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Biobehavioral Consequences of Prenatal Exposure to A Matrilineal Overthrow and Relocation in Captive Infant Rhesus (*Macaca mulatta*) Monkeys

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Abstract

There is a general consensus that perinatal experiences help to shape infant behavior; however, relatively little is known about the effects of prenatal experience on postnatal phenotype in non-human primates. The current study sought to take advantage of a naturally-occurring incident in a captive population of rhesus monkeys. Following a matrilineal overthrow in an outdoor field cage, pregnant female rhesus macaques were relocated from outdoor to indoor housing. Using data collected from the California National Primate Research Center's Biobehavioral Assessment Program, we assessed infants born to mothers that were in their first or second trimester of pregnancy during the overthrow and relocation, and compared their data with that of animals from two control groups born in the same year: indoor mother raised infants and field cage reared infants. Our results suggest that the experience of an overthrow and relocation during the first trimester elevated postnatal emotional responsiveness, while the same experience in the second trimester resulted in modified HPA axis regulation, elevated glucocorticoid output following maternal separation, and lower hematocrit levels compared to control groups. These data add to a growing body of literature that prenatal experiences represent a significant contribution to postnatal phenotypic variability. Findings such as ours have implications for studies in captive management and the management of captive rhesus monkey populations.

Keywords

Prenatal stress; Cortisol; Infant Behavior; Temperament

Introduction

It is well-known that early experiences can contribute to individual phenotypic variability [Lumaa and Clutton-Brock, 2001; Wells, 2003, 2007; Kuzawa, 2007; Harris and Seckl, 2011], and in non-human primates, postnatal rearing conditions of macaque infants can strongly influence individual variation in biobehavioral characteristics. For example, in an early study of 6-month old macaques, Meyer, Novak, Bowman, and Harlow [1975] found that infants reared by their mothers had an elevated cortisol response to capture and removal of animals in an adjacent cage, compared to surrogate-peer-reared infants [Meyer, Novak, Bowman, and Harlow, 1975]. A large literature exists showing the effects of variation in

postnatal experience on indicators of biobehavioral organization [e.g., Capitanio, 1986; Mason & Capitanio, 1988; Mason, 2000].

Although there is good evidence of a relationship between infant postnatal experience and biobehavioral organization in non-human primates, we know relatively little about the role of prenatal experience on postnatal behavior and physiology. It is well-known that the prenatal environment consists of a series of sensitive periods of development, where experiences can influence infant behavior and physiology [Carmichael, 1970; Kuo, 1976]. Over the past two decades, a significant amount of non-primate research has been conducted to study the influence of maternal stress during pregnancy on offspring neurobiological and behavioral outcomes. Studies in rats, for example, have shown that exposure of a pregnant dam to environmental stressors (e.g. noise, capture, immobilization, crowding, introduction of a strange male) often results in altered physiological function, decreased birth weight, and a shift in male/female ratio in their litters [Barlow, Knight, & Sullivan, 1978; deCatanzaro & Macniven, 1992]. Although rodent studies represent a significant contribution to our understanding of maternal stress effects during the prenatal period on offspring behavior and physiology, there have been only a few studies conducted with non-human primates.

Nonhuman primates are an excellent model system to study the effects of prenatal stress due to long gestational periods with slow-paced fetal growth rates, enriched placental nourishment, and high rate of single births, which makes them more similar to humans than are rodent models [Newell-Morris & Fahrenbruch, 1985]. A majority of the studies of prenatal stress conducted in non-human primates have used an acoustic startle paradigm to induce stress: pregnant females are acutely disturbed (for a 10-min period) five days per week by a 1-sec broadcast of a 110-dB horn, randomly at 1 – 4-min intervals (see Coe et al., 2003 for a detailed description of the procedure). Schneider, Roughton, Koehler, and Lubach [1999] found that pregnant macaques that were exposed to the acoustic startle paradigm were more likely to give birth to offspring that show delayed motor development, and more emotional and anxious reactions to novel stimuli. Repeated acoustic startle during pregnancy has also been shown to alter the newborn's emotional reactivity to stress [Clarke et al., 1994], affect changes in brain monoamine levels in juvenile monkeys [Schneider et al., 1998], and reduce the total amount of anaerobic lactobacilli in the gut of infant monkeys [Bailey, Lubach, Coe, & Christopher, 2004].

There is mounting evidence to suggest that the timing of prenatal stress has a significant impact on both behavior and physiology. Pregnant female rhesus macaques exposed to acoustic startle in mid-gestation produce offspring that have an especially high risk of developing significant behavioral, neural, and physiological abnormalities [Clarke & Schneider, 1993; Coe et al., 2003]. Coe and colleagues have found in rhesus macaques that infants born to mothers that were prenatally disturbed in mid-gestation displayed low levels of blood iron concentrations [Coe et al., 2007] and lower cytokine production [Coe, Kramer, Kirschbaum, Netter, & Fuchs, 2002], compared to infants born from mothers with late gestation disturbances and control infants. In contrast, rhesus macaque infants that experience stress in late pregnancy have been found to display lower bifidobacteria concentrations [Bailey, Lubach, & Coe, 2004] compared to mid-gestation stress infants and controls.

While laboratory studies have demonstrated how a pregnant female's experience of stress can impact her offspring's development, little is known about how more naturally occurring stressors during pregnancy might affect offspring. Given that rhesus monkeys, in particular, are a highly social species, with their groups comprising multiple matrilineal lines that form a dominance hierarchy, one might expect that social stressors could be potent influences on prenatal development. One experience that can have significant consequences for group stability is a matrilineal overthrow, which results when members of multiple matrilineal lines band together to attack the alpha matriline [Bernstein & Sharpe, 1966]. Due to their often violent nature, matrilineal overthrows usually result in the deposition of the female alpha line, with the next-lowest ranked females taking their place at the top of the hierarchy. Matrilineal overthrows can cause a great deal of disturbance in a group of rhesus macaques, and in some cases, females can be physically injured or killed following the disintegration of a group's social order.

The literature describing matrilineal overthrows is small. Overthrows have been observed in both wild and captive populations of primates [Chance, Emory, & Payne, 1977; Samuels & Henrickson, 1983; Ehardt & Bernstein, 1986], and there is evidence that such events can lead to elevated cortisol concentrations [Engh et al., 2005]. We believe that matrilineal overthrows in outdoor field cages represent a significant, semi-naturalistic stressor for captive colonies of rhesus macaques, and that pregnant females and their unborn offspring represent a potentially sensitive population. To date, we are aware of no study that has examined the consequences for infant development of having experienced a matrilineal overthrow *in utero*. Moreover, because one goal of captive care is to enhance well-being of animals, the potential negative consequences of a matrilineal overthrow may be compounded by relocation of the animals to different housing in order to stop the aggression. At our facility, such relocations are common responses to overthrow attempts. Demonstration of the consequences for the fetus of experiencing a matrilineal overthrow compounded by relocation may result in proactive strategies to minimize such consequences when future overthrows occur.

The primary goal of the present study was to test the hypothesis that there are biobehavioral differences in rhesus macaque infants whose pregnant mothers were relocated from an outdoor field cage to an indoor housing facility following an overthrow attempt. An additional goal of the study was to test the hypothesis (based on previously described studies of laboratory stressors) that pregnant female macaques that experienced an overthrow/relocation in their second trimester will produce infants with markedly different biobehavioral profiles than infants born to mothers that had this experience during their first trimester of pregnancy. We expected that cortisol output and infant emotionality would be particularly sensitive to prenatal experience, as previous studies have shown [Clarke et al., 1994; Schneider, Moore, Kraemer, Roberts, & DeJesus, 2002; Buitelaar, Huizink, Mulder, de Medina, & Visser, 2003; Pryce, Aubert, Maier, Pearce, & Fuchs, 2011]. We tested our hypotheses using data collected from an ongoing biobehavioral assessment program at the California National Primate Research Center, which was designed to study variation in behavior and physiology in young macaque infants across the colony.

Methods

The current study complied with protocols approved by the University of California, Davis, Animal Care and Use Committee (IACUC), and adhered to the legal requirements of the country in which the research was conducted, and also complies with the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Non-Human Primates.

Subjects

We examined four groups of animals in the current study. The two experimental groups were field cage reared animals that experienced a matrilineal overthrow and relocation in either the first trimester (TRI1) or the second trimester (TRI2). The two comparison groups were typical field cage reared infants (FCR), and typical indoor mother reared infants (IMR) from the same birth year as the TRI groups. Demographic information for each group can be found in Table 1. We found no significant between-group differences in age or weight at testing. Our methods for group selection are described below. The TRI1 and TRI2 subjects comprised 20 infants whose mothers were relocated in December 2012 from an outdoor 0.2 ha field-cage to indoor individual housing following a matrilineal overthrow. (We note that a total of 24 pregnant females were relocated from the field cages to indoor housing; one female [which would have delivered a TRI2 animal] had a stillbirth, two females rejected their infants [which would have been TRI1 animals], and one female could not be followed up due to a record-keeping problem.) Infant subjects were born in 2013. We estimated conception date by assuming each animal experienced a full 165 day gestation, and counted backwards from the birth date. The 165 day period of gestation was divided into three 55-day trimesters, and each subject was identified as having experienced the overthrow/relocation either in the first (TRI1) or second (TRI2) trimester. We assessed 13 TRI1 (date of the relocation occurred between 26 and 49 days of gestation) and 7 TRI2 (range of gestational age is 56 to 69 days) infants.

One comparison group (FCR) comprised infants that were conceived, gestated, and born into one of the 24 half-acre outdoor enclosures at the CNPRC. Each cage is enclosed by chain-link at the sides and top with a natural substrate, several A-frame structures that provide areas for sitting and protection from the elements, a number of physical structures that provide climbing opportunities, and several food hoppers. Purina chow is provided twice daily, with fresh fruit and vegetable supplements provided twice weekly. Water is available *ad libitum* from Lixit devices that are located along the periphery of the enclosure. Approximately 60–150 animals reside in each field cage. In the current study, we assessed infants born in 2013 to mothers in a field cage that was comparable (in terms of group composition) to the cage in which the matrilineal overthrow took place. We assessed a total of 25 FCR infants.

The second comparison group (IMR) were infants of mothers that participated in CNPRC's timed-mating program. These females are housed in standard-sized individual cages that are located indoors; consequently, these infants were conceived, gestated, and born indoors. Animals are fed twice-daily, water is available *ad libitum*, and fresh produce is provided twice weekly. Infants are raised with their mothers from birth in single cages. We assessed 13 IMR infants. IMR, TRI1, and TRI2 mothers all received similar socialization experiences

prior to giving birth (i.e., most were pair-housed by removing the divider between two individual cages).

General Procedures

Procedures for the BBA program have been described in detail elsewhere [Capitanio et al., 2005; Capitanio et al., 2006; Golub et al., 2009]. Briefly, in this program, 90–120 day old infants are removed from their living cages, relocated to a testing area, and housed in individual, standard-sized cages for a 25-hr period, during which they experience multiple behavioral assessments and blood sampling (see below) before being returned to their mothers and living cages. Animals in the BBA program are tested in cohorts of up to 8 individuals at a time; for the present study, the TRI1, TRI2, IMR animals were tested in two cohorts each, and FCR animals were tested across 8 cohorts.

Holding Cage Observations

To assess individual responses to separation and relocation, one co-author (LDR) observed each infant for a 5-min period beginning 15 min after initial relocation (Day 1) and approximately two hours from the end (Day 2) of the 25-hr BBA period. For these assessments, an individual observer sat in front of the row of cages at a distance of 2.4 m. Focal animal sampling was used, and data were recorded with the use of the Noldus Observer program (Noldus, 1991). Exploratory and confirmatory factor analyses of the behavioral data (Golub et al., 2009) identified two factors, Activity and Emotionality. In general, these measures are interpreted as reflecting behavioral responsiveness to the immediate separation and relocation (Day1 Activity, Day1 Emotionality) and adaptation to the novel testing situation (Day 2 Activity, Day 2 Emotionality).

Human Intruder Test

The human intruder test involves a standardized assessment of infants' responsiveness to specific challenging stimuli. Testing took place in a test cage in a room adjacent to the animals' holding cages approximately 5 hours after the animals' arrival in the testing room. Procedures are described in detail elsewhere [Gottlieb & Capitanio, 2013]. In brief, a technician presents her profile and her frontal face from a near (0.3m) and far (1.0m) position, and the animal's behavior is recorded. Exploratory and confirmatory factor analyses [Gottlieb & Capitanio, 2013] revealed a four factor structure to the data: Activity, Emotionality, Aggression, and Displacement.

Temperament Ratings

Temperament ratings provide an overall picture of each infant's behavioral characteristics during the period of testing. At the end of the 25-hr. assessment, each animal was rated on a 1–7 Likert-type scale for 16 broad behavioral traits (e.g. active, aggressive, fearful, gentle). Ratings on each descriptive adjective were z-scored across all subjects and subject to exploratory and confirmatory factor analysis (described in detail in Golub et al., 2009). Four factors were identified (named for the trait adjective that had the highest positive loading): Vigilant, Confident, Gentle, and Nervous.

Blood Sampling

Blood was sampled via femoral venipuncture on four occasions during the 25-hr period, to assess infants' responses to separation and relocation, and to determine the regulatory characteristics of the hypothalamic-pituitary-adrenal (HPA) axis. The first sample (1ml) was drawn approximately 2-hr. after the infants' arrival in our testing area, and was used for assessment of plasma cortisol concentrations and a complete blood count. The second sample (0.5 ml) was taken at the end of testing on Day 1 at approximately 1600 h. Immediately after the second sample was taken, each animal was injected intramuscularly with dexamethasone (500ug/kg) in order to suppress endogenous glucocorticoid output. Sample 3 (0.5 ml), which reflects the efficiency of negative feedback, was taken at 0830 h on Day 2, after which each animal received an injection of 2.5 IU of ACTH. The final sample (0.5 ml), which reflects adrenal responsiveness, was taken thirty minutes after the injection.

For all samples, blood was drawn into non-heparinized syringes and aliquotted into tubes containing EDTA. One tube (0.5 ml) of whole blood from Sample 1 was given to CNPRC's Clinical Laboratory for performance of a complete blood count using a Pentra 60C+ analyzer. The remaining tubes were centrifuged at 1900 G at 4°C for 10 min. Plasma was aliquotted into cryotubes for storage at -80C until assay. Cortisol concentrations were assessed via RIA using commercially available kits (Siemens Medical Solutions Diagnostics, Los Angeles, CA). Coefficients of variation were 4.20% (within-assay) and 6.10% (between-assay).

Statistical Analysis

Holding cage behavior (Activity, Emotionality) was analyzed using two-way analysis of variance (ANOVA) for group by day. A one-way analysis of variance for group was performed to examine the human intruder data and the temperament ratings. Data were found to be non-normal for the activity and displacement factors in the human intruder test, so a transformation was performed: we scaled the data by adding 1.82 to every measure, and then calculated the reciprocal of each variable. For the displacement data, most of the individuals had the same value, a likely cause of the observed non-normality. As such, we ran a non-parametric Kruskal-Wallis test with pairwise comparisons for the displacement data. Finally, we used one-way ANOVA to test whether measures of HPA-axis regulation (cortisol output) and hematology measures differed between groups. A more appropriate repeated-measures ANOVA for the cortisol data was not performed due to reduced sample size resulting from occasional missing samples. Following identification of a significant group effect, we used Bonferroni-corrected paired samples t-tests to delineate within-group differences in blood cortisol levels across the 4 samples.

Results

Holding Cage Observations

TRI1 animals displayed significantly higher levels of Emotionality compared to FCR animals; levels for the other groups were intermediate and not significantly different from either the TRI1 or FCR groups (Figure 1). This result was indicated by a significant main

effect of group $F_{(3,50)}=4.25$; $P<0.01$, for the Day 1 and Day 2 Emotionality scores; there was no significant main effect of day or the group by day interaction. No significant effects were found for Activity.

Human intruder

Group differences were evident for the Activity, Emotionality, and Displacement factors. For both the Activity and Emotionality measures, the one-way ANOVAs revealed that the three indoor-reared groups (TRI1, TRI2, IMR) had significantly higher levels compared to FCR animals (Activity: $F_{(3,54)}=21.21$; $P<0.001$; Emotionality: $F_{(3,54)}=4.89$; $P<0.05$); differences among the three groups were not significant (Figure 2). A Kruskal-Wallis test revealed a significant overall effect of group on Displacement behaviors in the human intruder test, $H_{(3,54)}=13.015$; $P<0.01$; pairwise comparisons showed that Displacement behaviors were significantly higher in the TRI2 group compared to the IMR ($P < 0.01$) and FCR ($P < 0.01$) groups.

Temperament

We did not find any significant main effects of group on the Vigilant, Confident, Gentle, or Nervous temperament factors (all $P > 0.50$).

Hypothalamic-pituitary-adrenal regulation

In general, the TRI2 animals had the highest concentrations of plasma cortisol (Figure 3). One-way ANOVAs revealed a significant group effect for sample 1 ($F_{(3,50)}=5.07$; $P<0.01$) and a near-significant effect for sample 2 ($F_{(3,50)}=2.71$; $P=0.054$), but not for samples 3 or 4. Follow-up tests revealed that, for samples 1 and 2, TRI2 plasma cortisol levels were significantly higher than the TRI1 group ($P<0.05$ for each sample) and the FCR animals ($P<0.01$ for sample 1; $P<0.05$ for sample 2). For sample 1, IMR animals also had significantly higher cortisol levels compared to FCR animals ($P<0.01$).

Inspection of means, however, suggested that TRI2 animals may not have been showing clear differentiation between the four blood samples, unlike animals in the other three groups. To explore this possibility, we conducted separate post-hoc repeated measures one-way ANOVAs for each of the four groups; analyses revealed significant sample effects for TRI1 (Wilks' Lambda = .137, $F_{(3,12)}=12.22$; $P=.001$), IMR (Wilks' Lambda = .122, $F_{(3,12)}=9.83$; $P=.001$), and FCR (Wilks' Lambda = .195, $F_{(3,21)}=23.99$; $P=.001$) groups, but the sample effect was not significant for the TRI2 group (Wilks' Lambda = .176, $F_{(3,5)}=.430$; $P=.734$). Our data suggest that the TRI1, IMR, and FCR infants had the expected significant reduction of plasma cortisol following dexamethasone suppression, while the TRI2 animals did not. Three post-hoc paired samples t-found a significant reduction between samples 2 and 3 (before and after dexamethasone suppression) for the TRI1 (sample 2 $M=87.28$, $SD=31.02$, sample 3 $M=56.67$, $SD=20.65$; $t(12)=3.68$, $P=.003$), IMR, (sample 2 $M=95.89$, $SD=28.47$, sample 3 $M=62.66$, $SD=25.30$; $t(12)=3.14$, $P=.009$) and FCR groups (sample 2 $M=81.26$, $SD=17.67$, sample 3 $M=64.10$, $SD=18.61$; $t(24)=5.331$, $P=.000$). The same comparison for the TRI2 sample was not significant (sample 2 $M=110.11$, $SD=35.40$, sample 3 $M=99.93$, $SD=114.15$; $t(5)=.264$, $P=.802$).

Hematology

Groups differed significantly for hematocrit; $F_{(3,51)}=5.93$; $P<0.001$) (see Figure 4). TRI2 animals had the lowest hematocrit compared to the other groups; the value for this group was significantly different from values for the TRI1 ($P<0.05$) and IMR groups ($P<0.05$), but not from the FCR group. Hematocrit was also significantly lower in the FCR animals when compared to the IMR animals ($P<0.05$). All other measures from the complete blood count (total leukocytes, neutrophils, lymphocytes, monocytes) were not significantly different among the four groups.

Discussion

Our results suggest that the TRI1 and TRI2 groups displayed significant differences in behavioral and physiological measures compared to controls, confirming our primary hypothesis; moreover, we also found evidence supporting our secondary hypothesis that TRI1 and TRI2 animals would differ. Finally, we also found a pattern of results suggesting that indoor-reared animals (TRI1, TRI2, and IMR) were different from the outdoor-reared (FCR) animals. We discuss these three patterns of results below.

Our principal hypothesis was that the experience of a matrilineal overthrow combined with a relocation during the prenatal period would result in behavioral (principally in the domain of emotionality) and physiological changes. Our analyses revealed several results in which one or both of the prenatally-exposed groups differed from one or both of the control groups. For example, TRI2 animals showed significantly more displacement activity in the human intruder test compared to IMR and FCR groups (TRI1 levels were intermediate). Displacement comprises tooth grind and yawn, both of which are behaviors suggestive of anxiety. Importantly, this result was not due to only one or two outlier animals on this measure in the TRI2 group. Of the 58 total subjects, only seven animals had a z-score greater than 1.0 on this measure, and of those seven, four were in the TRI2 group (note there were only seven animals in this group total). It is possible that the high levels of plasma cortisol observed in the TRI2 infants for sample 2 may be associated with the elevated levels of anxious behavior in the TRI2 animals, inasmuch as the human intruder test occurred within the two hours preceding the sample 2 blood collection.

A second result that support our primary hypothesis was that TRI1 animals showed significantly more emotionality in the holding cage on day 1 and day 2 compared to FCR animals (TRI2 levels were intermediate). Emotionality comprises vocalizations, threats, lipsmacks, and scratches. While all three indoor-raised groups had mean levels above zero on this z-scored scale (Figure 1), only TRI1 animals differed from the FCR animals, suggesting that heightened emotional reactivity may be a consequence of experiencing prenatal stress during the first trimester of gestation. There is little pertinent data in the nonhuman primate literature, inasmuch as experimental studies of prenatal stress in primates have only systematically examined the effects of mid-to-late gestation stress on infant emotional reactivity. However, a rodent study found that only first trimester (first week) and not later prenatal stress results in a reduction of rat pup ambulatory behavior in an open field (i.e. elevated emotional reactivity) [Suchecki & Neto, 1991].

Our secondary hypothesis was that the timing of the experience of matrilineal overthrow/relocation was important, and this result was supported principally by our physiological data. One such result was that TRI2 animals had significantly higher plasma cortisol concentrations for samples 1 and 2 compared to TRI1 animals (Figure 2). This finding of elevated cortisol in the TRI2 group is consistent with previous findings in the primate literature. For example, Coe and colleagues found increased HPA axis activity reflected by higher blood cortisol after an acute stressor (5 min blood draw from an awake monkey), higher elevation of cortisol after a dexamethasone suppression test, and elevated expression of emotionality in rhesus macaques prenatally stressed in their second trimester [Coe et al., 2003]. Moreover, our post-hoc analyses indicated that the TRI2 animals did not show clear cortisol differences across the four blood samples, unlike the animals in the TRI1, IMR, and FCR groups. Taken together, our plasma cortisol findings in the TRI2 infants add to a growing body of literature that maternal stress experienced in later gestational time periods results in markedly different HPA axis hormone-output profiles compared to individuals exposed to maternal stress in early pregnancy [Clarke et al., 1994; Coe et al., 2003; Huizink, Mulder, & Buitelaar, 2004; O'Regan, Kenyon, Seckl, & Holmes, 2004].

A second result that supported our secondary hypothesis was that TRI2 infants had significantly lower hematocrit compared to the TRI1 (and IMR) animals, though the mean value did not meet the requirements for anemia in macaques. Our findings were comparable to a previous study where maternal stress in the second trimester resulted in low levels of blood iron concentrations and hematocrit in 4 month old infant rhesus monkeys [Coe et al., 2007]. An earlier study in our laboratory, using a large sample, found hematological differences between indoor and outdoor-raised rhesus macaque infants [Capitanio et al., 2006]. In the present study, however, hematocrit levels in the indoor-raised TRI1 animals and outdoor-raised FCR animals did not differ significantly from each other, despite the fact that the TRI1 animals experienced gestation indoors for longer than the TRI2 animals; in fact the TRI1 animals did not differ significantly from the IMR animals. Our results suggest a more complex picture for this measure beyond simply being raised indoors versus outdoors; alternatively, we recognize that our small sample size may be partially responsible for the discrepant results. This is an idea that needs further study.

Finally, as noted above, we found a pattern of results in which the two TRI groups were similar to the IMR monkeys, and all three groups were significantly different from FCR animals. We observed this pattern for the Emotionality and Activity data from the human intruder test. The fact that the three indoor groups were also not significantly different from each other suggests that their responses may be more tied to their common postnatal, and not their different prenatal, experiences. In particular, it's possible that this pattern arises from the greater postnatal experience of the indoor animals (TRI1, TRI2, IMR) with humans who might stare at them regularly multiple times per day. Whereas in the animals' living cages, mother's presence might buffer the animals' behavioral responses, in the absence of mother, such as during the BBA program, the animals may display a more agitated response, characterized by activity, vocalization, etc. In contrast, outdoor-reared animals, which are much less experienced with either indoor housing conditions or the close presence of a human, might respond in a more inhibited style, involving reduced activity and emotionality.

In sum, the results from our study suggest that exposure in the second trimester to the presumed stress of experiencing a matrilineal overthrow followed by relocation to indoor housing is associated with: 1) greater anxiety when challenged, as shown by the high level of displacement behavior in the human intruder test, 2) greater glucocorticoid responsiveness, as seen in the elevated sample 1 and sample 2 blood cortisol levels, 3) significantly less suppression of glucocorticoid output following dexamethasone suppression of the HPA axis, as seen in sample 3, and 4) significantly lower hematocrit. It also appears that the TRI1 animals show more similarities to the IMR animals than to the TRI2 animals, as no significant differences were revealed for any of the outcome measures between the IMR and TRI1 animals. It's possible that the TRI2 animals were more strongly affected by the overthrow/relocation than the TRI1 animals because they were (presumably) exposed to a stressful social environment for a larger proportion of their gestation, as events in the field cage that culminated in the overthrow may have been occurring for a relatively lengthy period of time. Alternatively, if the overthrow was not preceded by sustained conflictual interactions (there is simply not much known about how overthrows arise and develop), then the more acute disturbance of the overthrow and relocation may have occurred at a critical time of development of body systems. Synaptogenesis in cortical regions, for example, is extensive during the second trimester (Granger et al., 1995). It is also possible that the naturally-occurring higher levels of circulating maternal glucocorticoids during the 2nd and 3rd trimesters in mammals (see Pepe & Albrecht, 1995; Beehner, Nguyen, Wango, Alberts, & Altmann, 2005) combined with the stress of an overthrow and relocation elevated fetal exposure to maternal glucocorticoids above a threshold that could have affected fetal development of the HPA axis. Much more research is needed in this area.

We believe our data suggest that efforts should be made to mitigate the effects of having experienced *in utero* a matrilineal overthrow/relocation event, particularly if the event occurs in the second trimester. We acknowledge the difficulty of this, however, as little is known about the dynamics that lead to an overthrow in the first place. Is there an extended period of social stress that precedes an overthrow attempt? If so, it is certainly not obvious to the casual observers who monitor cages, do morning health, etc. And what might be a successful mitigation strategy? In many, perhaps all, cases of an overthrow, relocation for at least some animals may be necessary to prevent injury. Relocation, by itself, however, has been shown to have biobehavioral consequences (Capitanio, Kyes, Fairbanks, 2006). Are there strategies that would minimize the consequences of overthrows/relocations that could keep the resulting offspring in the research pool? For example, could a feasible solution be to restrict the targeted animals (the alpha matriline) to a separate portion of a cage, or to relocate them together to a separate cage? This would at least minimize the social restriction, and the greatly changed environmental conditions, associated with a relocation to indoor housing. Obviously it would be unethical to induce an overthrow to study this phenomenon; but it might be useful to have plans in place for how such events could be handled, and to follow-up on such animals in light of our results.

Limitations

Finally, we acknowledge several limitations to the current study. The overall experimental control was not optimal, as data for the present study were analyzed after the matrilineal

overthrow and relocation took place; as mentioned, we know nothing about how long there may have been tension in the group prior to the actual overthrow, for example. Also, precise control over date of conception would have been ideal; however, this is not routinely assessed using, for example, ultrasound, in monkeys conceived in our outdoor colony (which would be FCR, TRI1, and TRI2 animals). The only proxy measure we have that might be pertinent to the question of whether TRI1 or TRI2 animals experienced early birth (which would make our trimester estimates less accurate), is postnatal weight, which was, unfortunately, not collected at consistent postnatal ages. Nevertheless, we examined this measure, using age at first weight as a covariate, and found no significant effects ($P=0.278$). Mean weights adjusted for age were 0.89, 0.85, 0.83, and 0.81 kg for TRI1, TRI2, IMR, and FCR animals, respectively. Moreover, research described earlier focusing on experimentally induced stress in pregnant rhesus monkeys has reported that the procedure does not produce obstetrical complications, alter gestation length, or result in reduced infant birth weight (Coe & Lubach, 2005). Thus, while we cannot be sure of the precise day of conception, we suspect that our infants were born at (or near) term and that our estimates when the overthrow/relocation occurred (i.e., first vs. second trimester) are reasonably accurate. We also acknowledge the possibility that males and females may differ on some of the measures. Due to our relatively low sample size in the TRI2 group, we did not have the statistical power necessary to determine if there were any sex differences in the current study.

Finally, we acknowledge that our principal assumption, namely that the group differences we reported are due specifically only to prenatal experiences, may be incorrect. Unfortunately, we were unable to collect physiological or behavioral data on the mothers to determine the extent to which they were indeed experiencing stress due to the overthrow/relocation event. Nor did we examine mother-infant interactions to determine whether the effects that we saw might have been a result of altered maternal behavior postnatally; such an effect has been reported for rodents (Patin et al., 2002), and in our cohort, as we mentioned earlier, two females did reject their infants right after birth. Future research in this area should focus on revealing how the offspring's biological systems, mother-infant interactions, and environmental factors interact and contribute to the emergence of biobehavioral variability in non-human primates following socially-derived prenatal stress.

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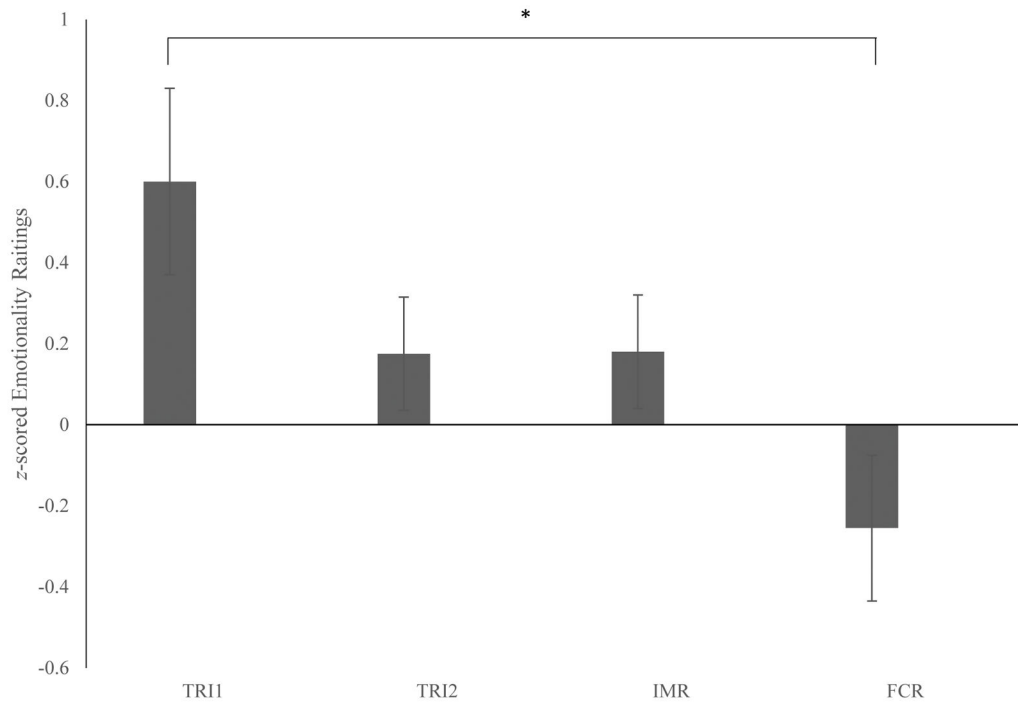


Figure 1.

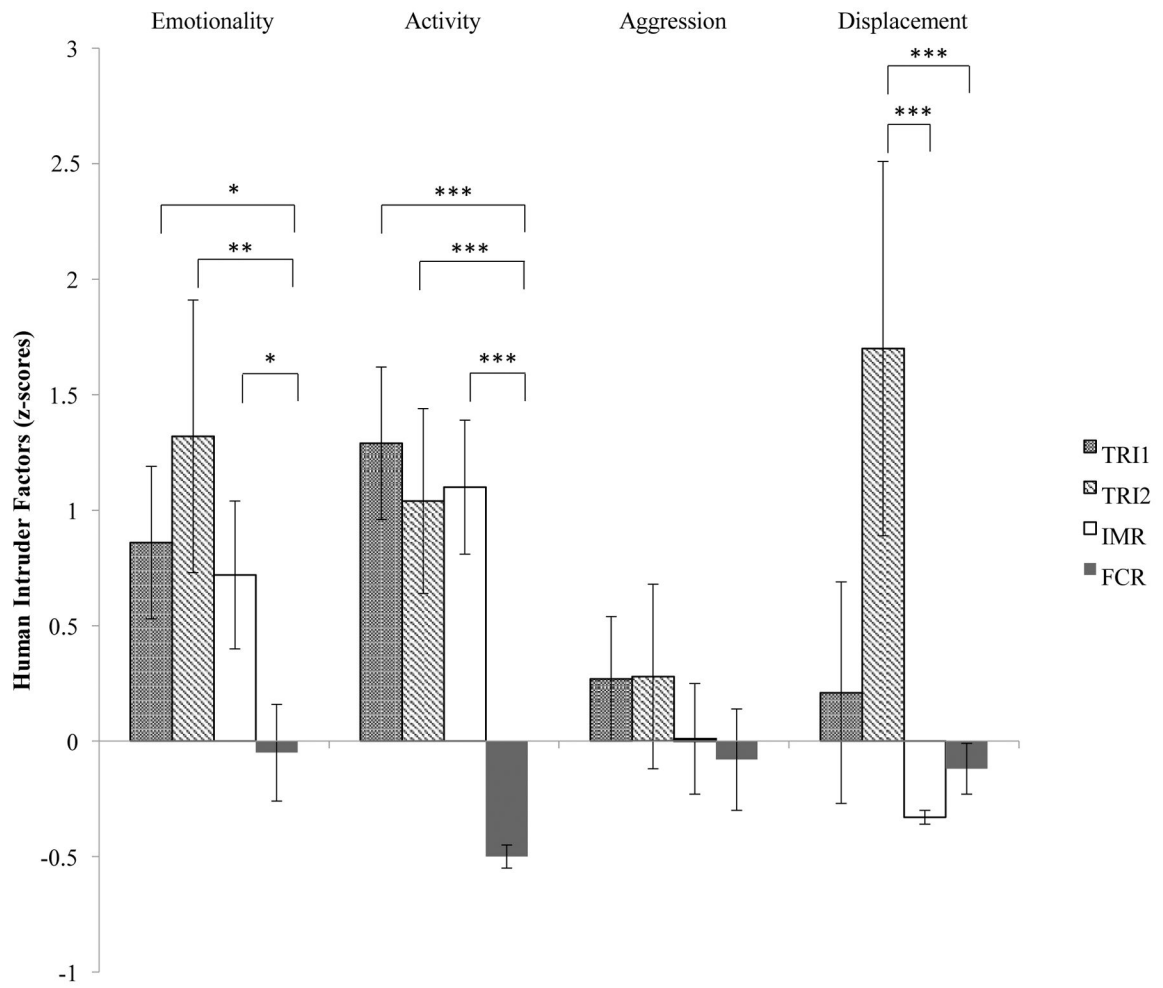


Figure 2.

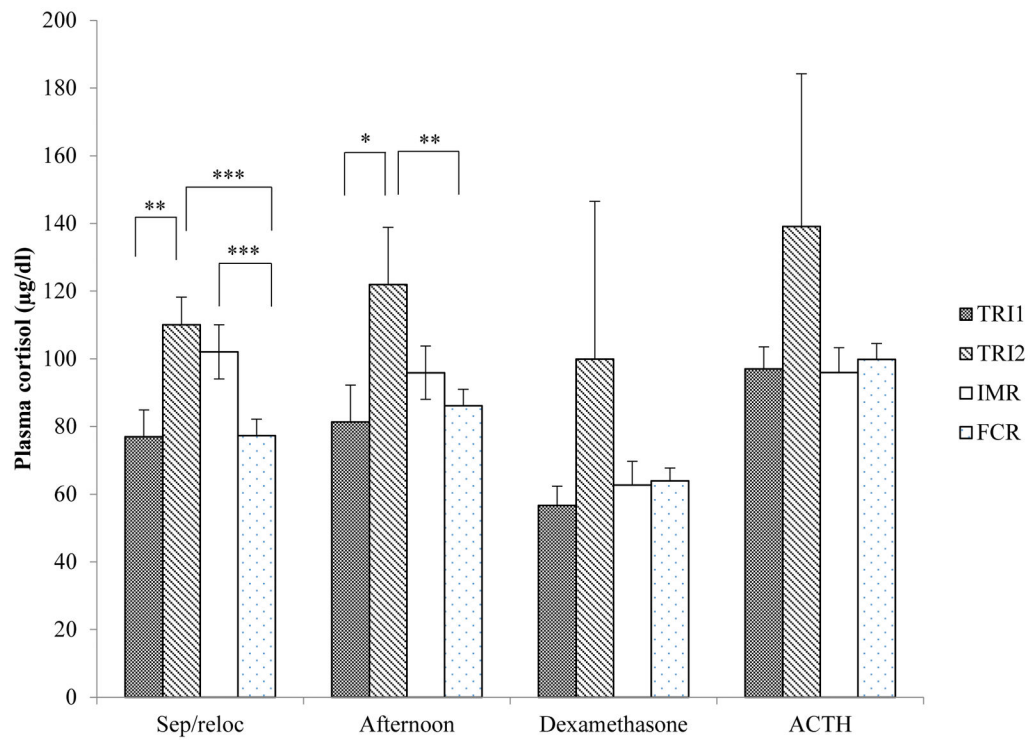


Figure 3.

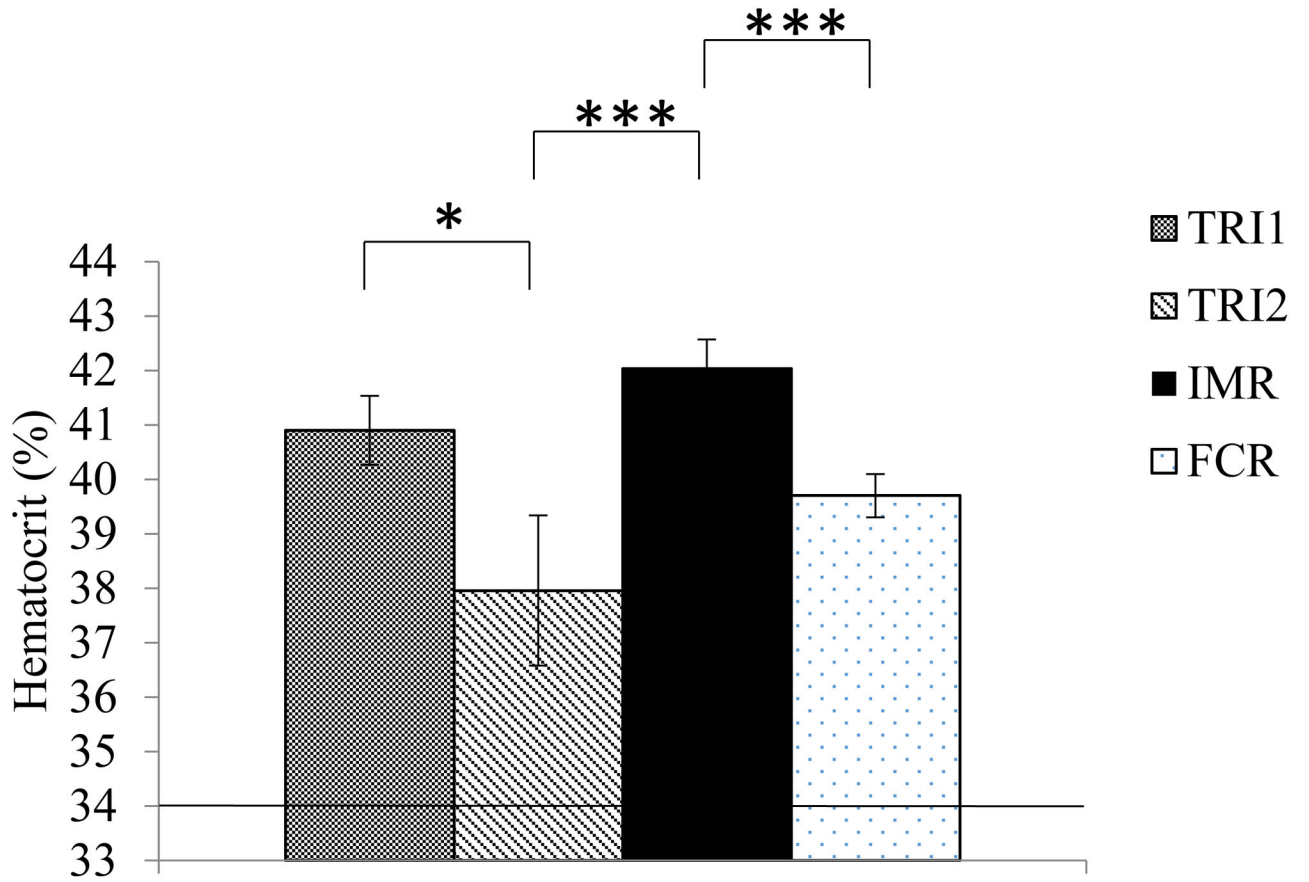


Figure 4.

Table 1

Group	N=	Days Old at Test: M (range)	Weight at Test (kg): M (range)
TRI1	13 (6 male)	108.54 (96 – 120)	1.14 (.90 – 1.29)
TRI2	7 (3 male)	102.00 (89 – 115)	1.10 (.96 – 1.20)
IMR	13 (6 male)	106.38 (93 – 119)	1.01 (.80 – 1.20)
FCR	25 (11 male)	102.92 (92 – 120)	1.04 (.77 – 1.25)

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