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Intergenerational Transmission of Maternal Behavior in Rhesus Macaques and Its Underlying Mechanisms

ABSTRACT: *Thirteen group-living rhesus macaque females that were cross-fostered shortly after birth were followed longitudinally until they gave birth for the first time. Their maternal behavior was compared to the behavior of both their foster and their biological mothers, and analyzed in relation to the cerebrospinal fluid (CSF) concentrations of serotonin, dopamine, and norepinephrine metabolites (5-HIAA, HVA, and MHPG) measured in their second year of life. Crossfostered females were similar to their foster mothers in their rates of maternal rejection and grooming, whereas their contact-making behavior was more similar to that of their biological mothers. Crossfostered females with lower CSF concentrations of 5-HIAA exhibited higher rates of maternal rejection than females with higher CSF 5-HIAA. In a related article (Maestripieri et al., 2006), we reported that rhesus infants reared by highly rejecting mothers had lower CSF 5-HIAA in their first 3 years of life. Taken together, these findings suggest that early social experience and experience-related long-term changes in serotonergic function may play a role in the intergenerational transmission of maternal rejection from mothers to daughters.* © *2007 Wiley Periodicals, Inc.* Dev Psychobiol 49: 165–171, 2007.

Keywords: *maternal behavior; intergenerational transmission; crossfostering; early experience; serotonin; primates*

INTRODUCTION

A mother's phenotype, for example, her nutritional status or behavior, can directly affect many aspects of her offspring's phenotype, for example, body size and growth, neuroendocrine reactivity to stress, reproduction, or behavior, independent of the offspring genotype. These ''maternal effects'' may represent a mechanism for nongenetic transmission of traits from one generation to the next and have recently been the focus of a great deal of research in evolutionary biology (Bernardo, 1996; Kirkpatrick & Lande, 1989; Mousseau & Fox, 1998). Maternal effects are expected to be particularly strong in mammals because mammalian mothers and offspring have an intimate and extended association with each other during gestation and lactation, and in some cases, also for many years after weaning (Reinhold, 2002).

Research with laboratory rats by Meaney and collaborators has shown that natural variation in maternal care patterns results in dramatic and long-lasting differences in the behavior, responsiveness to stress, and reproduction of the offspring, and that these maternal effects are mediated by specific molecular and neuroendocrine mechanisms including altered gene expression and glucocorticoid receptor density in particular areas of the brain (Cameron, Champagne, Fish, Ozaki-Kuroda, & Meaney, 2005; Champagne, Francis, Mar, & Meaney, 2003; Meaney, 2001). One of the findings of this research is that

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crossfostered female rats reared by mothers with high and low levels of licking, grooming, and arched-back-nursing displayed patterns of maternal care in adulthood that matched those of their foster mothers, thus demonstrating that early experience plays an important role in the transmission of variation in maternal behavior from one generation to the next (Francis, Diorio, Liu, & Meaney, 1999).

Maternal effects similar to those demonstrated in rats should be prominent in human and nonhuman primates as well, given the occurrence of marked intraspecific variability in parenting behavior and the prolonged association between parents, particularly mothers, and their developing offspring (Pryce, 1996). Indeed, nonhuman primate researchers have reported that the maternal care patterns of young monkey females (e.g., the amount of time spent in contact with offspring and the rate at which the infants' attempts to make contact are rejected by the mother) are correlated with those of their mothers (Berman, 1990; Fairbanks, 1989). These correlations, however, were obtained from observations of unmanipulated animals, and it was not clear whether they were the result of genetic similarities between mothers and daughters or the effects of early experience. The infant crossfostering approach already used in studies of maternal effects in rats and other animals (e.g., Francis et al., 1999; Krist & Remes, 2004) offers a promising opportunity to study intergenerational maternal effects in nonhuman primates as well (Maestripieri, 2005a,b), and elucidate some of their underlying mechanisms.

In this study, we investigated the relative role of genetic factors and early experience in the intergenerational transmission of maternal behavior in rhesus macaques by comparing the parenting behavior of crossfostered females to that of their foster and their biological mothers. In cercopithecine monkeys, most interindividual variation in parenting style occurs along the two orthogonal dimensions of Maternal Protectiveness and Rejection (e.g., Altmann, 1980; Fairbanks, 1996; Schino, D'Amato, & Troisi, 1995). The Protectiveness dimension includes variation in the extent to which the mother physically restrains infant exploration, initiates proximity and contact, and cradles and grooms her infant. The Rejection dimension includes variation in the extent to which the mother limits the timing and duration of contact, suckling, and carrying. Given that a previous study of rhesus macaques reported a significant correlation between maternal rejection rates of mothers and daughters but no significant correlations for maternal protective behaviors (Berman, 1990), we hypothesized that the crossfostered females may be similar to their mothers in their maternal rejection but not necessarily in their protectiveness.

In a related article, we reported that rhesus infants reared by mothers with high (above the median) levels of maternal rejection had lower CSF concentrations of the serotonin metabolite (5-hydroxyindoleacetic acid or 5-HIAA) and the dopamine metabolite (homovanillic acid or HVA) in the first 3 years of life than infants reared by mothers with low (below the median) levels of rejection (Maestripieri et al., 2006). Long-term alterations in monoaminergic function induced by early experience could be one of the physiological mechanisms underlying the intergenerational transmission of maternal rejection. In other words, exposure to high rates of maternal rejection early in life could result in reduced monoaminergic function in the offspring, and this in turn could contribute to the expression of high rates of maternal rejection when daughters become adults and rear their own offspring. In this study, we tested two predictions of this hypothesis: (1) the maternal rejection rates of adult crossfostered females should be more similar to those of their foster mothers than to those of their biological mothers, and (2) the maternal rejection rates of adult crossfostered females should be negatively correlated with their CSF levels of monoamine metabolites.

METHODS

Subjects

This study was conducted with rhesus macaques from a population of over 1,500 individuals living at the Field Station of the Yerkes National Primate Research Center in Lawrenceville, GA. 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 30–35 adult females with their immature offspring and 2–5 unrelated adult males. All groups had a stable matrilineal structure and a linear dominance hierarchy. Female dominance ranks were assessed using data on unidirectional aggression and submission collected during previous studies.

Study subjects were 13 females that were successfully crossfostered between abusive and nonabusive mothers within 24–48 hr after birth (see Maestripieri, 2005a, for definitions of infant abuse, relevant information on it, and details of the crossfostering procedure). Crossfostered infants were reared in groups different from those in which their biological mothers resided. The control and the abusive mothers did not differ significantly in their age and dominance rank (Maestripieri, 2005a).

Procedures

All 13 crossfostered females were focally observed (Martin & Bateson, 1986) 1 hr per week during their first 3 months of life and the behavior of their foster mothers was recorded. When they gave birth to their first offspring (eight males and five females), around 4–5 years of age, they were observed again with similar procedures and their own maternal behavior was recorded. The

observers were the same and had been tested for reliability prior to the beginning of data collection. Data on maternal behavior during the first 3 postpartum months were also obtained for nine of the crossfostered females' biological mothers (six of them served as foster mothers in this study and three others were observed with their own infants and with similar procedures the year before or the year after the crossfostering experiment). Average hourly rates of behavior across 3 months were used for the purposes of data analysis.

Data analysis focused on 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).

All subjects were captured and anesthetized twice in their second year of life, at 18 and 24 months of age, for the collection of CSF samples. All samples were obtained between 10 am and 12 pm. Prior to the 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 ketamine hydrochloride IM, 15 mg/kg) and the time of sampling was recorded for each subject. One 2–3 ml CSF sample was collected from the cisterna magna using a 5 ml syringe with a 1-inch, 22-gauge, bevel-tipped needle (Higley, Suomi, & Linnoila, 1992). CSF samples were analyzed using liquid chromatography with electrochemical detection (Seppala, Scheinin, Capone, & Linnoila, 1984) and assayed for concentrations of the serotonin metabolite, 5-HIAA, the dopamine metabolite, HVA, and the norepinephrine metabolite, 3-methoxy-4-hydroxyphenylgycol (MHPG). For the purpose of data analysis, the average CSF concentration of monoamine metabolites of the two samples was used (previous studies of rhesus macaques showed that CSF levels of 5-HIAA, HVA, and MHPG are highly stable across the first 3 years of life; Higley et al., 1992; Maestripieri et al., 2006). All inter- and intra-assay variabilities were less than 10%.

Behavioral similarities between offspring and their foster and biological mothers were assessed with linear regression analyses (Falconer & Mackay, 1996). Correlations between behavioral variables, or between behavioral and neurochemical variables, were assessed with the Pearson's product moment correlation coefficient. Other behavioral comparisons involved the Student's *t*-test for paired samples. Whenever the data were nonnormally distributed or the variances were nonhomogeneous, the data were log transformed and normality of distribution was checked again. All tests were two-tailed and probabilities $\leq .05$ were considered statistically significant.

RESULTS

The rates of contact-breaking and rejection of the adult crossfostered females were significantly lower than those of their foster mothers (contact-breaking, $t = 3.12$, $N = 12$, $p = .009$; rejection, $t = 2.36$, $N = 12$, $p = .03$; Fig. 1), whereas those of the other maternal behaviors were not significantly different (contact-making, $t = .21$, $N = 12$, $p = .84$; cradling, $t = -.47$, $N = 12$, $p = -.65$; restraining, $t = .86$, $N = 12$, $p = .40$; grooming, $t = .65$, $N = 12, p = .53$; Fig. 1; one pair of subjects was excluded from these analyses because the data on the foster mother were incomplete; she had to be temporarily removed from the group due to health issues).

Three of the six maternal behavior measures of adult crossfostered females were significantly predicted by the behavior of their foster mothers. Specifically, the rates of maternal rejection and maternal grooming of the adult crossfostered females were positively associated with the rates of rejection ($r = .84$, $r^2 = .71$, $N = 12$, $p = .0006$; Fig. 2a; the regression is statistically significant at $p = .01$ also without the individual with the highest rejection scores) and grooming ($r = .57$, $r^2 = .33$, $N = 12$, $p = .05$; Fig. 2b) of their foster mothers, whereas the association between rates of cradling was significant but negative ($r = -.66$, $r^2 = .44$, $N = 12$, $p = .01$; Fig. 2c; the regression is statistically significant at $p = .05$ also without the outlier). There was no significant correlation between these three maternal behaviors among the crossfostered females or their foster mothers. There was no significant association between the rates of contact-making ($r = .07$, $r^2 = .00$, $N = 12$, $p = .82$), contact-breaking ($r = .11$, $r^2 = .01$, $N = 12$, $p = .71$), or restraining ($r = -.06$, $r^2 = .00$, $N = 12$, $p = .84$) between daughters and their foster mothers. The rate of contact-making by adult crossfostered females, however, was positively correlated with that of their biological mothers ($r = .64$, $r^2 = .43$, $N = 9, p = .05$; Fig. 3). This correlation, however, was no longer significant if the individual with the highest scores

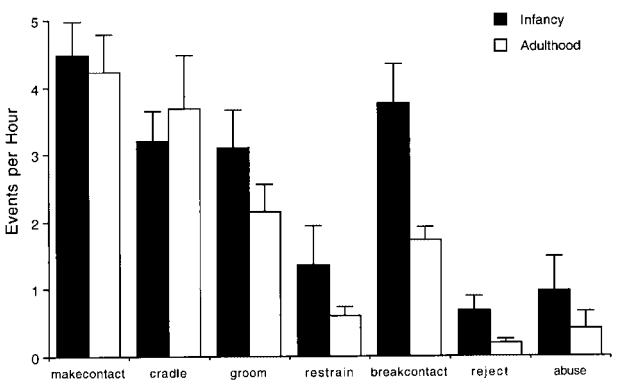

FIGURE 1 Mean (and SEM) hourly rates of maternal behaviors the crossfostered females experienced in infancy and exhibited in adulthood. Data refer to average scores across the first 3 months of life and the first 3 postpartum months. Differences are statistically significant for rates of contact-breaking and rejection.

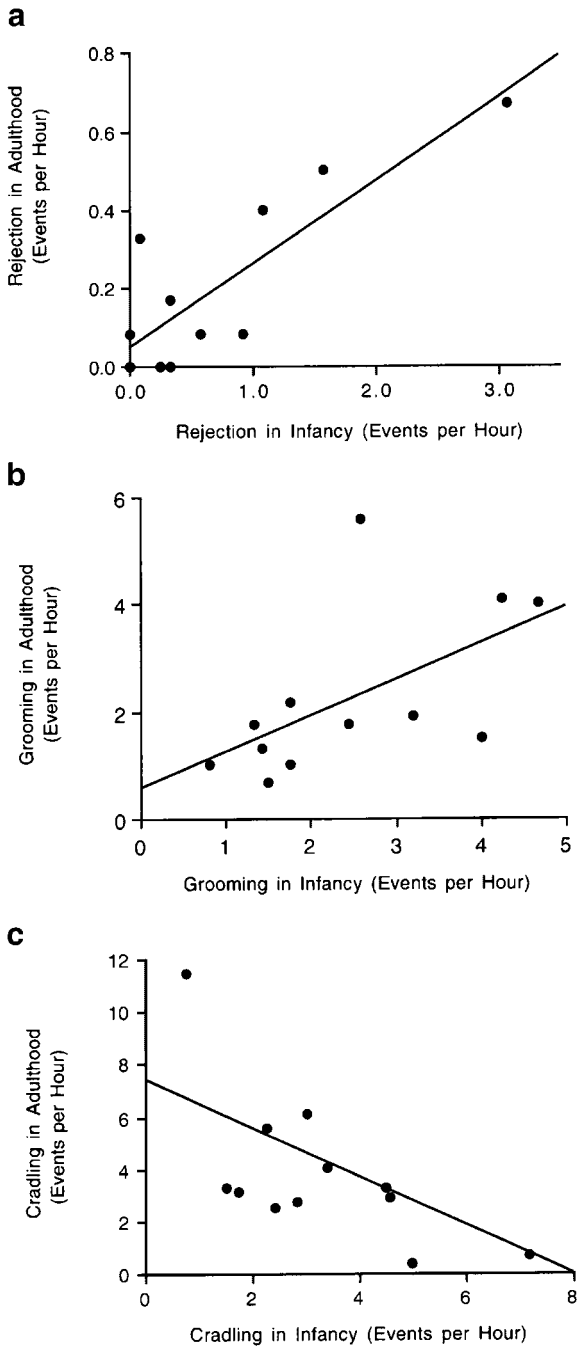

FIGURE 2 (a) Linear regression of rates of maternal rejection in infancy and adulthood. (b) Linear regression of rates of maternal grooming in infancy and adulthood. (c) Linear regression of rates of maternal cradling in infancy and adulthood.

of contact-making was excluded from the analysis. No other significant association was found between the daughters' behavior and that of their biological mothers.

The crossfostered females' CSF concentrations of 5-HIAA were negatively correlated with their rates of maternal rejection ($r = -.59$, $r^2 = .35$, $N = 13$, $p = .03$; Fig. 4) such that the individuals with lower CSF 5-HIAA exhibited higher rates of rejection with their infants. There was no significant association between CSF concentrations of 5-HIAA and other maternal behaviors. There were no significant correlations between CSF concentrations of HVA or MHPG and any maternal behaviors of the crossfostered females.

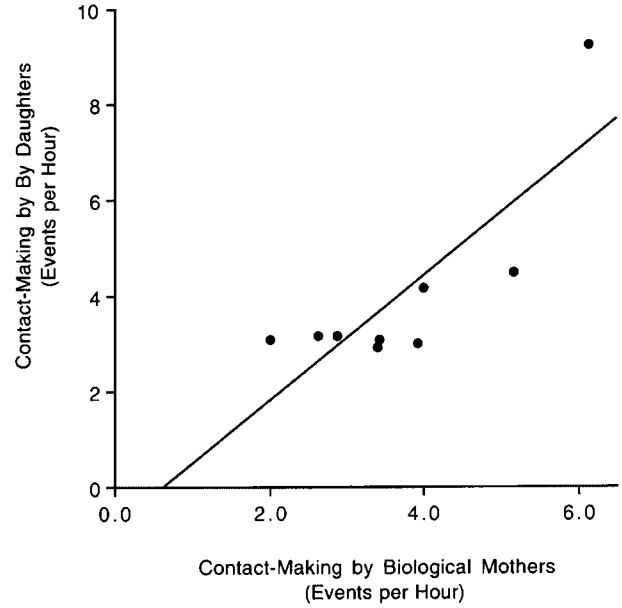

FIGURE 3 Linear regression of rate of maternal contact-making by adult crossfostered females and that of their biological mothers.

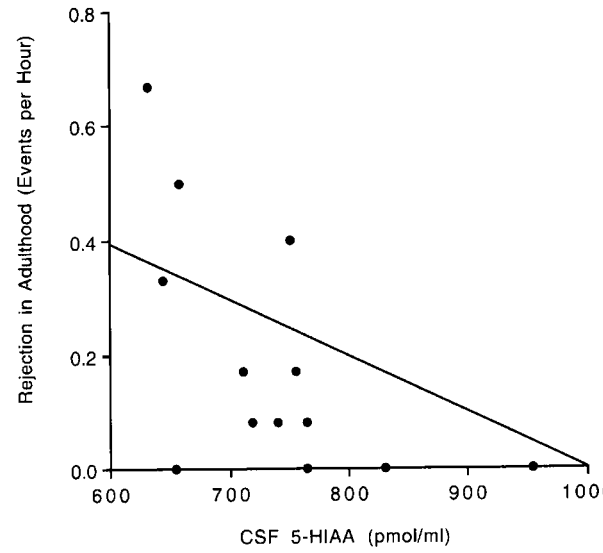

FIGURE 4 Correlation between CSF 5-HIAA concentrations in the second year of life and average hourly rates of maternal rejection by crossfostered females in adulthood.

DISCUSSION

The rates of maternal rejection and grooming exhibited by rhesus females that were crossfostered at birth and reared by unrelated females were positively correlated with those experienced in their first 3 months of infancy, that is, with the behavior of their foster mothers, while there was a significant but negative correlation between the rates of cradling of the crossfostered females and those of their foster mothers. These findings are consistent with a previously reported positive correlation between the rejection rates of rhesus macaque mothers and daughters (Berman, 1990) and suggest that this correlation is the result of early experience rather than genetic similarity.

The maternal rejection rates of crossfostered females were also significantly correlated with the CSF concentrations of 5-HIAA measured in their second year of life. Females with lower CSF 5-HIAA rejected their infants at higher rates than females with higher CSF-HIAA. There were, however, two individuals who had a low and an intermediate CSF 5-HIAA concentration but exhibited no maternal rejection in adulthood, suggesting that the relation between 5-HIAA and rejection is complex and potentially affected by other variables.

Although in this study the CSF concentrations of monoamine metabolites were only measured in the subjects' second year of life, other studies have shown that these concentrations are highly stable across the first 3 years of life (Higley et al., 1992; Maestripieri et al., 2006). Data presented elsewhere also showed that males and females reared by high-rejection mothers exhibit lower CSF concentrations of 5-HIAA in their first 3 years of life than individuals reared by low-rejection mothers (Maestripieri et al., 2006). Furthermore, individuals carrying the short and the long allele for the serotonin transporter gene did not differ significantly in their CSF 5-HIAA concentrations (Maestripieri et al., 2006). One possible interpretation of these findings is that exposure to relatively high rates of maternal rejection early in infancy results in long-lasting reduction in serotonergic function and that this, in turn, may contribute to the expression of high rates of maternal rejection with one's own offspring later in life. We have no direct evidence, however, demonstrating these causal mechanisms, and other explanations for these results are also possible.

In addition to maternal rejection, the crossfostered females were also similar to their foster mothers in their rates of infant grooming, whereas the correlation between rates of infant cradling was significant but negative. These behaviors were not correlated with each other, with other maternal behaviors, or with the CSF concentrations of monoamine metabolites of the crossfostered females. Therefore, although there may be a relationship between exposure to maternal grooming and cradling early in life and the manifestation of the same behaviors in the mother's role later in life, the specific mechanisms underlying this relationship remain unclear.

The rate with which the crossfostered females made contact with their own infants was predicted by the rate of contact-making, not of their foster mothers, but of their biological mothers. The association between the daughters' contact-making behavior and that of their biological mothers, however, was not strong and mostly driven by one individual. Although this result is consistent with a previously reported correlation between the rate at which crossfostered females made contact with other group members in their first 3 years of life and the rate of affiliative contact-making by their biological mothers (Maestripieri, 2003), the current data do not allow us to conclude that contact-making behavior is transmitted from mothers to daughters through genetic mechanisms (see also Fairbanks, 1989). The possible genetic basis of this maternal behavior trait should be further investigated in future studies, for example, by analyzing with quantitative genetic methods interindividual variation in rates of contact-making behavior in large populations of monkeys of known pedigree.

In addition to early experience and genetic factors, the maternal behavior of female monkeys can be potentially affected also by other variables such as the parity and caregiving experience of the mother, her dominance rank, the quality of the surrounding social environment (e.g., risk of aggression or availability of social support), and the sex of the infant (Hinde & Spencer-Booth, 1971). Young and inexperienced mothers are typically more protective and less rejecting than older and multiparous mothers (Hinde & Spencer-Booth, 1971; Maestripieri, 1993). In this study, the crossfostered females broke contact and rejected their offspring at lower rates than their foster mothers did with them in infancy, but there were no significant differences in measures of protectiveness such as rates of contact-making or restraining, or any other maternal behavior. The crossfostered females in this study were generally similar to their foster mothers in their dominance rank and in the characteristics of their social environment but differed from them in one important respect; 8 of the 13 crossfostered females in this study gave birth to a male infant whereas the maternal behavior of all of their foster mothers was recorded with female offspring (i.e., the crossfostered females themselves). Although the effects of infant sex on some measures of maternal behavior are only apparent with very large sample sizes (Maestripieri et al., 2006), it is possible that the similarities in maternal behavior between the crossfostered females and their foster mothers might have been even more marked if all the crossfostered females had given birth to female infants. These similarities might also have been stronger if a larger number of subjects had been

available. Although a small sample size is one of the methodological limitations of this study, successful infant crossfostering experiments are difficult in primates and previous studies using this procedure involved even fewer subjects (e.g., Owren, Dieter, Seyfarth, & Cheney, 1992; Suomi, 1987).

In conclusion, the results of this study show that nongenetic maternal effects play an important role in the intergenerational transmission of maternal behavior in primates and suggest that some of these effects may be mediated by specific physiological mechanisms. The ontogeny and intergenerational transmission of complex phenotypes such as maternal behavior in primates are complex processes, which are affected by multiple variables. These complexities may be even greater in humans than in monkeys. Nevertheless, nonhuman primates provide an excellent model for studying maternal effects on a variety of complex phenotypes and could make an important contribution to our understanding of the causes and consequences of individual differences in a wide range of adaptive and maladaptive behaviors.

NOTES

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