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Birth timing and the mother-infant relationship predict variation in infant behavior and physiology

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Abstract

The current study explored whether birth timing, known to influence the mother-infant relationship, also affected infant physiology up to 9 months later and infant behavior at weaning. Infant blood samples were collected at 5.75 and 8.75 months of age to assess functioning of the hypothalamic-pituitary-adrenal axis as well as the antibody response to a Cholera vaccination. Path analysis indicated infants born late in the birth season had less *Relaxed* relationships with their mothers. A less-*Relaxed* relationship was associated with greater infant *Positive Engagement* and *Distress*, which were negatively correlated, suggesting infants may have different strategies of coping with this type of relationship. Low *Relaxed* scores were also associated with higher infant cortisol concentrations at 5.75 months, which was associated with a reduced immune response to a vaccination 3 months later. Together these results indicate that the influence of birth timing on the mother-infant relationship may have consequences for infant development.

Keywords

Birth timing; mother-infant relationship; coping; cortisol; immune system; *Macaca mulatta*; rhesus monkey

Many factors relating to the social and non-social environment are known to impact mother-infant interactions in primate species. These include size and composition of the social group (Berman, 1992; Hooley & Simpson, 1981; Schino, d'Amato, & Troisi, 1995), and foraging demand on the mothers (Rosenblum & Paus, 1984). Recent research suggests that birth timing, the relative timing of births within a birth season, may also affect the mother-infant relationship. In a seasonally breeding species, mothers that give birth late in the birth season are likely to have infants that are younger, smaller, and not fully weaned at the start of the breeding season. Due to the effects of lactational suppression of estrus, females that are still nursing their infants frequently at the start of the breeding season are likely to resume reproductive cycling later in the breeding season and may be less likely to conceive (Johnson, Berman, & Malik, 1993). These mothers of late born infants may engage in two different strategies to maximize their reproductive output. First, mothers may focus on imparting resources to the current offspring and delay reproduction thereby resulting in a longer interbirth interval. On the other hand, mothers may limit investment in the current offspring by weaning their infants early in order to produce other infants in the upcoming breeding season. The effects of these tradeoffs may be most visible during naturally-occurring transitions, such as weaning and resumption of breeding, as the mother changes

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her focus from rearing her current infant to producing the next year's infant. In rhesus monkeys, this time period occurs when infants are approximately 4–8 months of age.

Although little research has been conducted on birth timing, there is preliminary evidence that birth timing influences the mother-infant relationship in macaques. In wild Assamese macaques (*Macaca assamensis*), mothers that gave birth early in the birth season had shorter interbirth intervals (1-year) than those giving birth late in the birth season (2-years) (Fürtbauer, Schülke, Heistermann, & Ostner, 2010). Recent evidence from our research program also suggests that birth timing may impact mother-infant interactions: infants born early in the birth season had more conflictual relationships with their mothers during the breeding season than did late born infants (Vandeleest & Capitanio, 2012).

What might be the consequences for the infant of birth timing-related variation in its relationship with mother? Considerable evidence from both experimental and naturalistic studies suggests that variation in maternal behavior is associated with offspring behavioral and physiological responsiveness. Early rodent studies demonstrated that repeated, brief maternal separation and manipulation of rat pups resulted in alterations in maternal behavior that led to reduced behavioral and physiological reactivity to stress among offspring (Levine, Haltmeyer, Karas, & Denenberg, 1967; Liu et al., 1997). Further exploration of these effects indicated that natural variation in maternal care could also impact the development of offspring behavioral and physiological systems; mothers rats that naturally licked, groomed, and archback nursed their offspring more had offspring that demonstrated reduced behavioral and physiological stress reactivity (Cameron et al., 2005; Francis & Meaney, 1999). Similar to the rat studies, evidence from experimental primate studies also suggests that higher levels of maternal care were associated with reduced behavioral and physiological stress reactivity in infants (Coplan et al., 1996; Levine & Mody, 2003; Rosenblum & Paully, 1984). While the effects of natural variation in maternal care in primates on infant stress physiology have yet to be thoroughly explored, evidence suggests that maternal style is associated with infant behavioral development. In general, it is thought that maternal protectiveness results in infants that exhibit greater behavioral reactivity to stressful events (Bardi & Huffman, 2002; Fairbanks & McGuire, 1988), and there is some evidence that maternal rejection may lead to greater infant independence (Bardi & Huffman, 2002; Rosenblum & Paully, 1984). Still unexamined, however, is whether variation in the mother-infant relationship due to birth timing would have consequences for infant behavior and physiology.

One physiological system that is very sensitive to developmental perturbations in general, and that might be affected by birth timing, is the hypothalamic-pituitary-adrenal (HPA) axis (Francis & Meaney, 1999; Levine & Mody, 2003). Cortisol, the end product of the HPA axis, is a hormone released by the adrenal cortex. Although mainly a metabolic hormone, cortisol has widespread effects throughout the body including effects on the cardiovascular system, fluid volume, immunity and inflammation, metabolism, and reproductive physiology (Sapolsky, Romero, & Munck, 2000). In relation to immunity, glucocorticoids like cortisol have a generally suppressive effect on this system (but see Dhabhar, 2009) as they are potent inhibitors of cytokine synthesis and release, inhibit antigen presentation, and reduce the activation of T and B cells (see Sapolsky, et al., 2000; or Webster Marketon & Glaser, 2008 for a detailed review). In an acute stress situation these effects are thought to be protective in that they prevent an overshoot of immune system activation. In chronically stressful situations, however, there is evidence that the normal regulation of the HPA axis is altered (Capitanio, Mendoza, Lerche, & Mason, 1998). These effects of chronic stress on the regulation of the HPA axis have also been associated with altered immune function (Cole, Mendoza, & Capitanio, 2009) and therefore could have implications for a variety of negative health outcomes. Although the immunosuppressive effects of cortisol are well known, it is

unclear whether the effects of maternal care on HPA-axis function described above could lead to negative health outcomes. Studies in human preschoolers have shown that qualities of the care environment can impact daily secretion patterns of cortisol; children at daycare often fail to exhibit the normal afternoon fall in cortisol (Dettling, Parker, Lane, Sebanc, & Gunnar, 2000; Watamura, Coe, Laudenslager, & Robertson, 2010), and high afternoon cortisol has been shown to be related to both reduced salivary antibody levels and a greater incidence of respiratory illness (Watamura, et al., 2010).

In our earlier report, we demonstrated that early-born infants had more conflictual relationships with their mothers during the breeding season, and trends were also found for relationship measures during weaning. In the present report, we were able to add additional animals to the cohort to examine more closely the trends found during weaning. Most importantly, however, we now examine whether this variation is associated with physiological outcomes near the end of weaning (5.75 months of age), and at a later point in infancy (8.75 months of age). We were especially interested in examining a health-related outcome; consequently, in addition to measuring plasma concentrations of cortisol, we immunized our animals with cholera toxin vaccine, and assessed variation in immune response.

METHODS

Subjects and Housing

Subjects were 41 rhesus monkeys (21 Male; *Macaca mulatta*) housed at the California National Primate Research Center (CNPRC). All animals were born to multiparous mothers between the ages of 5 and 15 years, that had reared at least 1 prior infant to at least 9 months of age. Mothers were of variable rank (15 high, 12 middle, and 14 low). Infants were observed in two cohorts with 20 observed in 2008 and 21 observed in 2009. Infants were born and reared in one of four large naturalistic social groups that were housed outdoors in 0.2 ha field cages consisting of 125–175 animals with age and sex compositions similar to that in the wild. Cages contained multiple perches, climbing structures, and small shelters to provide protection from the rain and wind. Food was provided twice daily and water was available ad libitum.

Data collection

Birth Timing—At the CNPRC the outdoor housed animals breed seasonally during the fall (September–December) and have a gestation of approximately 165 days leading to a birth season in the spring. The start of the birth season was determined for each of the 4 field cages separately and was considered to start when the first infant was born in each cage. The birth timing measure was then calculated by determining the number of days each infant was born after the start of the birth season in that cage (date of first birth ranged from January 16 – February 25 for the four cages). Subjects were born an average of 50.7 days after the start of the birth season (range: 3–91 days). The current analysis used a categorical variable, identical to that used in our earlier work (Vandeleest & Capitanio, 2012), in which the earliest 25% of infants born in a cage, the middle 50%, and the latest 25% of animals were grouped together to form early- (N = 10; Range: 3–32 days), peak- (N = 20; Range: 35–68 days), and late-born (N = 11; Range: 69–91 days) groups. One female subject from the early-born group had incomplete physiological data and was excluded from the current analyses resulting in final sample size of 40. Interbirth interval was not examined in the current paper as most females in the colony conceive each year (95% of females in our sample conceived another infant during the study period).

Behavioral Observations—Ten minute focal animal observations were conducted on each infant twice per week when the infant was 4.5–5.0 months of age, which provided a snapshot of the patterns of mother-infant interactions and infant behavior during weaning. Observations were conducted between 9:00 am and 1:00 pm and time of day was balanced across subjects. A total of 30–40 minutes of data was available for all subjects and all data were collected by a single observer. At the end of each focal observation adjective ratings were also completed to get an overall picture of the events and themes from that observation period. These adjective ratings were not designed to assess stable personality characteristics (Capitanio, 1999) but instead to assess the more labile attitude of an individual using the observers' impressions of the animals during a specific observation session. The adjective ratings consisted of 4 maternal attitude adjectives (nurturing, restrictive, indifferent, and aggressive), 4 mother-infant interaction theme adjectives (conflictual, tense, calm or relaxed, aggressive) and 8 infant attitude adjectives (fearful, relaxed, depressed, nervous, active, confident, affiliative, playful). Adjective ratings involved a 7-point Likert scale with 1 indicating a total absence of the attitude and 7 indicating an extremely large amount of the attitude was present. Reliability was assessed at the start of the study and for the adjective ratings inter-rater agreement was 92.2% when ratings were allowed to vary by 1 point.

Factor analysis was performed (see Vandeleest & Capitanio, 2012, for details) on adjectives relating to 1) qualities of the mother-infant relationship and 2) infant attitude. The factor analyses resulted in the formation of two mother-infant relationship factors, *Relaxed* (mother was nurturing and not indifferent, relationship was relaxed and not tense or conflictual) and *Aggressive* (mother was aggressive and the relationship was characterized by aggression), and two infant attitude factors, *Positive Engagement* (playful, affiliative, active, and confident) and *Distress* (fearful and nervous). Notably, the *Positive Engagement* factor was constructed of adjective ratings that reflected infant activities when away from the mother and therefore reflects an infant's engagement with the social and non-social environment *outside* the context of the mother-infant relationship. Factor scores were constructed by adding together the scores from the adjectives that had loadings greater than 0.30, and were then expressed as a proportion of the maximal possible score for that factor, resulting in each factor having a minimum score of zero and maximum of one (see Table 1 for descriptive statistics). Scale reliability was good (Cronbach's alphas ranged from 0.62–0.89).

Blood Sampling and Immunization—Two blood samples were collected from infants to assess cortisol levels and immune responses to an immunization. The first sample was collected when infants were 5.75 months of age, and the second at 8.75 months of age. For all blood samples, animals were captured in their home cages and 1.5 ml of blood was drawn from the femoral vein of each infant between 9:00–10:00 am on a day when behavioral data were not being collected. On some occasions multiple animals in the same cage had samples collected, thus for all samples the disturbance time (i.e. the time from cage entry to blood sample completion) was recorded (mean = 10.8 minutes, range: 4.28 – 27.03 minutes) to later control statistically for the effects of cage disturbance, capture, and venipuncture on hormone levels. Immediately following the blood draw at 5.75 months of age infants received an immunization with 0.1 mg of cholera toxin B subunit (List Biological Laboratories, Campbell, CA) subcutaneously between the shoulder blades. Blood samples were drawn into 3 ml syringes and transferred to sterile tubes and allowed to clot at room temperature. Serum was then extracted and stored at –80°C until assay.

Cortisol Assay—Prior to assay, samples were diluted 1:4 in PBS gel buffer. Plasma concentrations of cortisol were estimated in duplicate using commercial radioimmunoassay kits (Siemens Medical Solutions Diagnostics, Los Angeles, CA). Assay procedures were

modified with the addition of 0.5 and 2.5 $\mu\text{g/dL}$ standards. Assay sensitivity was 0.26 $\mu\text{g/dL}$. Intra- and inter-assay coefficients of variation were 2.52 and 3.21, respectively.

Anti-Cholera Antibody Assay—Anti-cholera immunoglobulin G (IgG) was assayed using enzyme-linked immunoassay (ELISA) methods described elsewhere (Capitanio, et al., 1998; Otsyula et al., 1996; Van Rompay et al., 1996). Briefly, microtiter plates were coated with cholera toxin B subunit at a concentration of 2.0 $\mu\text{g/ml}$ and stored overnight at 4°C. Before the samples were added, plates were washed, block buffer was added to each well, and plates were incubated for 1 hour at 37°C. A series of six 4-fold dilutions were prepared for each sample (starting at 1:100) for each test sample, and ten 4-fold dilutions were prepared for the positive control sample (starting at 1:100). After the removal of the block buffer, 50 μl of each dilution of test and control serum was added to each well and plates were incubated at 37°C for 1-hour and 4°C overnight. After incubation, plates were again washed and 50 μL of goat anti-monkey IgG-horseradish peroxidase conjugate (Accurate Chemical & Scientific Corp., Westbury, NY) diluted 1:4000 was added to each well and plates were incubated at 37°C for 1 hour. O-phenylenediamine dihydrochloride (OPD) substrate (Sigma Chemical Co., St. Louis, MO) was added and plates were incubated at room temperature for 5 minutes before stop buffer (6N H_2SO_4) was added. Plates were then read on a plate reader at 490nm. All samples were run in duplicate. The intra-assay coefficient of variation was 2.67%; the inter-assay coefficient of variation was 9.48%. Due to low variability in titer points, antibody levels were expressed as the proportion of plate positive control on each plate at the 1:400 dilution (Capitanio, et al., 1998; Maninger, Capitanio, Mendoza, & Mason, 2003). This dilution was chosen as it showed the greatest inter-individual variability.

Data analysis

Bivariate correlations were calculated to examine the relationships between birth timing, qualities of the mother-infant relationship, infant attitude, and infant physiological measures (cortisol and antibody response). Infant sex and maternal rank were not associated with birth timing or any of the behavioral or physiological measures. All variables with significant correlations were used to construct a path analysis (Mplus 3.0), which allows for the examination of several related regression equations simultaneously. Disturbance time was also included in the path model as a covariate to control for the potential effects of capture stress on cortisol concentrations (Capitanio, Mendoza, & McChesney, 1996). Disturbance time was calculated from the time of entry into the housing cage until completion of the blood sampling for each animal, and was significantly correlated with plasma cortisol ($r = 0.494$, $p = 0.001$). Paths were included in the model if the variables were significantly correlated with each other and the direction of the path was determined such that group level variables (i.e. birth timing) predicted relationship level variables (i.e. mother-infant relationship factors) and individual level variables (infant attitude and physiology), and relationship level variables predicted individual level variables. Paths were removed from the model if they were non-significant and removal did not result in a decrease in model fit. Model fit was evaluated using the comparative fit index (CFI), the Chi-Square goodness-of-fit test (good fit is indicated by a non-significant test), the root mean square error of approximation (RMSEA), and the standardized root mean square residual (SRMR). A CFI > 0.90 generally indicates an adequate fit while a CFI > 0.95 is regarded as indicating a good fit. The RMSEA indicates mediocre fit if values are between .08 and .10, adequate fit if values are between .05 and .08, and close fit if values are less than .05. Finally, the SRMR indicates good fit if the value is less than .08 (Hu & Bentler, 1995). The CFI was used as the primary fit index due to the small sample size (Bentler, 1990), however all indexes are presented.

RESULTS

Significant correlations were found between birth timing, mother-infant relationship factors, and infant attitude and physiology (See Table 2). These significant correlations determined which relationships (paths) to include in the path analysis. Infants born early in the birth season had significantly more *Relaxed* relationships with their mothers during weaning (4.5–5.0 months of age) than infants born late in the birth season, a result that was only a trend in our earlier paper. As previously reported, mother-infant pairs that were more *Relaxed* were less *Aggressive*, and had infants that exhibited lower levels of infant *Positive Engagement* and *Distress* (Vandeleest & Capitanio, 2012). The *Aggressive* relationship factor was significantly related to greater infant *Distress*. The correlational analysis also indicated that while there were no significant correlations between infant attitude and infant physiology, there was a negative association between the relationship factor *Relaxed* and cortisol levels at 5.75 months of age. Finally, higher cortisol levels at 5.75 months of age were associated with a lower antibody response to vaccination at 8.75 months of age, but cortisol concentrations at 8.75 months were not related to the antibody response. All significant bivariate correlations were used in the construction of the path model.

The initial path analysis indicated direct effects of birth timing on the mother-infant relationship factors and infant attitude, and direct effects of mother-infant relationship factors on infant attitude and physiology. Model fit statistics for the full model indicated poor fit, however (significant Chi-Square test, CFI<0.90, RMSEA> 0.10, SRMR>0.08). The path in the full model from the mother-infant relationship factor *Aggressive* to infant *Distress* was non-significant and so was removed from the subsequent model. Fit statistics indicated good model fit for the reduced model (Chi-Square (11) = 6.307, $p = 0.85$; CFI = 1.0; RMSEA = 0.00; SRMR = 0.075). The final path model is presented in Figure 1. This model indicates that infants born early in the birth season had more *Relaxed* relationships with their mothers. Infants in a *Relaxed* relationship showed less *Positive Engagement* with others and exhibited less *Distress*. Infants from a *Relaxed* dyad had lower cortisol concentrations at 5.75 months of age, which was associated with a better immune response to immunization 3 months later. Although uncorrelated in the bivariate analysis ($r = -0.097$), path modeling indicated a significant negative correlation between *Positive Engagement* and *Distress* ($r = -0.307$, $p < 0.05$) suggesting that, within the context of a relationship low on the *Relaxed* factor, infants were likely to display either *Positive Engagement* or *Distress* but not both. (Although the *Distress* factor was not normally distributed, further examination of our model suggests that this variable did not unduly influence the results of our final model. Approximately 55% of the infants did not display *Distress*, but inspection of the distribution revealed there were no obvious outliers.)

DISCUSSION

The current analysis confirms earlier trends that suggested that birth timing influences the mother-infant relationship during weaning; our analysis reveals new information on how variation in the mother-infant relationship is associated with infant behavior and physiology. When the mother-infant relationship was not *Relaxed* during weaning, as was the case for infants born relatively late in the birth season, infants were more likely to display one of two response patterns, *Positive Engagement* or *Distress*. In addition, a less-*Relaxed* mother-infant relationship was also associated with higher plasma cortisol concentrations at approximately 6 months of age and a weaker immune response to a vaccination 3 months later. These data provide preliminary evidence that the effects of birth timing on the mother-infant relationship during weaning may have influences on infant behavior and physiology.

Although our previous analysis did not report significant effects of birth timing during weaning, the results from the current analysis are consistent with trends present in our earlier analysis (which had fewer animals owing to the longitudinal nature of the earlier analysis, and some mothers failing to breed). Our previous paper also showed that late born infants and their mothers had more *Relaxed* relationships during the breeding season than did early born infants and their mothers (Vandeleest & Capitanio, 2012). Together these analyses suggest that while late born infants have less *Relaxed* relationships with their mothers during weaning, the effect of birth timing may actually reverse during maternal breeding: the weaning effects presented here are consistent with our original hypothesis that late born infants may experience early weaning as the mother prepares for the oncoming breeding season and therefore may have a more conflictual relationship with their mothers during this time. Interestingly, this conflict may not persist and mothers of late born infants may resume a higher level of maternal care once they successfully begin reproductive cycling. Therefore, weaning may be a greater challenge for late born infants while our previously reported analysis suggests that maternal breeding poses a greater challenge for early born infants. We present this suggestion provisionally, however, owing to the differences in sample size, and we recognize that the question of birth timing and changes in relationships between weaning and breeding should be confirmed in a larger sample.

The negative associations between a *Relaxed* mother-infant relationship, and infant *Positive Engagement* and *Distress* outside the mother-infant relationship have been reported previously (Vandeleest & Capitanio, 2012); the current path analysis, however, revealed a negative relationship between *Positive Engagement* and *Distress*. This result suggests that these different patterns of infant attitude reflect two different ways infants may cope with a relationship that is not *Relaxed* and instead is characterized by conflict and tension. Infants that exhibit greater engagement with members of the social group when away from their mothers, those scoring high on *Positive Engagement*, may be engaging in more active or proactive coping strategies. Although proactive coping has most often been explored in rats and is often associated with increased aggression, it has been suggested that social activity may also reflect a proactive coping style (Koolhaas et al., 1999). In addition, a general proactive coping strategy would likely lead to different specific behaviors for each species that reflect the life history of that animal. Animals scoring high on the *Positive Engagement* factor also exhibit behavior profiles that are similar to stress-inoculated squirrel monkeys (Lyons & Parker, 2007). As weaning is likely one of the first major challenges these infants face, it is possible that their reactions to a less *Relaxed* relationship may influence how they are able to cope with future stressors. Previously, we found that, during maternal breeding early born infants had significantly less *Relaxed* relationships with their mothers and significantly higher *Positive Engagement* scores than late born infants (Vandeleest & Capitanio, 2012). In addition, *Positive Engagement*, assessed during the breeding season was predicted by infant temperament (measured at 3–4 months of age) but not concurrent qualities of the mother-infant relationship. Together, these results suggest that although adverse conditions of the mother-infant relationship may require infants to employ coping strategies, the use of active coping or social engagement strategies may be influenced by temperamental characteristics of the infant.

Distress, on the other hand, exhibited by animals in less *Relaxed* relationships, may reflect a more reactive or passive style of coping. In rats, studies based on the selective breeding for individuals that exhibit either high or low frequencies of anxiety vocalizations in response to maternal separation have suggested that individuals that exhibit high rates of distress vocalizations in infancy tend to exhibit more behaviors consistent with a reactive coping style (Brunelli & Hofer, 2007). This possibility, however, requires further data as other indexes of proactive versus reactive coping styles were not measured in the current study. In addition, caution was used in the interpretation of this variable due to violations of the

assumption of multivariate normality. Removal of the *Distress* factor from the model, however, did not change path coefficient estimates (changes of only 0.001) and did not increase in model fit. Therefore, it does not appear that the non-normality of this variable impacted our overall analysis.

Most importantly, not only did variation in the qualities of the mother-infant relationship influence infant coping, but variation was also related to infant physiological responses. The current study indicated that late born infants were likely to have a less *Relaxed* relationship with their mothers during weaning, and that this quality of the mother-infant relationship was related to higher plasma cortisol concentrations 3 weeks later. Although this effect was seen at 5.75 months of age, it was not apparent at 8.75 months of age, which suggests that the effect seen in the current study may be primarily a reflection of recent patterns of interaction with the mother. It is also possible that the higher cortisol concentrations seen in infants with less *Relaxed* relationships with their mothers were due mainly to the metabolic demands of being more independent of the mother: a relationship that is less *Relaxed* is one in which the infant is spending more time away from the mother (Vandeleest & Capitanio, 2012). This suggests a greater responsibility for the infant in thermoregulation and may be associated with greater energy demands, especially in those infants exhibiting greater activity and *Positive Engagement*. Thus the high cortisol seen in these infants could be a reflection of cortisol's role as a metabolic hormone.

The variation in mother-infant interactions during weaning was also demonstrated to have consequences for immunity through its effects on HPA axis activity. Variation in mother-infant interactions was not only associated with infant cortisol as much as 3 weeks later, but the cortisol differences were associated with the specific antibody response nearly 4 months later. Similar to the current findings, Laudenslager and colleagues (1993) reported that during maternal breeding infants that received more grooming from their mothers had higher tetanus specific antibody levels, and those that were rejected more had lower antibody levels. Unlike the Laudenslager (1993) study, however, the current analysis did not find any associations between either infant play (a component of *Positive Engagement*) or infant *Distress* and immune function. Importantly, whether or not the differences in cortisol were due to patterns of mother-infant interactions, stress, or metabolic requirements, they were still associated with differences in immune function. It is also important to note that between the first and second blood samples the infants experienced their mothers' resumption of mating activity, which has been suggested to be a natural form of mother-infant separation (Berman, Rasmussen, & Suomi, 1994). The fact that cortisol concentrations prior to the breeding season and at the time of immunization were associated with antibody response, but that cortisol concentrations concurrent with the measurement of antibody were not, emphasizes the fact that conditions at the time of a vaccination, such as greater conflict or stress, can have long lasting effects on immunity (Merlot, Moze, Dantzer, & Neveu, 2004). Although the current data differences in immune function, we acknowledge that it is unclear whether these differences would translate into actual differences in disease risk or severity upon exposure to a pathogen.

The exploration of the effects of birth timing on mother-infant interactions and infant development remains in its early stages. We provide evidence that birth timing influences how mothers and infants interact, and that this could have consequences for both infant physiological and behavioral development. The current paper presents data captured during only a small portion of the weaning process; future studies are needed to fully explore the effects of birth timing throughout the weaning process to determine if mothers of late born infants are in fact weaning their infants differently than mothers of early born infants. The reversal of direction for the effects of birth timing on the mother-infant relationship from weaning to maternal breeding indicates that birth timing may have a complex and possibly

changing influence on mother-infant interactions across these developmental periods. In addition, although we present data indicating that the effect of birth timing on the mother-infant relationship has consequences for infant behavior and physiology, it is still unclear how long the effects presented persist. More data are needed to examine if these effects are evident beyond the first year of life and what impact they might have on infant outcomes later in life.

Birth timing appears to have effects on infant behavior and physiology through its effects on mother-infant interactions. Previous analysis has indicated that birth timing impacts the mother-infant relationship during the breeding season; the current study extends those findings to indicate that effects may also be present during weaning and may have important consequences for both infant behavior and physiology. When the mother-infant relationship is not *Relaxed* infants may employ different strategies to cope with the tension and disruption of the mother-infant relationship. In addition, the fact that immune effects were seen up to 4 months after variation in mother-infant relationships was measured suggests that variation in the mother-infant relationship may have important consequences for infant health.

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Figure 1.

Significant paths coefficients are indicated with a solid line with a unidirectional arrow. Correlations are indicated by a solid line with bidirectional arrows. Dotted lines indicate the coefficients for the residuals (R). * $p < 0.05$, ** $p < 0.01$. ^a Model also controls for the effects of disturbance time on basal cortisol levels.

Table 1

Descriptive Statistics by birth timing group

	Birth timing			
	Early Born (n= 9) Mean (SD)	Middle Born (n = 20) Mean (SD)	Late Born (n=11) Mean (SD)	Total (n=40) Mean (SD)
Relaxed Relationship Factor	0.745 (0.095)	0.698 (0.078)	0.639 (0.138)	0.693 (0.105)
Aggressive Relationship Factor	0.000 (0.000)	0.008 (0.020)	0.010 (0.025)	0.007 (0.019)
Infant Positive Engagement	0.500 (0.173)	0.476 (0.126)	0.565 (0.164)	0.506 (0.149)
Infant Distress	0.012 (0.021)	0.022 (0.046)	0.051 (0.061)	0.028 (0.048)
Cortisol ^a (5.75 mo)	28.677 (6.721)	34.087 (10.214)	34.887 (11.309)	33.089 (9.940)
Cortisol ^a (8.75 mo)	41.627 (7.038)	40.090 (7.749)	35.436 (5.849)	39.156 (7.345)
Antibody ^b (8.75 mo)	0.856 (0.248)	0.785 (0.313)	0.692 (0.310)	0.775 (0.297)

^a
μg/dL^b
Expressed as the proportion of the positive control value.

Table 2

Bivariate Correlations Among Predictors

	Birth Timing	Relaxed Relationship Factor	Aggressive Relationship Factor	Infant Positive Engagement	Infant Distress	Cortisol (5.75 mo)	Cortisol (8.75 mo)
Relaxed Relationship Factor	-0.362*	-----					
Aggressive Relationship Factor	0.170	-0.509**	-----				
Infant Positive Engagement	0.169	-0.412**	0.175	-----			
Infant Distress	0.303	-0.515**	0.388*	-0.097	-----		
Cortisol (5.75 mo)	0.215	-0.352*	-0.031	0.103	0.282	-----	
Cortisol (8.75 mo)	-0.309	-0.033	0.160	-0.124	0.035	0.030	-----
Anti-Cholera IgG (8.75 mo)	-0.199	0.225	0.206	0.019	-0.082	-0.478**	0.269

*p<0.05,

**p<0.01