as a covariate did not alter the pattern of results in the resident–intruder test.

AVP-ir Staining

As predicted based on our previous work (Bester-Meredith *et al.*, 1999), we found species differences in AVP-ir staining. Across all four groups of control and cross-fostered mice, the California mouse showed a greater maximum percentage of staining than the white-footed mouse in the BNST (Figs. 2 and 3; $F(1, 20) = 45.34$, $P < 0.0001$). In addition to this difference in the maximum percentage of staining, the two species differed in three parameters related to the intensity of AVP-ir staining. On the section with the maximum percentage of staining, the California

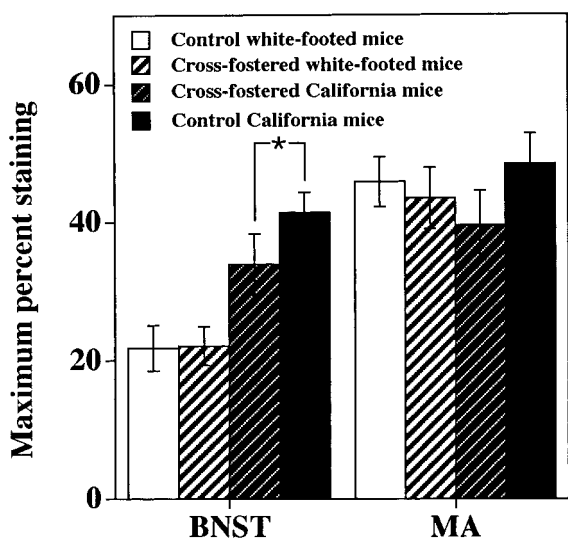


FIG. 3. Maximum percentage of staining as a measure of AVP-immunoreactive staining in the BNST and medial amygdala (MA) in sexually naïve male mice (control white-footed mice, $n = 6$; cross-fostered white-footed mice, $n = 7$; cross-fostered California mice, $n = 7$; control white-footed mice, $n = 7$). The two species differ in AVP-ir staining only in the BNST ($F(1, 20) = 45.34$, $P < 0.0001$). Data are presented as adjusted means \pm standard errors. * $P < 0.05$.

mouse showed a greater number of cells, larger cell size, and higher optical density of cells (Fig. 4; Wilk's $\lambda(3,17) = 0.58$, $P = 0.02$).

In addition, cross-fostering altered this species-typical pattern of AVP-ir staining (Figs. 2 and 3). In the male California mouse, cross-fostering decreased the maximum percentage of AVP-ir staining in the BNST ($F(1, 20) = 5.43$, $P = 0.03$). Cross-fostering also altered the combination of three parameters related to the intensity of AVP-ir staining in the California mouse. Overall, fewer cells, smaller cells, and cells with a lighter optical density existed on the section in the BNST with the maximum percentage of staining in the cross-fostered California mice compared to control California mice (Fig. 4; Wilk's $\lambda(3,17) = 0.54$, $P = 0.01$). In contrast, cross-fostering did not alter the maximum percentage of staining or the three cell parameters (cell number, cell area, or cell optical density) on the section in the BNST with the maximum percentage of staining in the white-footed mouse.

Contrary to our predictions, we measured no statistically significant species differences or cross-fostering effects in the MA. On the section with the maximum percentage of staining, we observed no species differences in the percentage of staining or in the three cell parameters. However, although no significant effect of

cross-fostering was observed in the percentage of staining or cell parameters in the MA in the white-footed mouse, a marginally nonsignificant trend toward a decrease in AVP-ir staining in the MA of cross-fostered California mice was observed on the section with the maximum percentage of staining ($F(1, 20) = 4.16$, $P = 0.055$). Despite this trend toward a change in the predicted direction in the maximum percentage of staining in the MA, cross-fostered California mice showed no changes in the measured cell parameters that contribute to the maximum percentage of staining. Therefore, this trend instead may be caused by changes in fiber density.

In the PVN and SON, two brain areas used as controls for general differences in AVP-ir staining, we tested for species differences or cross-fostering effects. As predicted, we did not measure any statistically significant species differences in AVP-ir staining in these two areas (Fig. 5). The two species did not differ in maximum percentage of staining in the PVN or the SON. As expected, cross-fostering did not alter the maximum AVP-ir staining in the PVN or SON in the white-footed mouse or the PVN in the California mouse. However, in contrast to our predictions, cross-fostered California mice differed from control California mice in maximum percentage of staining in the SON ($F(1, 20) = 8.09$, $P = 0.01$). In addition to a decrease in maximum percentage of staining in the SON, cross-fostered California mice showed a nonsignificant trend toward a decrease in the three cell parameters in the SON (Wilk's $\lambda(3,17) = 0.70$, $P = 0.10$). This trend toward a decrease in cell measurements in the SON due to cross-fostering occurred despite the absence of a species difference in the cell parameters in the SON.

Testosterone and Corticosterone

None of the variables that were examined correlated significantly with adult plasma T concentrations (date of sacrifice, age of sacrifice, weight, anogenital distance, date of birth, number of brothers, number of sisters, number of siblings, or sex ratio of the litter in which the animal was raised). Although there was a trend toward T concentrations being significantly higher in the white-footed mouse than the California mouse, this trend was not statistically significant (Fig. 6; $F(1, 23) = 3.60$, $P = 0.07$). Despite this nonsignificant trend toward a difference in species levels of T, cross-fostering did not significantly alter T concentrations in the California mouse [control, 0.46 ng/ml with a 95% confidence interval of (0.27,0.77); cross-fostered,

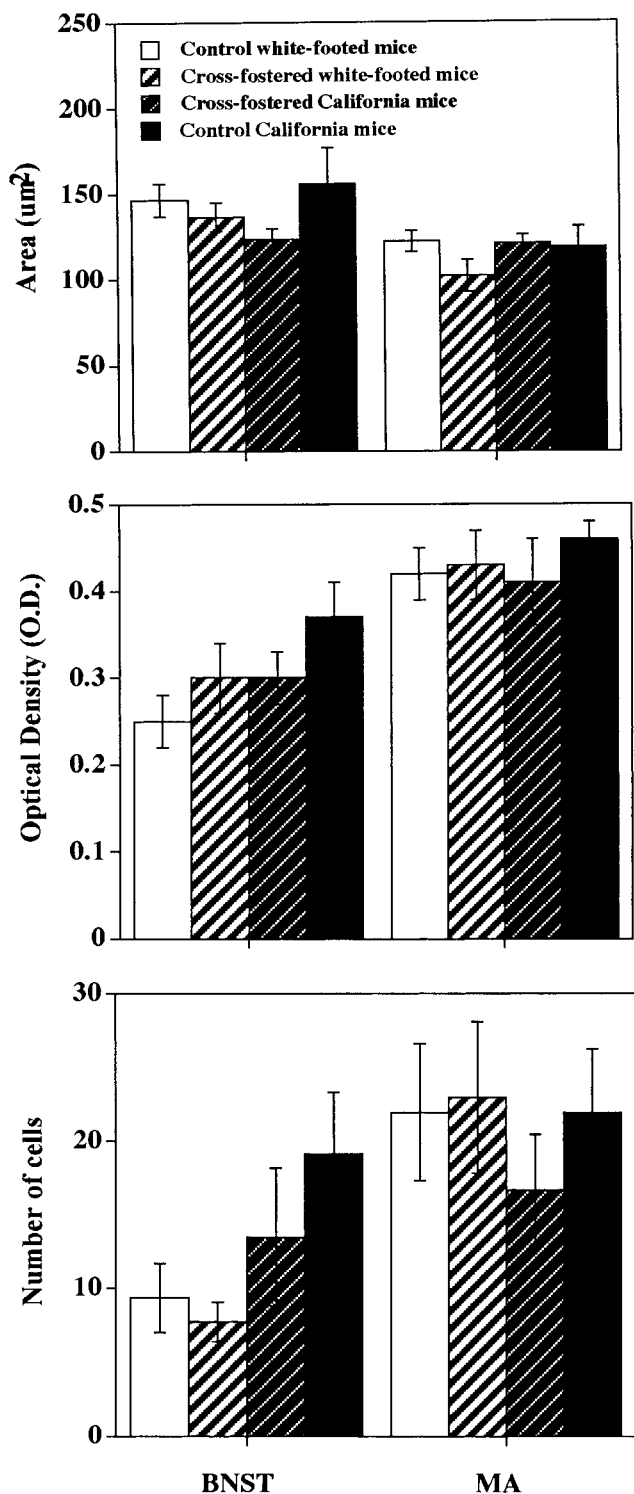


FIG. 4. Average cell area, optical density, and number of neuronal cells on the section with the maximum percentage of staining in the BNST and MA in sexually naïve male mice (control white-footed mice, $n = 6$; cross-fostered white-footed mice, $n = 7$; cross-fostered California mice, $n = 7$; control white-footed mice, $n = 7$). Data are presented as adjusted means \pm standard errors. Using a multivariate

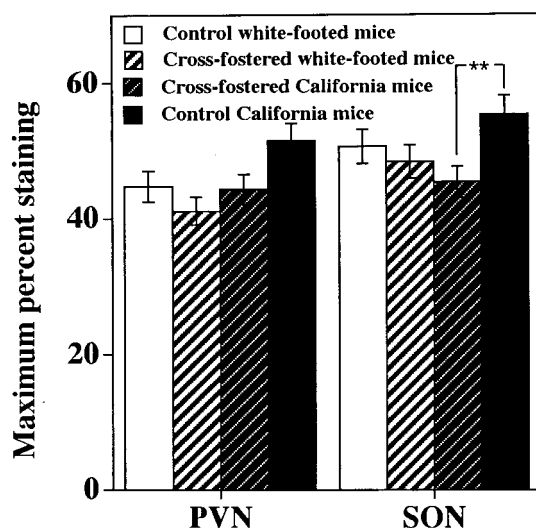


FIG. 5. Maximum percentage of staining as a measure of AVP-immunoreactive staining in the paraventricular nucleus of the hypothalamus (PVN) and supraoptic nucleus (SON) in sexually naïve male mice (control white-footed mice, $n = 6$; cross-fostered white-footed mice, $n = 7$; cross-fostered California mice, $n = 7$; control white-footed mice, $n = 7$). Data are presented as adjusted means \pm standard errors. $**P = 0.01$.

0.30 ng/ml with a 95% confidence interval of (0.17,0.52)]. Cross-fostering also did not alter T concentrations in the white-footed mouse [control, 0.70 ng/ml with a 95% confidence interval of (0.48,1.02); cross-fostered, 1.14 ng/ml with a 95% confidence interval of (0.81,1.60)]. In addition, the observed changes in AVP-ir staining do not appear to be caused by changes in adult baseline concentrations of T because T was not associated with any of the AVP-ir staining measures in either species.

Because there was a significant positive correlation between B concentrations and the number of siblings when control (number of siblings varied only in this group) and cross-fostered white-footed mice are combined ($r = 0.60$, $P = 0.04$), number of siblings was used as a covariate in all analyses involving B. Baseline concentrations of B were significantly higher in the California mouse than the white-footed mouse (Fig. 6; $F(1, 21) = 8.88$, $P = 0.007$). However, there

analysis, California mice show more staining on these three measures than white-footed mice (Wilk's $\lambda(3,17) = 0.58$, $P = 0.02$), but cross-fostered California mice show less staining on these three measures than control California mice (Wilk's $\lambda(3,17) = 0.54$, $P = 0.01$).

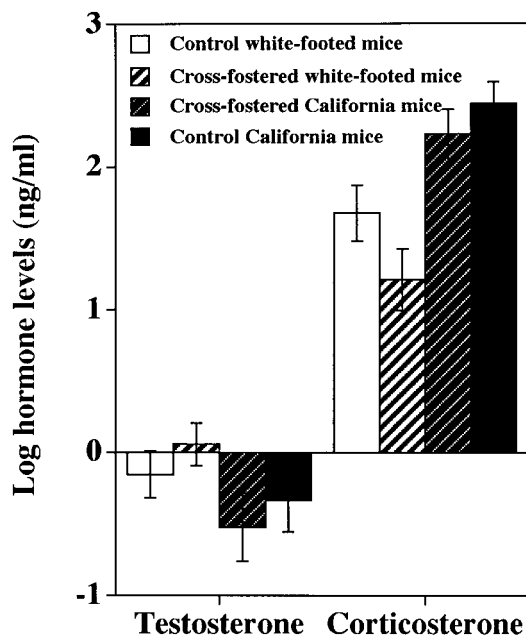


FIG. 6. Log testosterone and corticosterone levels in sexually naïve male mice (control white-footed mice, $n = 6$; cross-fostered white-footed mice, $n = 7$; cross-fostered California mice, $n = 7$; control California mice, $n = 7$). Data are presented as adjusted means \pm standard errors.

was no difference in B concentrations of control and cross-fostered California mice [control, 277.44 ng/ml with a 95% confidence interval of (196.46,391.80); cross-fostered, 170.39 ng/ml with a 95% confidence interval of (115.23,251.95)]. There were also no significant differences in B concentrations of control and cross-fostered white-footed mice [control, 47.57 ng/ml with a 95% confidence interval of (30.33,74.61); cross-fostered, 16.27 ng/ml with a 95% confidence interval of (9.88,26.79)]. These hormone concentrations are comparable to B concentrations previously collected for both species in the laboratory via the retroorbital sinus (means, 20–397 ng/ml; Trainor and Marler, unpublished data). In addition, the average B levels found in each group are near the middle of the previously documented B range in our laboratory. B was not associated with any of the AVP-ir staining measures in either species.

DISCUSSION

Cross-Fostering and Aggression as Measured by Attack Latency

Cross-fostering between species with differing levels of parental care and aggression significantly al-

tered the behavior of the next generation. After cross-fostering, the offspring became more like their foster parents: white-footed mice raised by the more aggressive California mice became more aggressive, whereas California mice raised by the less aggressive white-footed mice became less aggressive. Interestingly, however, testing conditions appear to be critically important in determining whether cross-fostering alters aggression. Cross-fostering altered attack latencies only in a neutral arena in the white-footed mouse and only in the resident-intruder test in the California mouse.

The disparate responsiveness of the two species to cross-fostering in the two aggression tests may be related to the differing ecology of the two species. Whereas a field study has suggested that the California mouse is a territorial species (Ribble and Salvioni, 1990), the white-footed mouse shows territoriality only under high population densities (Metzgar, 1971). Because the resident-intruder test may measure a form of territorial aggression, the California mouse may have responded to cross-fostering only in this more ecologically relevant test. In contrast, the white-footed mouse responded to cross-fostering only in the test of aggression in a neutral arena, a measure of aggression in a neutral environment. For the white-footed mouse, cross-fostering may alter dominance interactions only in the absence of territorial defense. Further studies will need to investigate this possibility.

Which aspects of the environment provided by the parents cause this change? We can only speculate at this point. Previously, we showed that the two species differ in male parental care (Bester-Meredith *et al.*, 1999) and we have unpublished data suggesting that parents show similar levels of parental care regardless of whether they are caring for their own or foster pups. It is therefore possible that male or female parental behavior may be important in shaping the species' difference in and effects of cross-fostering on offspring attack latency. Other possibilities range from other behaviors of the parents to differences in milk composition (e.g., Rose and McCarty, 1994; Smotherman and Robinson, 1994; Blass, 1994; Koldovský, Illnerová, Macho, Štrbák, and Štěpánková, 1995). Regardless of the cause of this behavioral difference, these data clearly show the importance of the rearing environment in determining adult patterns of social behavior.

Cross-Fostering and AVP-ir Staining in the BNST

The most intriguing finding was that in another group of sexually naive males that had never been exposed to aggression tests, cross-fostering altered AVP-ir staining in the BNST in a way that corresponded to the changes in resident-intruder attack latency described above. In the California mouse, which showed a decrease in resident-intruder aggression as indicated by longer attack latencies after cross-fostering, we observed a decrease in AVP-ir staining in the BNST. In the cross-fostered white-footed mouse, which showed no change in resident-intruder attack latency after cross-fostering, we observed no changes in AVP-ir staining in the BNST. These findings suggest that alterations in the environment provided by the parents can directly alter neurobiological pathways that are associated with social behavior. In addition, these results suggest that the responsiveness of the AVP pathway associated with social behaviors differs between the two species.

This association between resident-intruder attack latency and vasopressin adds to a large body of evidence demonstrating a role for vasopressin in the modulation of aggression. For example, administration of vasopressin has been associated with changes in aggression and/or dominance behaviors in prairie voles (Winslow *et al.*, 1993), golden hamsters (Ferris *et al.*, 1997), and Syrian hamsters (Bamshad and Albers, 1996). Previous studies in house mice and *Peromyscus* mice have identified differences in AVP-ir staining that are associated with differences in attack latency (Bester-Meredith *et al.*, 1999; Compaan *et al.*, 1992, 1993). The results of this study add to the few studies demonstrating that the environment provided by the parents can have a significant impact on both the neurochemistry and social behavior of their adult offspring. Throughout development, social experience appears to shape adult behavior and vasopressin levels. In the present study, early experience prior to weaning can shape adult patterns of vasopressin distribution within the brain. Similarly, social experience later in development also can alter aggression and adult levels of vasopressin, as socially subjugated golden hamsters show changes in their aggressiveness and vasopressin levels (Delville *et al.*, 1998). At this time, it is not known specifically how social cues alter adult vasopressin levels and aggressiveness. However, because the foster parents in this study provide such significant differences in social environments due to the varying degrees of male parental care (Bester-Meredith *et al.*, 1999; Bester-Meredith and

Marler, unpublished data), we eventually will be able to determine whether parental behaviors shown by fathers or mothers are shaping these different behavioral responses.

Cross-Fostering and AVP-ir Staining in the SON and MA

In addition to altering AVP-ir staining in the BNST, cross-fostering also led to a decrease in AVP-ir staining in the California mouse in the SON and was associated with a nonsignificant trend toward a decrease in the MA. However, we did not find any species differences in these two brain areas in control animals, nor did we find changes in AVP-ir staining in these areas in the white-footed mouse. One possible explanation for the similarity between these findings in the BNST, MA, and SON is that these three areas may be linked through their association with social behavior. In the California mouse, as environmental manipulations alter social behavior, they also may cause fluctuations in the amount of AVP in all three brain areas. Similarly, in the white-footed mouse, linkage between these three areas may explain the consistent absence of a measurable effect of cross-fostering on AVP-ir staining in any of these three areas. Supporting this hypothesis is the fact that functions related to the regulation of species-specific social behavior have been reported for AVP neurons in the BNST, MA, and SON. For example, AVP-ir neurons in the BNST and MA have been associated with parental care in voles (Wang, 1995). In addition, AVP in the BNST has been associated with attack latency in house mice (Compaan *et al.*, 1993) and *Peromyscus* mice (Bester-Meredith *et al.*, 1999). AVP cell populations in the SON also may be involved in aggression in golden hamsters because there is evidence that they project to the anterior hypothalamus (Ferris *et al.*, 1997) and that they increase in c-Fos immunoreactivity after participation in an aggressive encounter (Delville, De Vries, and Ferris, 2000). Therefore, fluctuations in AVP-ir staining may be consistent across all three brain areas due to their common role in regulating species-specific social behavior.

Another, though possibly less likely, explanation for the decrease in AVP-ir staining in the SON and the trend toward a decrease in the MA is that changes in osmoregulation or increased stress may be lowering overall levels of AVP-ir staining in cross-fostered California mice while having no effect in the white-footed mouse. Most studies investigating the link between AVP and stress or AVP and osmoregulation have been

more likely to focus on the role of the PVN than the BNST or MA (e.g., Wotjak, Kubota, Liebsch, Montkowski, Holsboer, Neumann, and Landgraf, 1996; Ma, Lightman, and Aguilera, 1999; reviewed in Landgraf, Wotjak, Neumann, and Engelmann, 1998). In this study, since cross-fostering did not alter AVP-ir staining in the PVN, it seems less likely that the changes in AVP-ir staining in the SON or MA may be associated with stress. However, Koolhaas, Everts, de Ruiter, de Boer, and Bohus (1998) recently proposed that AVP in these brain areas might be important in regulating anxiety-related behaviors. Although subordinate rats in a social stress paradigm show a decrease in AVP mRNA in the MA, the authors suggest that this decrease probably occurs in response to lower T concentrations and not as a direct result of the stressor (Albeck, McKittrick, Blanchard, Blanchard, Nikulina, McEwen, and Sakai, 1997). In our study, cross-fostering did not alter T and therefore the trend toward a decrease in AVP-ir staining observed in the MA would not likely be in response to a similar mechanism.

In the present study, two additional lines of evidence suggest that changes in AVP-ir staining in cross-fostered mice do not occur in response to increased stress levels. Although both T and B concentrations can change in response to stress (Sapolsky, 1992), B and T concentrations do not differ between adult control and cross-fostered mice of either species. In addition, our study found no correlation between hormone concentrations and AVP-ir staining even though adult concentrations of T (van Leeuwen *et al.*, 1985; De Vries, Duetz, Bujis, Van Heerikhuizen, and Vreeburg, 1986; De Vries and Al-Shamma, 1990; Wang and De Vries, 1993; De Vries, Wang, Bullock, and Numan, 1994; Wang, 1994; Lonstein and De Vries, 1999) and B (Watters *et al.*, 1996) have been shown to influence adult AVP pathways. However, it is possible that stress could alter hormone concentrations early in development differentially between the groups and could be critical in shaping adult patterns of AVP and attack latency (i.e., Compaan *et al.*, 1992, 1993). It is not known whether the two species show different patterns of release of T and B earlier in development. Alternately, other factors associated with cross-fostering, including the amount of male parental care received by the pups, may have altered T early in development and caused organizational changes in AVP-ir staining.

Our results demonstrate that manipulating the environment provided by the parents during development can alter both a neurotransmitter system and

social behavior. In future papers we will examine which behavioral traits of the parents of the cross-fostered offspring are consistent with the offspring behavioral findings and AVP-ir staining patterns presented in the current study.

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