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Early Maternal Rejection Affects the Development of Monoaminergic Systems and Adult Abusive Parenting in Rhesus Macaques (*Macaca mulatta*)

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This study investigated the effects of early exposure to variable parenting style and infant abuse on cerebrospinal fluid (CSF) concentrations of monoamine metabolites and examined the role of monoaminergic function in the intergenerational transmission of infant abuse in rhesus monkeys (*Macaca mulatta*). Forty-three infants reared by their biological mothers and 15 infants that were cross-fostered at birth and reared by unrelated mothers were followed longitudinally through their first 3 years of life or longer. Approximately half of the infants were reared by abusive mothers and half by nonabusive controls. Abused infants did not differ from controls in CSF concentrations of 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), or 3-methoxy-4-hydroxyphenylgycol (MHPG). Abused infants, however, were exposed to higher rates of maternal rejection, and highly rejected infants had lower CSF 5-HIAA and HVA than low-rejection infants. The abused females who became abusive mothers in adulthood had lower CSF 5-HIAA than the abused females who did not. A similar trend was also observed among the cross-fostered females, suggesting that low serotonergic function resulting from early exposure to high rates of maternal rejection plays a role in the intergenerational transmission of infant abuse.

Keywords: early experience, monoamine metabolites, development, primates

Child maltreatment has been shown to result in long-lasting impairments of affective, cognitive, and social development as well as alterations in a number of physiological systems that regulate stress reactivity and social processes, including neuropeptides and hormones of the hypothalamic–pituitary–adrenal (HPA) axis, the monoaminergic systems, and other neuropeptides such as oxytocin and vasopressin (Pollak, 2005; Wismer Fries, Ziegler, Kurian, Jacoris, & Pollak, 2005). Research with animal models of child maltreatment can provide an opportunity to examine the long-term consequences of maltreatment with longitudinal studies, to tease apart experimentally the role of genetic and experiential variables in the development of abused children, and to obtain accurate measures of biological variables under controlled experimental conditions (Maestripieri & Carroll, 1998; Sanchez, Ladd, & Plotsky, 2001). For example, a recent longitudinal study of rhesus macaques involving an infant cross-fostering experiment highlighted the importance of early experience in the intergenerational transmission of abuse (Maestripieri, 2005).

Maltreatment is one extreme and abnormal expression of parenting behavior, but considerable interindividual variation also exists within the normal range of parenting. A growing number of studies in rodents and human and nonhuman primates has shown that variation in the quality of parental care received early in life results in long-term differences in the offspring's neuroendocrine reactivity to stress (rodents: Cameron, Champagne, Fish, Ozaki-Kuroda, & Meaney, 2005; Meaney, 2001; nonhuman primates:

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Dettling, Pryce, Martin, & Dobeli, 1998; humans: Gunnar & Donzella, 2003; Hane & Fox, 2006). Experimental manipulations of the early rearing environment in nonhuman primates such as separation from the mother and surrogate- and peer-rearing are also associated with long-term developmental alterations in a number of physiological variables such as plasma concentrations of HPA axis hormones and cerebrospinal fluid (CSF) concentrations of serotonin, dopamine, and norepinephrine metabolites (Higley, Suomi, & Linnoila, 1992; Shannon et al., 2005; see Pryce et al., 2005, for a recent review). Some of these studies have shown that individual differences in vulnerability to adverse early experience are modulated by genetic factors, such as the polymorphism in the serotonin transporter gene. In rhesus monkeys, a 21-base pair insertion/deletion polymorphism analogous to the human 5-hydroxytryptamine transporter gene (5-HTT) length variant of the gene-linked polymorphic region (rh5-HTTLPR) has been found in the same transcriptional region, resulting in similar allelic variation and reductions in transcriptional efficiency (Bennett et al., 2002). Rhesus monkeys with the short (*s*) allele are more vulnerable to the developmental dysregulation of the HPA axis induced by early social deprivation than are monkeys with the long (*l*) allele (Barr, Newman, Lindell, et al., 2004; Barr, Newman, Shannon, et al., 2004; Bennett et al., 2002).

In the present study, we investigated the relationship between exposure to different parenting styles early in life, including abusive parenting, and CSF concentrations of serotonin, dopamine, and norepinephrine metabolites in group-living rhesus monkeys during their first 3 years of life. Our subjects included male and female infants that were reared by their biological mothers as well as female infants that were cross-fostered at birth and reared by unrelated mothers. Approximately half of our subjects were reared by abusive mothers and half by nonabusive controls. Previous studies have shown that the parenting style of macaque mothers consists of two independent dimensions, Maternal Protectiveness and Maternal Rejection, and that abusive mothers reject their infants at higher rates than nonabusive mothers (Maestripieri, 1998a; McCormack, Sanchez, Bardi, & Maestripieri, in press). Both abuse and rejection are stressful experiences for monkey infants; therefore, we predicted that these infants might exhibit lower CSF concentrations of monoamine metabolites similar to the individuals exposed to other early stressors (Higley et al., 1992; Shannon et al., 2005). We also hypothesized that some of the alterations in monoaminergic systems potentially associated with infant abuse may play a role in the transmission of abusive parenting across generations. A previous study showed that approximately half of the rhesus macaque females who are physically abused by their mothers in infancy exhibit abusive parenting with their first-born offspring, and half of them do not (Maestripieri, 2005). Therefore, we predicted that the abused females who became abusive mothers might show greater physiological alterations than abused females who did not become abusive mothers. The relative role of experience and genetic characteristics in accounting for individual differences in CSF concentrations of monoamine metabolites was addressed by comparing cross-fostered and non–cross-fostered individuals as well as individuals that carried different alleles for the serotonin transporter gene.

Method

Subjects

This study was conducted with rhesus macaques (*Macaca mulatta*) from a population of over 1,500 individuals living at the Field Station of the Yerkes National Primate Research Center in Lawrenceville, Georgia. The subjects lived in several different social groups and were housed in 38 × 38 m outdoor compounds with indoor housing areas. The groups consisted of 30 to 35 adult females with their immature offspring and 2 to 5 unrelated adult males. All groups had a stable matrilineal structure and a linear dominance hierarchy. Female dominance ranks were assessed with data on unidirectional aggression and submission collected during previous studies.

One set of subjects included 43 infants reared by their biological mothers in their natal groups. Twenty-one of these infants (9 males and 12 females) were born to and reared by multiparous mothers with a history of abusive parenting, whereas 22 of them (9 males and 13 females) were born to and reared by nonabusive controls. Another set of subjects consisted of 15 females that were successfully cross-fostered between abusive and nonabusive mothers within 24 to 48 hr of birth (see Maestripieri, Megna, & Jovanovic, 2000, for details of the cross-fostering procedure). Specifically, 7 female infants born to multiparous mothers with a history of abusive parenting were adopted and reared by unrelated control mothers, whereas 8 female infants born to control mothers were adopted and reared by abusive mothers. One additional cross-fostered female was excluded from this study because no relevant behavioral or physiological data were available. Cross-fostered infants were reared in groups different from those in which their biological mothers resided. The abusive mothers that served as study subjects had been observed in previous years and their abusive behavior had been documented (Maestripieri, 1998a; Maestripieri, Tomaszycki, & Carroll, 1999). Only mothers whose frequency and severity of abuse did not jeopardize their infant's life were included in this study. These abusive mothers were typically consistent in the frequency and severity with which they abused offspring born in successive years (Maestripieri et al., 1999). Control mothers for the first set of subjects were females without a history of abusive parenting who had characteristics (e.g. age, parity, dominance rank, and infant sex) similar to the abusive mothers and who gave birth in the same time period and in the same social groups as the abusive mothers. Control mothers for the cross-fostered subjects were selected opportunistically among multiparous females from other social groups who gave birth to a female infant within 24 to 48 hr of the abusive mothers' delivery and had no previous record of abusive parenting. In one case, a primiparous mother was used for lack of alternatives. In this case, we made sure that no instance of infant abuse had been previously reported in the subject's matriline or observed in the time interval between the subject's parturition and the cross-fostering procedure. The control and the abusive mothers did not differ significantly in their age or dominance rank (Maestripieri, 2005).

Procedures

All 58 infants were studied longitudinally, in their own social groups, from birth to 36 months of age. The cross-fostered females and approximately half of the females reared by their biological mothers were also followed into their 4th or 5th year of life until they gave birth, so that the quality of their maternal behavior (i.e., abusive vs. nonabusive) could be assessed. Infants and their mothers were focally observed in weekly or monthly observations beginning the day after birth. Hours of observations ranged from 2 to 5 hr per week in the first month of life to 1 hr per month from the 4th month of life onwards. All behavioral data were converted into mean hourly rates of behavior per month for the purposes of data analysis. The observers were tested for reliability prior to the beginning of data collection.

Analysis of the focal behavioral data focused on hourly rates of maternal abuse as well as hourly rates of the following maternal behaviors: making contact (any physical contact with the infant lasting more than 5 s), breaking contact, cradling (holding one or both arms around the infant), grooming (common definition), restraining (preventing the infant from breaking contact by pulling its leg or tail), and rejection (preventing the infant from making contact by holding the infant at a distance with an arm or forcibly removing the infant from the nipple and pushing infant away). Infant abuse was operationally defined as the following: dragging: the mother drags her infant by its tail or leg while walking or running; crushing: the mother pushes her infant on the ground with both hands; rough grooming: the mother forces her infant onto the ground and pulls out the infant's hair with force, causing distress calls; throwing: the mother throws her infant a short distance with one hand while standing or walking; hitting: the mother violently slaps her infant with one hand or arm; biting: common definition; stepping or sitting on: the mother steps on her infant with one foot or both feet, or sits on her infant; abusive carrying: the mother carries the infant with one arm away from her body, preventing the infant from clinging. Every occurrence of infant abuse within the social groups in which the subjects lived was also recorded with the behavior sampling method (Martin & Bateson, 1986).

All infants that were reared by abusive mothers, whether biological or foster mothers, were abused by them, and all cases of infant abuse occurred in the first 6 months of life (Maestripieri, 2005). Analyses of other maternal behaviors were focused on the first 6 months of infant life because after this period mothers began to resume their mating activity and some measures of maternal behavior dropped to negligible rates. For two subjects, a cross-fostered female born to an abusive female and raised by a control and a second female born and raised by a control mother, no accurate behavioral data were available for the first 6 months of life other than information that they were not abused. Another cross-fostered female born to a control and reared by an abusive mother died at the age of 1.5 years as a result of injuries sustained in a major outburst of aggression within her group.

CSF Sample Collection and Assays

All 43 subjects reared by their biological mothers were captured and anesthetized once every 6 months, at 6, 12, 18, and 24 months of age for the collection of CSF samples. For a subset of the subjects ($n = 23$; 12 abused and 11 controls), CSF samples were also collected at 30 and 36 months of age. CSF samples from the cross-fostered females were collected only twice, in the subjects' second year of life. The procedures of sample collection were similar for all subjects. All samples were obtained between 0600 and 1200. Prior to sample collection, all animals had been trained to run into an indoor capture area, where they were transferred via a transfer box into a standard squeeze cage. CSF samples were obtained as soon as possible following anesthesia induction (with telazol, 5 mg/kg i.m.), and time to obtain the sample was recorded for each subject. One 2–3 ml CSF sample was collected from the cisterna magna with a 5-ml syringe that had a 1-in., 22-gauge, bevel-tipped needle (Higley et al., 1992). For some subjects, CSF was drawn into a sterile needle by pressure difference and collected by gravity (Winslow, Noble, Lyon, Sterk, & Insel, 2003). CSF samples were immediately frozen on dry ice and stored at −80°C until further analysis. Samples were analyzed by means of liquid chromatography with electrochemical detection (Seppala, Scheinin, Capone, & Linnoila, 1984) and assayed for concentrations of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA); the dopamine metabolite, homovanillic acid (HVA); and the norepinephrine metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG). For the cross-fostered females, the average CSF concentrations of monoamine metabolites of the two samples obtained in the second year were used for data analysis. All inter- and intra-assay variabilities were less than 10%.

Genotyping

Collection of 4-ml blood samples via saphenous venipuncture was conducted with 21 subjects reared by their biological mothers (4 abused males, 6 abused females, 4 control males, 7 control females) at approximately 36 months of age for genotyping purposes. Blood samples were collected in EDTA-containing tubes, and DNA was later extracted through standard extraction methods. The rh5-HTTLPR was amplified from 25 ng of genomic DNA with flanking oligonucleotide primers (stpr5, 5′-GGCGTTGCCG-CTCTGAATGC; intl, 5′-CAGGGGAGATCCTGGGAGGG), as described in Barr, Newman, Lindell, et al. (2004) and Barr, Newman, Shannon, et al. (2004). Amplicons were separated by gel electrophoresis on a 10% polyacrylamide gel, and the *s* (388-bp) and *l* (419-bp) alleles of the rh5-HTTLPR were identified by direct visualization after ethidium bromide staining. Genotypes most frequently detected in rhesus monkeys are *l/l, l/s, s/s* (although an extra-long allele can be detected infrequently).

Data Analyses

The subjects' CSF concentrations of monoamine metabolites measured at different ages were analyzed with an analysis of variance (ANOVA) for repeated measures. Correlations between measures of maternal behavior and CSF concentrations of monoamine metabolites measured at different ages were assessed with Pearson's correlation coefficients. The relationship among different measures of maternal behavior was assessed with the principal components analysis (PCA). The PCA is a statistical technique used to identify a small number of factors, or principal components, that can be used to represent relationships among sets of many variables (Maestripieri, 1998b; Schino, D'Amato, & Troisi, 1995). The assumption of this analysis is that correlations between variables result from their sharing these factors. In PCA, linear combinations of the observed variables are formed. The first factor is the combination that accounts for the largest amount of variance in the sample. The second factor accounts for the next largest amount of variance and is uncorrelated with the first. Factor loadings are coefficients of correlation between the factors and the variables. In this study, coefficients of correlation greater than +.75 or less than −.75 were chosen as a criterion for loading. Other statistical analyses involved mixed-design ANOVAs, Student's *t* tests for unpaired samples, and chi-square tests. Whenever the data were non-normally distributed or the variances were non-homogeneous, the data were log-transformed. All tests were two-tailed, and probabilities ≤.05 were considered statistically significant.

Results

Repeated measures ANOVAs revealed no significant differences between abused and control infants, or between male and female infants, in CSF concentrations of 5-HIAA, HVA, or MHPG in any age periods, whether the analysis was conducted over the 3 years ($n = 23$), the first 2 years only ($n = 43$), or the 2nd year only ($n = 58$). All subsequent data analyses were therefore focused on measures of maternal behavior other than infant abuse and their possible relationship with CSF concentrations of monoamine metabolites in the offspring.

Interindividual Variation in Maternal Behavior

Correlational analyses indicated that individual differences in maternal behavior over the first 6 months of infant life were generally stable. Table 1 shows that consistency over time was very high for measures of breaking contact and rejection; intermediate/high for measures of restraining, cradle and grooming; and lowest for making contact. The results of the PCA applied to the

average scores of maternal behavior across the 6 months are shown in Table 2. Making contact and restraining were positively correlated and loaded onto a first factor, labeled Protectiveness. Breaking contact and rejection were positively correlated and loaded onto a second factor labeled Rejection. The scores of making contact and restraining were added together to obtain a composite measure of Maternal Protectiveness, and the scores of breaking contact and rejection were added together to obtain a composite score of Maternal Rejection.

The mothers of 56 of the 58 subjects (no reliable information on maternal behavior was available for 2 subjects) were classified as high or low in Maternal Protectiveness and high or low in Maternal Rejection depending on whether their scores were above or below the median value for the composite measures. Abused infants did not differ significantly from controls in relation to Maternal Protectiveness by their mothers; abusive: high = 15 (52%); low = 14 (48%); control: high = 13 (48%); low = 14 (52%); $\chi^2(1) = 0.07$, *ns*. Abused infants, however, were significantly more likely than controls to have highly rejecting mothers; abusive: high = 21 (72%); low = 8 (28%); control: high = 8 (30%), low = 19 (70%); $\chi^2(1) = 10.25$, $p = .001$. Female infants were more likely to have highly protective mothers than were male infants; female: high = 24 (63%), low = 14 (37%); male: high = 4 (22%), low = 14 (78%); $\chi^2(1) = 8.18$, $p = .004$, whereas infant sex and Maternal Rejection were not significantly associated; female: high = 19 (50%), low = 19 (50%); male: high = 10 (55%), low = 8 (45%); $\chi^2(1) = 0.15$, *ns.*

Maternal Behavior and Offspring CSF Monoamine Metabolites

Correlations between CSF concentrations of 5-HIAA, HVA, and MHPG measured at 6-month intervals across the first 3 years of life were generally significant (Table 3), suggesting that these variables, and especially 5-HIAA and HVA, were stable over time.

Table 2
Factor Loadings of Principal Components Analysis for Six Measures of Maternal Behavior

Maternal behaviors	Factor 1 Protectiveness	Factor 2 Rejection
Make contact	0.83*	−0.19
Restrain	0.80*	−0.37
Break contact	0.48	0.75*
Reject	0.25	0.82*
Cradle	0.63	0.01
Groom	0.52	−0.22

* $p < .05$.

The infants exposed to high levels of Maternal Rejection in the first 6 months of life had significantly lower CSF concentrations of 5-HIAA and HVA than the infants exposed to low levels of Maternal Rejection across their first 2 years of life: 5-HIAA, $F(1, 40) = 10.10$, $p = .003$; HVA, $F(1, 40) = 7.18$, $p = .01$ (see Figures 1a and 1b). Differences in 5-HIAA and HVA were also significant across the 3 years for the subset of subjects ($n = 22$) for which all six age data points were available: 5-HIAA, $F(1, 20) = 8.47$, $p = .008$; HVA, $F(1, 20) = 6.02$, $p = .02$ (see Figures 1a and 1b). In contrast, differences in CSF concentrations of MHPG in relation to early Maternal Rejection were not statistically significant, whether the analysis was conducted over the first 2 years, $F(1, 40) = 1.71$, *ns,* or all 3 years, $F(1, 20) = 2.99$, *ns* (see Figure 1c). Data from the cross-fostered females were analyzed separately (see below). There were significant age effects on all three monoamine metabolites across the 3 years: 5-HIAA, $F(1, 5) = 6.89$, $p < .0001$; HVA, $F(1, 5) = 5.60$, $p < .0001$; MHPG, $F(1, 5) = 20.46$, $p < .0001$. 5-HIAA and HVA concentrations were generally highest in the first year of life and declined thereafter (Figures 1a and 1b), whereas MHPG had an opposite pattern (Figure 1c).

Table 1
Correlation Coefficients Between Average Hourly Rates of Six Measures of Maternal Behavior Measured in the First 6 Months of Infant Life Among Rhesus Monkeys

	Maternal behaviors					
Months	Make contact	Restrain	Break contact	Reject	Cradle	Groom
1–2	.22	.53***	.33	.46**	.50***	.37
1–3	.34**	.62***	.43**	.11	.24	.25
1–4	.09	.37**	.36**	.36	.33	.18
1–5	.09	.39**	.42**	.63***	.48***	.36
1–6	.01	.08	.05	.28	.03	.03
2–3	.53***	.84***	.52***	.55***	.56***	.59***
2–4	.16	.82***	.41**	.49***	.18	.42**
2–5	.19	.84***	.34	.38**	.22	.29
2–6	.26	.23	.25	.49***	.33	.03
3–4	.58***	.46**	.38**	.58***	.40**	.64***
3–5	.07	.46**	.55***	.22	.39**	.31
3–6	.44**	.18	.33	.36**	.31	.11
4–5	.17	.81***	.56***	.54***	.47***	.42***
4–6	.38**	.00	.49***	.44***	.25	.32
5–6	.00	.01	.50***	.42**	.16	.14

Note. Sample size range = 43–55. Alpha level was set at .01 because of the high number of correlations.
** $p < .01$. *** $p < .001$.

Table 3
Correlation Coefficients Between Cerebrospinal Fluid (CSF) Concentrations of 5-HIAA, HVA, and MHPG Measured at 6-Month Intervals in the First 3 Years of Infant Life

	CSF monoamine metabolites		
Months	5-HIAA	HVA	MHPG
6–12	.33	.22	.24
6–18	.68***	.50***	.02
6–24	.69***	.48**	.16
6–30	.69***	.66***	.55**
6–36	.67***	.51**	.51**
12–18	.54***	.47**	.10
12–24	.23	.64**	.02
12–30	.57**	.43	.44
12–36	.55**	.56***	.24
18–24	.57***	.59**	.35
18–30	.59**	.45	.57**
18–36	.44	.49	.53**
24–30	.78***	.49	.55**
24–36	.70***	.53**	.63**
30–36	.72***	.69***	.70***

Note. Sample size range = 23–43. Alpha level was set at .01 because of the high number of correlations. 5-HIAA = 5-hydroxyindoleacetic acid; HVA = homovanillic acid; MHPG = 3-methoxy-4-hydroxyphenylglycol. ** $p < .01$. *** $p < .001$.

There was no statistically significant interaction between early Maternal Rejection and age for any of the three monoamine metabolite measures, and there were no significant differences in any of these measures between infants reared by mothers high and low in Maternal Protectiveness.

To assess whether differences in CSF concentrations of monoamine metabolites between infants reared by mothers with high and low Maternal Rejection reflected effects of experience or genetic differences inherited from their mothers, these variables were compared among the cross-fostered females (1 subject was excluded from this analysis because no data on Maternal Rejection were available, and another 1 because she died during the course of the study). Although the cross-fostered females reared by high-rejection mothers had lower CSF concentrations of 5-HIAA, HVA, and MHPG in their second year of life than the cross-fostered females reared by low-rejection mothers (Table 4), the differences were not statistically significant: 5-HIAA, $t(11) = -.037$, *ns*; HVA, $t(11) = -1.01$, *ns*; MHPG, $t(11) = -0.58$, *ns.* To assess whether differences in the serotonin transporter genotype affected CSF concentrations of the serotonin metabolite, the individuals with the *l/l* ($n = 5$), *l/s* ($n = 13$), and the *s/s* ($n = 3$) genotype were compared, but no significant differences were found, $F(2, 90) = 0.22$, *ns*; Figure 2. Furthermore, there were no significant differences in CSF 5-HIAA concentrations between individuals carrying the long allele (*l/l* genotype) and those carrying the short allele (*l/s* and *s/s* genotype), $F(1, 95) = 0.66$, *ns.*

Offspring CSF Monoamine Metabolites and Adult Abusive Parenting

To assess whether differences in CSF concentrations of monoamine metabolites affected the probability of displaying adult abusive parenting among females that were abused in infancy, we analyzed the CSF concentrations of 5-HIAA, HVA, and MHPG measured at 6, 12, 18, and 24 months of age in females born to and reared by abusive mothers in relation to whether they became abusive mothers themselves. The abused females who became abusive mothers had significantly lower CSF 5-HIAA concentrations than did the abused females who did not become abusive mothers, $F(1, 12) = 7.18$, $p = .05$; Figure 3. There were no significant differences in HVA, $F(1, 12) = 0.10$, *ns,* or MHPG, $F(1, 12) = 0.01$, *ns,* between these two groups of subjects. To rule out possible genetic effects, the same analysis was conducted with the cross-fostered females who were reared by abusive mothers. Data on monoamine metabolite concentrations were available for 7 subjects, 3 of which became abusive mothers themselves and 4 of which did not. Although the cross-fostered abused females who became abusive mothers had lower CSF concentrations of 5-HIAA than the abused females who did not become abusive (Table 4), the difference was not statistically significant, $t(5) = -.92$, *ns.* Sim-

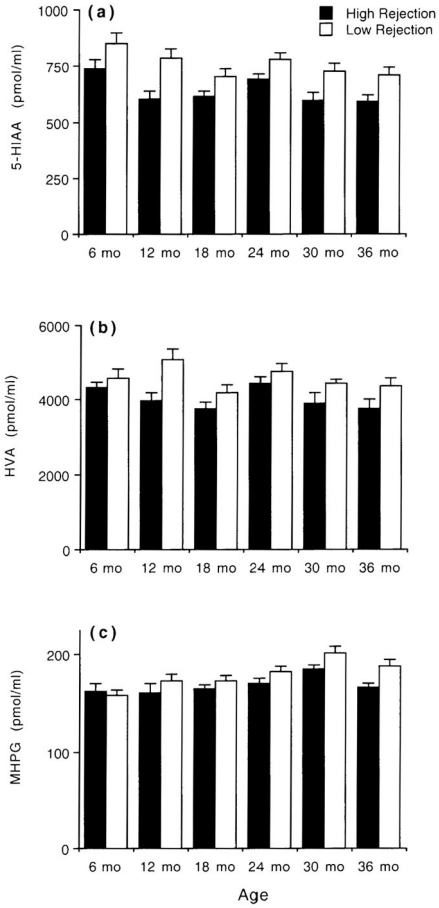

Figure 1. Mean (plus standard error of the mean) concentrations of (a) cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA), (b) homovanillic acid (HVA), and (c) 3-methoxy-4-hydroxyphenylglycol (MHPG) at 6, 12, 18, 24, 30, and 36 months (mo) of age in male and female rhesus monkeys exposed to higher (above the median) and lower (below the median) rates of maternal rejection in their first 6 months of life. Data from cross-fostered females are not included in the figure. Main statistical effects of maternal rejection rates are significant for CSF 5-HIAA and HVA but not MHPG.

Table 4
Mean (± Standard Error of the Mean) Cerebrospinal Fluid Concentrations (pmol/ml) of Monoamine Metabolites Among Cross-Fostered Females in Relation to Maternal Rejection Rates of Their Foster Mother and Whether They Became Abusive Mothers Themselves

Maternal rejection and abuse status	5-HIAA	HVA	MHPG
Females Reared by High Rejection Mothers ($n = 7$)	671.79 ± 24.76	4053.82 ± 170.99	172.91 ± 12.23
Females Reared by Low Rejection Mothers ($n = 6$)	695.02 ± 62.11	4657.18 ± 617.52	183.86 ± 14.48
Abused Females Who Became Abusive Mothers ($n = 3$)	635.48 ± 29.99	4004.77 ± 295.66	156.75 ± 16.17
Abused Females Who Did Not Become Abusive Mothers ($n = 4$)	680.87 ± 35.81	3876.89 ± 157.97	158.55 ± 10.57

Note. 5-HIAA = 5-hydroxyindoleacetic acid; HVA = homovanillic acid; MHPG = 3-methoxy-4-hydroxyphenylglycol.

ilarly, differences in CSF HVA and MHPG between these two groups were not significant: HVA, $t(5) = 0.41$, *ns*; MHPG, $t(5) = -0.09$, *ns* (see Table 4).

Discussion

Rhesus monkey infants who were exposed to higher levels of maternal rejection in their first 6 months of life exhibited significantly lower CSF concentrations of serotonin and dopamine metabolites in their 1st, 2nd, and 3rd years of life than did infants exposed to lower levels of maternal rejection. A tendency for highly rejected infants to have lower serotonin and dopamine metabolites was also observed among cross-fostered infants that were reared by unrelated females. Furthermore, there were no significant differences in CSF concentrations of 5-HIAA among infants with different alleles for the serotonin transporter gene. Although the possibility of genetic influences cannot be ruled out unequivocally (particularly considering our small sample size for the genetic analyses), these findings suggest that the developmental differences in CSF monoamine metabolites between the offspring of high-rejection and low-rejection mothers are primarily the result of early experience and not of genetic similarities between mothers and offspring. This is therefore the first demonstration that naturally occurring variation in maternal behavior in nonhuman primates is associated with differences in the neurobiological development of their offspring, similar to what has been reported in rodents (Meaney, 2001). Lower CSF 5-HIAA concentrations have also been reported in peer-reared and surrogate-reared rhesus monkeys (Higley et al., 1992), suggesting that early exposure to high levels of maternal rejection shares some characteristics with the experience of early maternal loss.

Individual differences in CSF concentrations of serotonin and dopamine metabolites measured every 6 months were highly stable across the 3 years (see also Higley et al., 1992), suggesting that the effects of early maternal rejection on these biological variables are potentially long-lasting. In fact, individual differences in CSF concentrations of serotonin metabolites during the first 3 years of life were associated with differences in maternal behavior with first-born offspring. Females who were abused by their mothers and became abusive mothers themselves had lower CSF 5-HIAA than did abused females who did not exhibit abusive parenting with their first offspring. This difference was observed among females reared by their biological mothers, but a similar trend was also apparent among the cross-fostered females, suggesting that the association between low 5-HIAA concentrations and the prob-

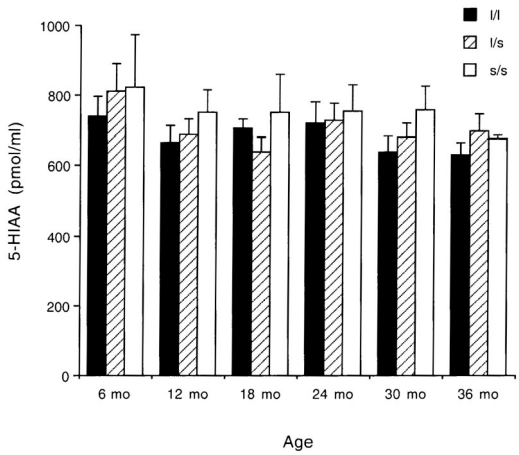

Figure 2. Mean (plus standard error of the mean) concentrations of cerebrospinal fluid 5-hydroxyindoleacetic acid (5-HIAA) at 6, 12, 18, 24, 30, and 36 months (mo) of age in individuals with the *l/l*, *l/s*, and *s/s* genotype, where *l* and *s* represent long and short alleles, respectively, for the serotonin transporter gene. Differences among the three genotypes are not statistically significant.

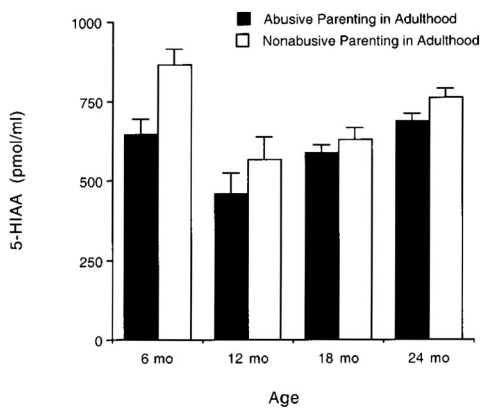

Figure 3. Mean (plus standard error of the mean) concentrations of cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA) at 6, 12, 18, and 24 months (mo) of age in females that were reared and abused by their biological mothers and who displayed or did not display abusive parenting in adulthood. The main effect of quality of adult parenting on CSF 5-HIAA is statistically significant.

ability of displaying abusive parenting is not inherited but may be the result of early experience.

Abused individuals as a whole did not differ from controls in any of the CSF monoamine metabolites at any age point, suggesting that there is no simple or direct relationship between early infant physical abuse and the development of the serotonergic, dopaminergic, and noradrenergic system, at least as reflected in our CSF measures. Abused infants, however, were exposed to higher levels of maternal rejection than controls in the first 6 months of their life, as abusive mothers typically exhibit higher levels of maternal rejection than do controls (Maestripieri, 1998a; McCormack et al., in press). Thus, it is possible that the high-rejection behavior of abusive mothers affects the behavioral development of their offspring, including the perpetuation of abusive behavior across generations, and that this contribution is mediated by long-term alterations of the serotonergic system and possibly also the dopaminergic system. The CSF concentrations of the norepinephrine metabolite MHPG had a different developmental pattern than those of 5-HIAA and HVA and were not significantly affected by early experience.

Previous studies of rhesus monkeys have shown that low CSF concentrations of the serotonin metabolite are associated with higher infant mortality, high impulsive behavior and aggression, early male emigration from the group, and poor reproductive success (Cleveland, Westergaard, Trenkle, & Higley, 2004; Mehlman et al., 1994, 1995). The association between low serotonin and impulsivity or violence has also been reported in humans (Gollan, Lee, & Coccaro, 2005). The causes of naturally occurring individual differences in serotonergic function, however, are not well understood. A previous study of rhesus monkeys found no significant variation in CSF 5-HIAA concentrations in relation to serotonin transporter gene polymorphism but reported that the individuals with the *s* allele were more vulnerable to the effects of early social deprivation on later neuroendocrine and behavioral responsiveness than the individuals with the *l* allele (Bennett et al., 2002). The sample size of our study was too small to permit investigation of a similar Genotype × Environment interaction, but nevertheless, our results suggest that early interactions with the mother are an important source of developmental variation in monoaminergic function.

In this study, male and female rhesus infants did not differ in rates of maternal rejection or in their CSF monoamine metabolites. Female infants were more likely to have highly protective mothers than were male infants, but differences in early exposure to maternal protectiveness were not associated with significant differences in measures of offspring neurotransmitter function. Maternal protectiveness and maternal rejection are relatively independent dimensions of maternal style in cercopithecine monkeys (Maestripieri, 1998b; Schino et al., 1995). Previous studies have suggested that differences in maternal protectiveness among macaque mothers have different determinants than differences in rejection (Maestripieri, 1994; Troisi & D'Amato, 1984), and this study suggests that their biological consequences for offspring development may be different as well. The investigation of the developmental consequences of variation in early mother–infant interactions in nonhuman primates is clearly a new but promising area of research, which may make an important contribution to our understanding of variation in adult psychological and neuroendocrine function and may have implications for developmental psychopathology as well. Research with this primate model of infant abuse can also further enhance our understanding of the contribution of biological and experiential variables to the intergenerational transmission of child maltreatment.

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