Stress-Induced Plasma Cortisol Concentrations in Infancy Are Associated with Later Parenting Behaviors in Female Rhesus Macaques (Macaca Mulatta)

Colt Halter
STRESS-INDUCED PLASMA CORTISOL CONCENTRATIONS IN INFANCY ARE ASSOCIATED WITH LATER PARENTING BEHAVIORS IN FEMALE RHESUS MACAQUES (*MACACA MULATTA*)

by
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Submitted to Brigham Young University in partial fulfillment of graduation requirements for University Honors

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Brigham Young University
August 2021

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ABSTRACT

STRESS-INDUCED PLASMA CORTISOL CONCENTRATIONS IN INFANCY ARE ASSOCIATED WITH LATER PARENTING BEHAVIORS IN FEMALE Rhesus Macaques (*Macaca mulatta*)

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Few studies have longitudinally assessed the relationship between infant stress reactivity and future parenting styles. Stress-induced plasma cortisol concentrations are stable over development and can be utilized as a marker for stress reactivity. This study investigates the relationship between stress-induced plasma cortisol concentrations in infancy and later parenting behavior in a translational nonhuman primate model. We hypothesized that higher stress-induced cortisol levels in infancy would predict impairments in maternal behaviors in adulthood. Subjects were rhesus macaque females (*N*=122; *Macaca mulatta*), assessed as infants and again as mothers. At three-to-four months of age, subjects underwent a standardized BioBehavioral Assessment during which blood samples were obtained and behavioral inhibition was assessed. Approximately seven years later, subjects were observed as they interacted with their own offspring for four 300-second sessions. Results showed subjects’ stress-induced cortisol concentrations and whether they exhibited behavioral inhibition as infants predicted later maternal behavior, with high cortisol concentrations and behavioral
inhibition predicting high rates of offspring approaches and leaves and low rates of maternal cradling. Results also showed higher stress-induced cortisol concentrations in infancy predicted higher scores on the Brown Index, an indication that the subjects’ offspring instigated changes in proximity. Taken together, these results suggest that higher stress-induced cortisol concentrations and behavioral inhibition at three-to-four months of age are a risk factor for engaging in less sensitive parenting behaviors as adults. To the extent these findings generalize to humans, they suggest a link between stress-induced cortisol concentrations and behavioral inhibition in infancy and parenting behavior later in life.
ACKNOWLEDGMENTS

I express my gratitude to the College of Family Home and Social Sciences for helping fund this research as well my thanks to Dr. J. Dee Higley and Dr. Elizabeth Wood for providing me with excellent mentorship.
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Introduction

Parenting styles are based on different dimensions of parental sensitivity, including parental responsiveness (warmth, sensitivity, and involvement) and parental demandingness (sometimes termed strict control, including active monitoring and restraint) (Baumrind, 1971; Maccoby & Martin, 1983), with such variation leading to long-term consequences for children. Research focusing on elucidating the association between different parenting styles and child outcomes shows that high quality, sensitive parenting is linked to positive peer relationships, academic achievement, and social adjustment; whereas, suboptimal, insensitive parenting styles are linked to poor child outcomes, such as disruptive behavior and drug abuse later in life (Chen et al., 1997; Dornbusch et al., 1987; Lamborn et al., 1991; Querido et al., 2002; Turner et al., 2009).

While it is well-established that early temperament predicts later-life personality, few studies have assessed the relationship between early-life temperamental stress reactivity and later parenting style. Temperament is biologically based (Rothbart & Ahadi, 1994). For example, studies show that the tendency to withdraw from novel situations and environments is trait-like (Coll et al., 1984) and is often referred to as stress reactivity (Talge et al., 2008), fearful temperament (Kalin et al., 1998), behavioral inhibition (Coll et al., 1984), anxious temperament, or simply reactivity (Suomi et al., 2011). This temperamental trait is mediated by an overactive sympathetic nervous system based on interindividually stable high levels of norepinephrine (Kagan et al., 1987; Rothbart, 1981), as well as high cortisol output in childhood (Kagan et al., 1987). Levels of cortisol are a traditional and well-studied biomarker of the tendency to withdraw from novel situations, (Kagan et al., 1987) and unfamiliar peers (Davis & Buss, 2012), as well
as the tendency to increase freezing during challenge (Shackman et al., 2017). Studies indicate that cortisol concentrations are trait-like, showing interindividual differences from childhood to adolescence in humans (Shirtcliff et al., 2012) and nonhuman primates (Higley et al., 1992; Wood et al., 2019). Other studies show that high cortisol is related to fearful temperament in preschoolers, as well as increased fear and anxiety across a number of provocative stimuli (Talge et al., 2008). Kagan and colleagues (1987) found that 21-month-old infants who were rated high in behavioral inhibition exhibited high cortisol as 5.5-year-olds. In that same study, high behavioral inhibition at 5.5 years of age was concurrently associated with high cortisol. Furthermore, Buss and colleagues (2003) found that, high basal and stress-induced cortisol were associated with high right frontal electroencephalography activity in six-month-old infants, a marker for behavioral inhibition and negative emotionality (Sutton & Davidson, 1997). These same six-month-old infants also showed a sad facial expression when an unfamiliar adult approached them (Buss et al., 2003). Another study showed that boys with negative affect as infants exhibited high basal cortisol levels four years later and showed high concurrent behavioral withdrawal (Perez-Edgar et al., 2008).

In nonhuman primates, cortisol is widely used as measure of stress reactivity. For example, high cortisol is positively correlated with a cluster of behaviors labeled as behavioral reactivity (cooing, scratching, distress calls, time spent alone, freezing, and lipsmacking) in nonhuman primates (Suomi et al., 2011; Kalin et al., 1998). This cluster of behaviors, referred to as behavioral inhibition by others (Chun & Capitanio, 2016) shows modest interindividual stability from infancy to later in life, with behavioral reactivity during infancy predicting more time spent alone as juveniles, as well as less
social grooming and more time alone as young adults (Chun & Capitanio 2016; Suomi et al., 2011). As cortisol is a biomarker of stress reactivity, and is stable across time, we propose that infants high in behaviors that characterize behavioral inhibition, with high stress-induced cortisol concentrations, would later exhibit less maternal cradling as mothers, and, as a consequence, their infants would leave and approach their mothers more often. The present study assesses this question, using a longitudinal design that utilizes a translational nonhuman primate model.

Stress reactivity plays a prominent role in the etiology of behavioral patterns and psychopathology. For example, individual stress reactivity is linked to notable pathological developmental outcomes, including post-traumatic stress disorder, schizophrenia, and impaired cognition (Corcoran et al., 2003; Elzinga et al., 2003; Kirschbaum et al., 1996; Steudte-Schmiedgen et al., 2015). As stress influences psychosocial functioning, it is possible that individual differences in stress reactivity may be linked to differences in parenting styles and sensitivity. For example, one study reports that variation in maternal stress reactivity mediates the relationship between maternal state, child temperament, and parental harshness (Matorell & Bugental, 2006). Given that stress-induced plasma cortisol concentrations in infancy are stable (Shirtcliff et al., 2012; Higley et al., 1992; Wood et al., 2019) and, given that high stress-induced plasma cortisol concentrations are linked to anxiety disorders and other internalizing behaviors (see, for example: Åhs et al., 2006; Henckens et al., 2016), they may also play a role in adult socioemotional outcomes, including parenting style.

To date, research investigating the role of temperamental stress reactivity as a predictor of parenting style is lacking. Long-term longitudinal studies in humans are
costly and time consuming, which may explain why, to our knowledge, studies have not assessed the relationship of early-life temperament and later adult parenting styles. Rhesus macaques (*Macaca mulatta*) are a translational nonhuman primate model that mature three-to-four times more rapidly than humans (Roth et al., 2004), allowing for such an assessment in a relatively short period. Moreover, the rhesus monkey has been widely used and validated as model of human development (Wood & Higley, 2018). As research subjects, rhesus monkeys have the added advantage that their environments can be closely controlled, leading to more homogeneous life histories. Moreover, recent studies show strong parallels between the structure of human and rhesus monkey infant temperament (Kay et al., 2010; Wood et al., 2020), which is likely due to their close genetic relatedness (Gibbs et al., 2007). Rhesus monkey mothers also exhibit individual differences in maternal sensitivity, paralleling that which is seen in humans (Fairbanks, 1996; Maestripieri, 1999; Sproul Bassett et al., 2020). As in human studies, optimal parenting styles in Old World monkeys are linked with positive developmental outcomes. For example, investigators found that mother-daughter contact in vervet monkeys, a marker of sensitive mothering, was predictive of the daughter’s future maternal behaviors (Fairbanks, 1989). Conversely, research assessing Old World monkey mothering shows that infant psychopathology and mortality are related to maternal rejection (Fairbanks, 1996; Hauser, 1993; Sproul Bassett et al., 2020). Given these parallels and the degree of experimental control that can be exerted in a translational nonhuman primate model, rhesus monkeys are an ideal model for elucidating the relationship between early temperament and adult parenting style.

In this study, we assess the relationship between early-life stress reactivity, as
measured by stress-induced plasma cortisol concentrations, and later parenting behavior. The paradigm used to induce stress involves a mother-infant separation and temporary relocation, known to be a potent stressor for infant rhesus monkeys (Smotherman et al., 1979). We hypothesize that high stress-induced cortisol concentrations early in life will predict less sensitive parenting behaviors later in adulthood.

**Methods**

Subjects were $N = 122$ rhesus macaque females housed at the California National Primate Research Center (CNPRC) in Davis, California. Subjects lived in large outdoor, half-acre (0.2 hectare) corrals in large social groups (50-to-100 monkeys of all age and sex classes) (Kanthaswamy et al., 2010) in social environments that closely-approximate the natural rhesus monkey social composition (matrilineal; female-headed extended families with multiple males, infants, and juveniles) (Lindburg, 1971). The study timeline is illustrated in Figure 1. Briefly, cortisol was obtained from subjects when they were three-to-four months old ($M_{age} = 105.37 \pm 0.91$ days) during testing in the CNPRC’s BioBehavioral Assessment program (Capitanio, 2017). Approximately seven years later ($M_{age} = 7.37 \pm 0.30$ years), subjects’ interactions with their own offspring (65 females, 57 males) were observed. Subjects’ offspring were approximately three months old at the time of observation ($M_{age} = 106.89 \pm 2.59$ days). All data were collected between the years of 2001-2019. All procedures were compliant with the protocols established by Institutional Animal Care and Use Committee at the University of California, Davis.
Figure 1. Depicts project timeline. At 3-4 months of age, the subjects were separated from their mothers and their larger social group and relocated for a 25-hour biobehavioral assessment. During the assessment, two blood samples were obtained. Approximately seven years later, the now-adult females were observed as they interacted with their own offspring for four 300 second sessions.

**Cortisol Sampling**

Two blood samples were examined in this study. The procedures for obtaining blood were described previously (Capitanio et al., 2005). Briefly, as infants, subjects were separated from their mothers and the larger social group for the 25-hour BioBehavioral Assessment (BBA), during which time they were individually housed in an unfamiliar location, away from their mothers and the larger social group. Because subjects had to be captured from half-acre, outdoor enclosures, obtaining a baseline sample was not possible. However, earlier work from our laboratory shows that infant social separation from mother and relocation results in robust infant cortisol increases. (Barr et al., 2004; Fahlke et al., 2000; Erickson et al., 2005; Higley et al., 1992). Thus, plasma cortisol concentrations were expected to be elevated, an indication that infants are experiencing some degree of stress that elevates the plasma cortisol above baseline.

Blood samples were collected via femoral venipuncture as subjects were manually restrained. Blood Sample 1 (1mL) was obtained at 1100, approximately two-hours after mother-infant separation and relocation. Blood Sample 2 (0.5mL) was obtained at 1600,
approximately seven-hours after separation and relocation. We note here that, as part of the BBA program, two additional samples were obtained; however, they are not considered in this work because they reflect pharmacological manipulations that are not germane to our hypotheses. Blood samples were obtained using unheparinized syringes and blood was immediately transferred to plastic EDTA tubes. Each blood sample was obtained in fewer than five minutes following removal from their holding cage. Samples were centrifuged at 4°C at 1277 g for 10 minutes. Plasma was pipetted into tubes and stored at -80°C until they were assayed for cortisol concentrations. All cortisol data were collected between 2001-2016. Samples collected before 2014 \((n = 108)\), were assayed using a commercial radioimmunoassay kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA). Samples collected after 2014 \((n = 14)\), were assayed using a quantitative competitive immunoassay (Siemens Healthcare Diagnostics, Tarrytown, New York, USA). For a description of each assaying procedure, see Vandeleest et al. (2019). For samples collected before 2014, assay sensitivity was 0.26069 μg/dL. Intra- and inter-assay coefficients of variability (CVs) were 5.18% and 8.30%, respectively. For samples collected after 2014, assay sensitivity was 0.2 μg/dL. Intra- and inter-assay CVs were 2.4% and 5.0%, respectively.

Subjects’ mean cortisol levels showed a significant increase from the first to the second sample \((t(118)=-2.43, \ p=.02; \ Sample \ 1: \ 86.85±2.26 \ μg/dL, \ Sample \ 2: \ 91.94±2.70 \ μg/dL)\). As sample 1 and sample 2 cortisol concentrations were highly correlated \((r=0.68; \ p<.0001)\), the concentrations were aggregated, and the aggregate scores were used in all analyses. This methodology has the advantage of reducing variance and increasing statistical power to detect a relationship between variables
Behavioral Inhibition

As part of the BBA, infant monkeys are assessed for their level of activity and emotionality over the two-day separation (see Golub et al., 2009 for a detailed description of the methodology and analytic procedures). Briefly, these measures are intended to reflect behavioral responsiveness to the separation and relocation (activity and emotionality scores on day-1) and adaptation to the situation (activity and emotionality scores on day-2). Day-1 observations were conducted approximately 15 minutes after subjects’ initial separation and relocation. Day-2 observations were conducted approximately 22 hours after subjects’ initial separation and relocation. For each of the assessments, an experienced observer sat 2.6 meters away from the subjects’ cage and recorded subjects’ behavioral reactions and changes in affect for 5 minutes. Factor analyses indicated two factors, Activity and Emotionality for day-1 and day-2. Behaviors that loaded into the Activity factor included proportion of total time locomoting, proportion of total time not hanging from the top or side of the cage, rate of environmental exploration, and the number of times the subject ate, drank, and crouched. An active state was recorded if the infant engaged in whole body movements, including steps and jumps. Environmental exploration was recorded if the infant discretely manipulated the physical environment or objects in the cage. Eating, drinking, and crouching were recorded dichotomously (i.e., whether the behavior was observed or not) as the mere occurrence of these behaviors indicates comfort in a new environment. Behaviors that loaded into the Emotionality factor included distress vocalizations, barking, scratching, threats, and lip-smacking. Distress vocalizations were recorded if the
infant made a medium-pitched, moderately intense, and clear call. Bark was recorded if the infant made a gruff and abrupt low-pitched vocalization. Scratching was recorded if the infant scratched itself—a common indication of anxiety. Threat was recorded if at least two or more of the following behaviors were observed: open mouth stare, head bob, ear flaps, and bark vocalizations. Lip-smacking was recorded if the infant engaged in rapid lip movement, usually with pursed lips, and accompanied by a smacking sound.

Activity and Emotionality scores were standardized and animals that received a score that was lower than zero on day-1 and day-2 Activity and day-1 and day-2 Emotionality were coded as behaviorally inhibited.

**Mother-Offspring Behavioral Observations**

When subjects reached adulthood and gave birth to their own offspring, they were observed as mother-offspring dyads in their social groups on four separate occasions. Each behavioral observation lasted 300 seconds and the behaviors were recorded by eight trained observers. Observers were assessed weekly during the study to ensure that an inter-rater reliability of $r > 0.85$ or above was maintained. Behaviors were recorded as frequencies using an established ethogram, comprising mutually exclusive and exhaustive behavioral categories, developed by our laboratory (see Table 1). Recorded behaviors included frequencies of offspring proximity and contact with mother, maternal approaches and leaves, offspring approaches and leaves, and maternal grooming of offspring (see Table 1 for detailed behavior definitions). To account for potential behavioral variability due to time of day (morning vs afternoon), the four observations were randomly distributed between morning and afternoon (i.e., two observations in the morning and two in the afternoon). All behavioral observations were conducted in the
summers months between 2016-2019 at the tail-end of the CNPRC rhesus monkey birthing season (Capitanio et al., 2006).

While the duration of focal sampling of mother-offspring behavior was relatively short, studies show that individual differences in maternal behaviors tend to be consistent over time, especially maternal cradling of offspring, the maternal role in contact initiations, and proximity indices, even when accounting for offspring age (Fairbanks, 1996; Hinde & Spencer-Booth, 1971). To assess whether the aggregate means across behavioral observations were interindividually stable and consistent, each of the behaviors were correlated across samplings. For each of the measured behaviors, a correlation between the average of the first two behavioral observations and the second two behavioral observations were positively correlated and statistically significant ($p < 0.03$), an indication of reliability of behavioral assessments. As the two means for each of the behaviors were correlated, all analyses used the overall mean of each behavior.

**The Brown Index**

To assess the subject-offspring relationship, the Brown Index (Brown, 2001), a modified version of the Hinde Index (Hinde & Spencer-Booth, 1971) developed to assess rhesus monkey mother-offspring relationships, was calculated for each dyad using the following formula: $100 \times \frac{(\text{offspring approaches} + \text{offspring leaves})}{(\text{offspring approaches} + \text{offspring leaves} + \text{mother approaches} + \text{mother leaves})}$. The Brown Index is intended to reveal which member of the mother-offspring dyad is responsible for maintaining the mother-offspring relationship, principally using the same proximity measures as the Hinde Index, but it has the advantage of not giving an ambiguous zero value if one member of the dyad has an equal number of entering and exiting proximity
of the other. The Brown Index ranges from zero to 100, with higher values indicating that the offspring is responsible for most of the changes in proximity and low values indicating that the mother is responsible for most of the changes in proximity.

**Mother-Infant Dyad Ethogram**

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximity</td>
<td>Mother is in physical proximity with offspring. Mother and offspring are not in contact, but offspring is within mother’s arm or leg’s reach.</td>
</tr>
<tr>
<td>Social Contact</td>
<td>Mother is in physical contact with offspring, but the ventrums of the mother and the offspring are not touching.</td>
</tr>
<tr>
<td>Mutual-Ventral Contact</td>
<td>The ventrums of the mother and the offspring are touching (the name cradling is used hereafter to provide a commonly used term to describe the relationship).</td>
</tr>
<tr>
<td>(Cradling)</td>
<td></td>
</tr>
<tr>
<td>Groom</td>
<td>Mother scratches, licks, or spreads her offspring’s fur with her fingers or mouth.</td>
</tr>
<tr>
<td>Mother/Infant Approaches</td>
<td>Mother/offspring approaches mother/offspring and is now within proximity.</td>
</tr>
<tr>
<td>Infant/Infant Leaves</td>
<td>Mother/offspring leaves proximity of mother/offspring.</td>
</tr>
</tbody>
</table>

*Table 1.* All behaviors were recorded using modified frequency sampling during four, 300-second observation periods.

**Data Analysis**

To assess the relationship between infant behavioral inhibition and stress-induced cortisol concentrations, an ANOVA was conducted, with whether the subject was behaviorally inhibited as the independent variable and mean cortisol concentration as the dependent variable, with year of data collection and age at BBA entered as covariates.

Multiple regression was first used to assess the relationship between mean early life cortisol concentrations and the later life parenting behaviors. Then, multiple regression was used to assess the relationship between mean early life cortisol
concentrations and the behavioral inhibition score and later life parenting behaviors.

Preliminary screening of the behavioral data was conducted and outliers greater than three standard deviations above/below the mean were removed. Preliminary analyses showed no effects of parity, subjects’ age, or subjects’ dominance rank on the outcome variables of interest ($p > .05$). Thus, these variables were excluded from formal analyses. There was, however, a significant year of data collection effect, therefore, year of data collection was controlled for in all analyses. Analyses of standard residuals was carried out, which showed that the data contained no outliers (Std. Residual Min > -2.18, Std. Residual Max < 3.05). Tests to see if the data met the assumption of collinearity indicated that multicollinearity was not a concern (Tolerance > 0.98, VIF < 1.14). The data met the assumption of independent errors (Durbin-Watson values range from 2.00-2.11). Scatterplots of standardized residuals showed that the data met the assumptions of homogeneity of variance and linearity, and the data met the assumption of non-zero variance. Because age of subjects’ offspring was correlated with maternal cradling, maternal grooming of offspring, and social contact, they were controlled for in these respective analyses. The mean of the two cortisol samples was used in all regressions, with mean cortisol the independent variable and the mean of each parenting behavior was used as the dependent variable. To assess which member of the mother-offspring dyad was responsible for maintaining proximity, a regression with mean infant cortisol concentrations as the independent variable and the Brown Index as the dependent variable was conducted. All analyses were conducted using SPSS, version 26 (IBM, 2018).
Results

Mean Cortisol Concentrations

Results showed that there was a significant difference in mean cortisol concentrations ($F(1,327)=5.227, p=.02$), with monkeys that were coded as behaviorally inhibited exhibiting higher mean cortisol concentrations ($M=93.81\pm3.85$), when compared to monkeys that were not behaviorally inhibited ($M=83.63\pm2.22$).

Mother-Offspring Behavioral Observations

Controlling for year of data collection and assay platform, results showed a significant positive relationship between subjects’ mean early-life plasma cortisol concentrations and subjects’ offspring approaches ($\beta = .231, p = .011$; Overall model: $R = .265; F(3,119) = 2.991, p = .034$; See Table 2 and Figure 2) and offspring leaves ($\beta = .212, p = .019$; Overall model: $R = .275; F(3,119) = 3.238, p = .025$; See Table 2 and Figure 3). After controlling for offspring age, assay platform, and year of data collection, results showed a significant negative relationship between the mean cortisol concentrations and maternal cradling ($\beta = -.190, p = .028$; Overall model: $R = .384; F(4,119) = 5.137, p = .001$; See Table 2 and Figure 4).
### Cortisol and mother-offspring behavior model summaries

<table>
<thead>
<tr>
<th>Model</th>
<th>Mean cortisol</th>
<th>$\beta$</th>
<th>$t$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Infant Approaches)$^a$</td>
<td>0.23</td>
<td>2.59</td>
<td>.01</td>
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<tr>
<td>Assay platform</td>
<td>-0.10</td>
<td>-1.05</td>
<td>.30</td>
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</tr>
<tr>
<td>Year of data collection</td>
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<td>.02</td>
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<td>Assay platform</td>
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<td>-0.93</td>
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<td>Year of data collection</td>
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<tr>
<td>Mean cortisol</td>
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<td>.03</td>
<td></td>
</tr>
<tr>
<td>Assay platform</td>
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<td>0.98</td>
<td>.33</td>
<td></td>
</tr>
<tr>
<td>Year of data collection</td>
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</tr>
<tr>
<td>Infant age</td>
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<td>.0001</td>
<td></td>
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<tr>
<td>Mean cortisol</td>
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<td>2.77</td>
<td>.007</td>
<td></td>
</tr>
<tr>
<td>Assay platform</td>
<td>-0.09</td>
<td>-0.92</td>
<td>.36</td>
<td></td>
</tr>
<tr>
<td>Year of data collection</td>
<td>0.01</td>
<td>0.06</td>
<td>.95</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Note: All significant $p$-values are bolded. $^a$ $R = .27; F(3,119) = 2.99, p = .034;$ $^b$ $R = .28; F(3,119) = 3.23, p = .03;$ $^c$ $R = .38; F(4,119) = 5.14, p = .001;$ $^d$ $R = .29; F(3,106) = 3.23, p = .03$

![Stress-Induced Cortisol Concentrations and Rates of Offspring Approaches](image)

Figure 2. Figure depicts the results from a linear regression, with the partial residuals on the y-axis and mean cortisol concentrations on the x-axis. The results show a significant positive relationship between mothers’ early-life plasma cortisol concentrations and rates of approaches by her infant ($\beta = .231, p = .011$).
Figure 3. Figure depicts the results from a linear regression, with the partial residuals on the y-axis and mean cortisol concentrations on the x-axis. Results show a significant positive relationship between mothers’ early-life plasma cortisol concentrations and rates of her offspring leaving close proximity ($\beta = .212, p = .019$).

Figure 4. Figure depicts the results from a linear regression, with the partial residuals on the y-axis and mean cortisol concentrations on the x-axis. Results show a significant negative relationship between mothers’ early-life plasma cortisol concentrations and rates of maternal cradling of her offspring ($\beta = -.190, p = .028$).
Controlling for year of data collection and assay platform, results showed a significant positive relationship between subjects’ mean early-life plasma cortisol concentrations and the Brown Index ($\beta = .261, p = .007$; Overall model: $R = .289$; $F(3,106) = 3.227, p = .025$; See Table 2 and Figure 5).

![Stress-Induced Cortisol Concentrations and the Brown Index](image)

*Figure 5.* Figure depicts the results from a linear regression, with the partial residuals on the y-axis and mean cortisol concentrations on the x-axis. Results show a significant positive relationship between mothers’ early-life plasma cortisol concentrations and the Brown Index ($\beta = .261, p = .007$). Higher Brown Index values indicate that the offspring is more responsible than the mother for maintaining the relationship, as measured by high rates of entering and exiting the mother’s proximity.

There was no relationship between subjects’ mean early-life plasma cortisol concentrations or behavioral inhibition and future rates of proximity, contact, grooming, maternal approaches and leaves of offspring ($p > .05$).

Results showed mean cortisol concentrations and behavioral inhibition scores were significantly related to later life parenting behaviors, including maternal cradling (cortisol: $B = -0.20, p = 0.02$; inhibition: $B = 0.19, p = 0.03$; infant age: $B = -0.32, p < 0.0001$;
overall model: $F(3,110)=8.00, p<.0001$), infant approaches (cortisol: $B=0.19, p=0.04$; inhibition: $B=0.22, p=0.02$; overall model: $F(2,103)=5.32, p=.006$), and infant leaves (cortisol: $B=0.26, p=0.04$; inhibition: $B=0.26, p=0.006$; overall model: $F(2,107)=6.54, p=.002$).

**Discussion**

Consistent with the overall hypothesis, results showed that the subjects’ early-life plasma cortisol predicted later adult parenting style, with high stress-induced plasma cortisol concentrations in infancy predicting low rates of maternal cradling later in life (see Figure 4), as well as high rates of offspring approaches (see Figure 2) and leaves (see Figures 3). Subjects’ early-life plasma cortisol concentrations showed a positive relationship with the Brown Index (Brown, 2001), a measure indicating which member of the mother-offspring dyad is initiating entering and leaving proximity to one another (see Figure 5). Given that dyads exhibiting high Brown Index scores involved subjects with high early life stress-induced cortisol concentrations (see Figure 5), these results suggest that the offspring of those subjects initiated the changes in proximity and took a more active role in the relationship than did the subjects themselves. It is unusual for infant rhesus monkeys at such an early age (approximately 3 months) to take a more prominent role than their mothers in maintaining proximity (Hinde & Spencer-Booth, 1967), and these findings are consistent with the hypothesis that mothers with high stress-induced cortisol concentrations in infancy would show less sensitive parenting behaviors later in life. That high stress-induced plasma cortisol in infancy predicted the low rates of maternal cradling, high rates of offspring approaches and leaves, as well as higher scores on the Brown Index in adulthood, suggests that females that showed high stress reactivity
in infancy, as measured by stress-induced plasma cortisol concentrations, are at risk to exhibit less sensitive parenting behavior as adults.

At an age when socially and motorically immature offspring attempt to explore, sensitive mothers play a primary role in retrieving and maintaining proximity to protect them from risky social interactions or physical injury and provide a secure base when they are overaroused (Wood & Higley, 2018). Studies show that mothers that fail to protect and provide a secure base for their offspring are prone to have offspring that exhibit anxiety and atypical socioemotional development (McCormack et al., 2006; Sproul Bassett et al., 2020). The association between high early-life cortisol and a lack of secure base, as measured by maternal cradling of offspring and a failure of the mother to approach and retrieve her immature offspring when it attempted to explore, (see Figures 2 and 3), is an indication of an aberrant mother-offspring relationship at this early age (Fairbanks, 1996). The greater rates of approaches and leaves by the offspring suggest the mother’s disengagement, perhaps letting the offspring exert more control over the relationship, which may lead to earlier offspring independence, but at the cost of poor infant outcomes, including indications of anxiety and aggression (Fairbanks, 1996; Sproul Bassett et al., 2020). Subjects with high stress-induced cortisol concentrations in infancy were part of the dyads that exhibited higher Brown Index scores later in life (see Figure 5), which is a measure of which member of the dyad has primary responsibility for maintaining proximity. While speculative, these findings suggest that individuals with high stress reactivity in infancy may be less sensitive to the needs of their future offspring. Therefore, the offspring of subjects that showed high stress reactivity in infancy may be engaging in high rates of approaches and leaves as a strategy to mitigate
low quality maternal behavior. Future studies should investigate the long-term outcomes of the offspring to determine whether offspring that use this type of strategy are protected against the consequences of lower quality parenting. While causation cannot be established from correlation, another possible explanation of these findings is that the offspring of mothers with trait-like high stress-reactivity exhibit frequent approaches and leaves because their mothers are inattentive to their needs. An alternative, but not inconsistent explanation, is that the offspring of mother with trait-like high stress reactivity may rely less on maternal support, leading to premature independence. Studies show that rhesus monkey infants that show premature independence due to maternal insensitivity tend to exhibit more aggression and anxiety (Sproul Bassett, et al., 2020). Future studies should investigate whether offspring of individuals with trait-like high stress reactivity show similar long-term differences in behavior.

Although the actual parenting behaviors of humans and rhesus monkeys are not identical, in both species parental behaviors focus on providing a secure base to reduce infant arousal and shaping the infant’s social behaviors, eventually leading to social competence. The behaviors that were measured in this study are typically used to characterize the quality of secure attachment (De Wolff & Van Ijzendoorn, 1997; Hinde & Spencer-Booth, 1967). Mothers that are sensitive to their infants’ state and needs are more likely to produce a secure attachment, which leads to positive adjustment and social outcomes in their developing offspring (Brunelli et al., 2014; Groh et al., 2014). The findings of this study suggest that stress reactivity in infancy, as measured by stress-induced plasma cortisol concentrations, may be a useful indicator of future parenting style. To the extent that interindividual differences in stress-induced cortisol
concentrations are a marker of stress reactivity, infant stress reactivity may be associated with long-term behavioral patterns, including parenting style. Kagan’s work and others show that early-life cortisol and anxiety-like temperament early in life is interindividually stable (Kagan et al., 1989; Suomi et al., 2011), increasing the risk for anxiety-related diagnoses (Hirshfeld et al., 1992; Sandstrom et al., 2020), and may be a marker for future parental insensitivity.

In this study, stress-induced cortisol concentrations were used as a proxy for stress reactivity. As noted earlier, the subjects were living in large outdoor corrals, it was not possible to obtain a baseline cortisol measure from the subjects in this study. However, the cortisol levels of the subjects in this study clearly reflect the stress of a mother-infant separation and relocation stressor, with mean cortisol levels of 90.14±2.14 μg/dL, a mean 2-3 times higher than baseline cortisol measures obtained in other studies or infant rhesus monkeys in our laboratory (Barr et al., 2004; Fahlke et al., 2000; Erickson et al., 2005; Higley et al., 1992). These cortisol values are indicative of the stressful nature of social separation, with the values for these infants higher than that seen in infants during laboratory and feral social separations (Higley et al., 1991). The time of the stress-induced cortisol peak is not certain, but the results showed that the mean of the second plasma cortisol sample was still elevated above the first sample mean, an indication that the separation and relocation-induced cortisol remained stressful seven hours after social separation. The findings of this study show that the stress response to mother-infant separation may have important implications for later life outcomes, particularly in terms of parenting style later in life. Another interesting question to consider is whether baseline levels of cortisol in infancy are similarly predictive of later
parenting style. Future studies would benefit from assessing the relationship between basal and stress-induced cortisol concentrations in infant rhesus monkeys, including whether the stress-induced change from baseline cortisol concentrations has a relationship with later life outcomes, particularly given that baseline plasma cortisol concentrations are correlated with stress-induced cortisol concentrations in human adults (Oswald et al., 2006). Future studies assessing the ongoing relationship between infant basal and stress-induced cortisol levels and Brown Index scores would also be of interest, as they may provide insight into whether less sensitive maternal behavior is a factor in chronic cortisol elevation. Some studies show long-term, stable, interindividual differences in cortisol (Higley et al., 1992; Wood et al., 2019). While somewhat speculative, as a hypothetical construct, early-life temperamental stress reactivity may form the basis of certain adult personality traits, which may underlie variation in parental behavior. As studies indicate that stress reactivity may be related to trait-like behavioral inhibition (Kagan et al., 1987), future studies should assess the relationship between infant stress reactivity and concurrent and later-life behavioral inhibition. Furthermore, future studies evaluating the relationship between stress-induced plasma cortisol concentrations in infancy and stress-induced plasma cortisol concentrations in adulthood, specifically in mothers, would, in part, validate this postulate. Subsequent studies may benefit from utilizing concurrent measures of cortisol and maternal temperament to clarify this relationship.

To our knowledge, this is the first study to longitudinally assess the relationship between infant stress-induced plasma cortisol concentrations and later maternal behaviors. This study provides an important insight into early-life variables that are
associated with later parenting styles. Measuring variables at an early age that are hypothesized to predict or lead to aberrant parenting styles is an important step in understanding and promoting positive long-term child outcomes (Chen et al., 1997; Lamborn et al., 1991; Querido et al., 2002), which could lead to interventions that reduce risky parenting behaviors and promote normative infant development. Given that early intervention is typically more effective than later interventions (Guralnick, 2004; Thomaidis, Kaderoglou, Stefou, Damianou, & Bakoula, 2000), this study identifies one factor that increases risk for later maladaptive parenting. To the extent that these findings generalize to humans, they suggest that cortisol as an early-life proxy for inhibited temperament is associated with later life parenting style, and that it may predict risk for later life insensitive maternal styles. Identifying early factors that modulate future healthy parenting is an important research goal, given research suggesting that parenting style is related to a myriad of long-term child outcomes (Chen et al., 1997; Lamborn et al., 1991; Querido et al., 2002). Taken together, these findings are an important step towards disentangling the underlying etiology of quality and high-risk parenting styles and may help promote healthy parent-infant relationships.
References

Åhs, F., Furmark, T., Michelgård, Å., Långström, B., Appel, L., Wolf, O. T., ... & Fredrikson, M.


Science, 305(5689), 1423-1426.


doi:10.1353/csd.0.0073


