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Prenatal Maternal Stress Programs Infant Stress Regulation

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Abstract

Objective—Prenatal exposure to inappropriate levels of glucocorticoids and maternal stress are putative mechanisms for the fetal programming of later health outcomes. The current investigation examined the influence of prenatal maternal cortisol and maternal psychosocial stress on infant physiological and behavioral responses to stress.

Methods—The study sample comprised 116 women and their full term infants. Maternal plasma cortisol and report of stress, anxiety and depression were assessed at 15, 19, 25, 31 and 36+ weeks' gestational age. Infant cortisol and behavioral responses to the painful stress of a heel-stick blood draw were evaluated at 24 hours after birth. The association between prenatal maternal measures and infant cortisol and behavioral stress responses was examined using hierarchical linear growth curve modeling.

Results—A larger infant cortisol response to the heel-stick procedure was associated with exposure to elevated concentrations of maternal cortisol during the late second and third trimesters. Additionally, a slower rate of behavioral recovery from the painful stress of a heel-stick blood draw was predicted by elevated levels of maternal cortisol early in pregnancy as well as prenatal maternal psychosocial stress throughout gestation. These associations could not be explained by mode of delivery, prenatal medical history, socioeconomic status or child race, sex or birth order.

Conclusions—These data suggest that exposure to maternal cortisol and psychosocial stress exert programming influences on the developing fetus with consequences for infant stress regulation.

The prenatal period is a time of enormous change during which organs and organ systems are forming and are susceptible to both organizing and disorganizing influences. These influences on the fetus have been described as programming; the process by which a stimulus or insult during a vulnerable developmental period has a long-lasting or permanent effect. The effects of programming are dependent on the timing (i.e. the developmental stage of organ systems and the changes in maternal and placental physiology) and the duration of exposure (E.P. Davis & Sandman, 2010; Nathanielsz, 1999). Compelling evidence from epidemiological studies indicates that adverse birth outcomes such as low birth weight and preterm birth are associated with a number of diseases of adulthood including heart disease and obesity (Barker, 1998, 2002) as well as psychological dysfunction (Bohner & Breslau, 2008; Costello, Worthman, Erkanli, & Angold, 2007). The influence of prenatal exposure to maternal stress signals on the developing fetal HPA axis has been proposed as one mechanism that underlies fetal programming of adult health outcomes (Kapoor, Petropoulos, & Matthews, 2008; Seckl, 2008).

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Glucocorticoids are steroid hormones that exert influences on nearly every organ and tissue in the body (Drake, Tang, & Nyirenda, 2007). Regulation of the maternal HPA axis changes dramatically during the course of normal human pregnancy. Maternal cortisol increases two to four-fold over the course of normal gestation (Sandman et al., 2006). Fetal exposure to the increasing concentrations of maternal cortisol is regulated by a placental enzyme, 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2), which oxidizes cortisol to its inactive form cortisone (Brown et al., 1996). Levels of placental 11 β -HSD2 also rise as pregnancy advances, providing partial protection for the fetus from maternal cortisol during critical stages of development (Murphy & Clifton, 2003). Because placental 11 β -HSD2 is only a partial barrier, active maternal cortisol passes through the placenta, and fetal cortisol levels are significantly correlated with maternal levels (Gitau, Cameron, Fisk, & Glover, 1998; Gitau, Fisk, Teixeira, Cameron, & Glover, 2001). Exposure to cortisol that passes the placental barrier can influence the fetal nervous system because cortisol easily passes through the blood-brain barrier and targets GC receptors that are present throughout the central nervous system (Jacobson & Sapolsky, 1991; Sanchez, Young, Plotsky, & Insel, 2000).

Results from animal models indicate that fetal exposure to both maternal stress and glucocorticoids disrupts the regulation of physiological and behavioral stress responses in the offspring (Abe et al., 2007; Kapoor et al., 2008; Maccari & Morley-Fletcher, 2007; Schneider, 1992). Similarly, in humans, prenatal treatment with synthetic glucocorticoids shapes the development of the fetal HPA axis (Ashwood et al., 2006; E. P. Davis, Townsend et al., 2004; E. P. Davis et al., 2006). Few human studies have evaluated the joint role of maternal cortisol and psychosocial stress for infant development (Sandman & Davis, in press). There is evidence that high levels of prenatal psychosocial stress (Bergman, Sarkar, O'Connor, Modi, & Glover, 2007; E. P. Davis, Snidman et al., 2004; B. Van den Bergh, 1990) and prenatal maternal cortisol (E. P. Davis et al., 2007; C. de Weerth, van Hees, & Buitelaar, 2003) are associated with fearful and reactive behaviors in the offspring. Less is known about the influence of prenatal maternal stress signals on the developing HPA axis (Sandman & Davis, in press). Several studies have shown that maternal *psychosocial* stress alters circadian regulation and laboratory levels of cortisol in the offspring (Grant et al., 2009; O'Connor et al., 2005; B. R. H. Van den Bergh, Van Calster, Smits, Van Huffel, & Lagae, 2007). To our knowledge, only Gutteling and colleagues (2004; 2005) have evaluated the influence of prenatal maternal *cortisol* on HPA axis functioning in the offspring. In samples of 24 and 29 children respectively, they found that elevated maternal cortisol and psychosocial stress measured at one time during gestation (15 to 17 gestational weeks) independently predicted higher cortisol levels on the day of an inoculation and on the first day of a new school year. These data suggest that the development of the fetal HPA axis is shaped by prenatal maternal stress signals.

The present study is unique in the evaluation of both prenatal maternal psychosocial stress and cortisol, at multiple gestational periods, in association with infant physiological and behavioral stress regulation. Multiple assessments of stress are essential for examining timing or critical period effects on the developing fetus and for determining the trajectory of stress measures over the course of pregnancy. Evaluation of infant behavioral and physiological stress regulation in the immediate neonatal period provides a unique opportunity to identify the consequences of prenatal programming independent of postnatal influences. This study will determine whether prenatal maternal psychosocial stress and cortisol exert a joint or independent influence on infant stress regulation and whether there are critical periods in gestation during which the fetus is more susceptible to these influences.

Methods

Study Overview

Study participants included mother-infant pairs from an ongoing longitudinal study of prenatal stress and development. Women with singleton pregnancies less than 16 weeks gestational age were recruited from obstetric clinics in Southern California and have been followed longitudinally.

Participants

The current sample comprised 116 full term infants (55 girls and 61 boys) and their mothers. Initial prenatal recruitment criteria were as follows: English speaking, non smoker, over the age of 18, no steroid medication, and no evidence for drug or alcohol use during pregnancy. Additional inclusion criteria for the current study were full term at birth, delivery at the University of California Irvine, Medical Center, admission to the Well-baby Nursery and complete data for the heel-stick procedure (Data collection was attempted for 126 infants. However, six infants did not provide sufficient saliva samples for cortisol analysis and four were missing behavioral data). Mothers gave informed consent for all aspects of the protocol, which was approved by the Institutional Review Board for protection of human subjects. Descriptive information for the study sample is shown in Table 1.

Maternal Data Collection

Maternal plasma samples were collected for cortisol analysis and maternal psychological state (state anxiety, perceived stress and depression) at five intervals during pregnancy (Time 1: 15.1 ± 0.9 , Time 2: 19.3 ± 1.0 , Time 3: $25.4 \pm .9$, Time 4: 30.9 ± 0.69 ; Time 5: 36.5 ± 1.2 weeks of gestation). The sampling intervals were selected based upon evidence that biological markers of stress (e.g., cortisol) increase over the course of human pregnancy (e.g., Sandman et al., 2006). We have adopted roughly equal intervals that represent early, mid and late pregnancy to capture the trajectories of these markers. The initial period is the earliest that we could reliably recruit women into the study. Prenatal medical history and risk for adverse obstetric outcomes were obtained from extensive structured medical interviews at each visit in combination with a thorough review of prenatal and hospital medical records.

Maternal Plasma Cortisol

In the afternoon, at least one hour after women had eaten (mean time of day ranged from 13:19 to 13:30 across the five assessment intervals), maternal blood samples were withdrawn by antecubital venipuncture into EDTA vacutainers. EDTA vacutainers were chilled on ice immediately, then centrifuged at 2000 xg (15 minutes), decanted into polypropylene tubes and stored at -70°C until assayed. Plasma cortisol levels were determined by a competitive binding solid phase enzyme-linked immunosorbent assay (IBL Immuno Biological Laboratories America, Minneapolis, MN). All samples were assayed in duplicate. The inter- and intra-assay coefficients of variance are reported as less than 8% with a minimum detectable level of $0.25 \mu\text{g/dL}$.

Maternal Psychological Assessments

Maternal psychosocial stress was evaluated using three standardized measures all of which have been used extensively with pregnant and non pregnant subjects (e.g., Glynn, Dunkel Schetter, Hobel, & Sandman, 2008; Marcus, Flynn, Blow, & Barry, 2003). Generalized or non-specific stress was evaluated using the 12-item version of Cohen's Perceived Stress Scale (PSS, Cohen, Kamarck, & Mermelstein, 1983). The PSS evaluated participants' feelings about how they were able to handle day-to-day problems and hassles, how often

they felt nervous and stressed and how often they felt things were going well during the past week. Responses were made on a 5-point Likert scale ranging from 0 (never) to 4 (almost always) and the final score could range from 0 to 48. The short form of the Center for Epidemiological Studies Depression Inventory was used to evaluate maternal depression (CES-D, Santor & Coyne, 1997). Responses to each of the 9 items in this measure were recorded on a four-point Likert scale with a range of 0 to 3. Anchor points, in terms of days per week during the last week, were “rarely or none of the time (less than 1 day)” to “most or all of the time (5-7 days)”. The final score could range from 0 to 27, with a higher score indicating greater impairment. The 10-item State Anxiety subscale of the State-Trait Personality Inventory (Spielberger, 1983) assessed the degree to which participants had experienced anxiety-related symptoms or emotions in the last few days. Responses were made using a 4-point Likert scale ranging from 1 (not at all) to 4 (very much) and scores could range from a minimum of 10 to a maximum of 40.

Prenatal Course and Birth Outcome

An extensive structured medical interview was conducted by a research nurse at each prenatal visit to assess maternal health and pregnancy related complications. Maternal and infant medical records were reviewed to assess pregnancy complications and birth outcome. A binary score assessing the presence or absence of prenatal obstetric risk was derived (Hobel, 1982). This obstetric risk score includes assessment of the presence or absence of infection, pregnancy induced-hypertension, gestational diabetes, oligohydramnios, polyhydramnios, preterm labor, vaginal bleeding, placenta previa and anemia, in the index pregnancy.

Infant Data Collection

Infant response to the heel-stick blood draw was evaluated between 13 and 35 hours after birth ($M = 23$ hrs 47 min, $SD = 4$ hrs 13 min). Infants were monitored continuously for one-hour prior to the assessment to ensure that they were not handled or fed. The assessment began with baseline measurements. During the ten-minute baseline period, behavioral state was recorded at 20-second intervals. Baseline salivary cortisol was then assessed, followed by a clinically indicated heel-stick blood draw performed by a neonatal nurse. For this protocol, the participant's nurse cleaned the heel with an alcohol swab, applied an automated lancet, and then repeatedly squeezed the heel until a sufficient blood sample was obtained. The median length of heel-stick was 4 minutes. Behavioral assessments continued during the heel-stick procedure and during the five minutes immediately following the heel-stick (recovery). To capture the peak cortisol response to the heel-stick, salivary cortisol samples were taken at 20 minutes and 40 minutes after the start of the heel-stick (E. P. Davis, Townsend et al., 2004; Gunnar, 1989; Gunnar, Hertsgaard, Larson, & Rigatuso, 1992). During the study period, infants were not fed or handled beyond the blood draw protocol.

Infant Salivary Cortisol Assessment

Saliva was obtained (without any stimulant) by placing a swab in the infant's mouth. Samples can be collected in this manner without disturbing or waking the infant (e.g., Davis et al., 2004). Salivary cortisol reflects the unbound or active fraction of cortisol and is highly correlated with plasma cortisol (Gunnar, 1989; Kirschbaum & Hellhammer, 1989). The collection swab was then placed in a saliva extraction tube (Roche Diagnostics). Saliva samples were spun and stored at -20 degrees C until assayed. Salivary cortisol levels were determined by a competitive luminescence immunoassay (LIA; IBL-America, Minneapolis, MN) with detection limits of 0.005 $\mu\text{g}/\text{dl}$. The intra- and inter-assay coefficients of variance are 5.5% and 7.6%, respectively. All samples were assayed in duplicate and averaged.

Infant Behavioral Responses to Stress

Behavioral state was assessed using a modified version of a coding system designed for the assessment of neonatal behavioral regulation (Als, Lester, Tronick, & Brazelton, 1988). This scheme was used to categorize each infant's state on a scale of 1 to 6: quiet sleep, active sleep, quiet awakeness/ drowsy, awake and alert, awake and fussy, and crying. Behavior was coded in real time in 20-second epochs. Codes recorded represented the highest state noted during each 20-second epoch. For 15% of the infants, two observers independently coded behavioral state. Percent agreement for state codes was 85.1%. Coders were blinded to all information about the mothers (e.g., maternal prenatal cortisol and psychological state).

Data Transformation and Analysis Plan

Analyses were performed using SPSS 17 and HLM6 software. Inspection of maternal cortisol data indicated that levels were normally distributed and analyses were performed with raw data. Summary scores were created to characterize infant cortisol and behavioral responses to the heel-stick. For infant cortisol, delta scores were calculated by subtracting baseline levels from values at 20 minutes (response) and 40 minutes (recovery) after the start of the heel-stick. Behavioral state composite scores were calculated for each of the three phases. Baseline was defined as the average behavioral state score during the 10-minute period prior to the heel-stick procedure. The heel-stick response score was defined as the average behavioral state score during the first minute of the heel-stick procedure. This interval was selected to ensure that all infants had complete data despite variability in length of heel-stick. Recovery was defined as the average behavioral state score during 5-minute period following the end of the heel-stick. Repeated measures ANOVAs, with post hoc as needed, were implemented to determine if cortisol or average behavioral state changed across the three assessment intervals (baseline, response and recovery).

Preliminary analyses were performed to identify variables that might influence infant cortisol or behavioral regulation including, sociodemographic factors (marital status, race/ethnicity, education, and household income), medical risk (prenatal medical risk, parity, maternal age, mode of delivery) and infant factors (gestational age at birth, birth weight, sex, Apgar score, and postnatal age in hours). None of these factors were associated significantly with infant cortisol or behavior at baseline, response or recovery and so none were included in the model as covariates (all p 's > 0.10). Additionally, aspects of the heel-stick event, time of day (p 's > .14) and length of the heel-stick procedure (p 's > .12) were not associated with patterns of infant cortisol or behavior. Even within the restricted range of sample collection, maternal cortisol was correlated with collection time (r ranged from -0.49 to -0.33). Thus, time of maternal sample collection was included as a covariate in all subsequent analyses. Additional correlational analyses determined that there were no significant association between prenatal maternal cortisol or psychosocial stress and baseline infant cortisol and behavioral state (all p 's > .15).

Hierarchical linear modeling (HLM) growth curve analyses (Raudenbush & Bryk, 2002) were used to describe the trajectory of prenatal maternal cortisol and psychosocial stress across pregnancy. HLM, when used with repeated measures, treats the data in a hierarchical fashion with observations nested within persons. This approach allows variance to be modeled at multiple levels and provides several advantages over ordinary least squares (OLS) regression. First, standard regression models are limited to one component of variability, the deviation of the individual from the group mean. In contrast, HLM includes the within-person variability assessed over time. Second, HLM uses precise measures of timing (i.e. gestational week) of data collection rather than nominal estimates of assessment intervals. Third, estimates of goodness of fit in modeling each individual's data are derived and the most reliable data are given greater statistical weight. Fourth, HLM produces robust

estimates despite missing values for the repeated measure. In HLM models cases with complete data are weighted more heavily, but all cases are included in the estimation of effects. Ninety-two mothers had complete cortisol data at all prenatal assessments. Twenty-one participants were missing data from one prenatal assessment and three participants were missing data from two assessments.

Separate two-level models were constructed to determine the association between the trajectory of maternal cortisol and maternal psychosocial stress across gestation and infant cortisol and behavioral responses to stress. A linear model was used for perceived stress, state anxiety and depression. For maternal plasma cortisol a quadratic function was a better fit ($p < .05$). In all cases predictor and outcome variables were modeled as continuous variables. Level 1 variables, or those evaluated longitudinally across the five prenatal assessment days, included: maternal cortisol, maternal psychosocial stress measures, time of day and gestational week. Infant cortisol levels (response-delta 20 min post and recovery-delta 40 min post) or behavioral composite scores (response and recovery) were the outcomes of interest and were modeled continuously at level 2. These analyses were used to identify the period during gestation during which maternal cortisol and psychosocial stress were most strongly associated with infant stress responses. The model was tested for differences in intercept at each gestational week within the range of actual endocrine and psychosocial assessments (13 to 38 weeks of gestation).

Results

Prenatal Maternal Cortisol

As anticipated, maternal cortisol increased from 10.7 $\mu\text{g/dl}$ at 15 gestational weeks to 25.5 $\mu\text{g/dl}$ at 36 gestational weeks (see Table 2). Further, as shown in Table 3, maternal plasma cortisol levels were moderately correlated across gestation.

Maternal Psychological State

As shown in Table 2, maternal perceived stress (r 's ranged from 0.44 to 0.67, p 's $< .001$), depression (r 's ranged from 0.44 to 0.67, p 's $< .001$) and state anxiety (r 's ranged from 0.41 to 0.58, p 's $< .001$) were relatively stable across gestation. Further, within each assessment time point the three measures were highly correlated (r 's ranged from 0.47 to 0.83, p 's $< .001$).

Prenatal Maternal Cortisol and Maternal Psychosocial Stress

Maternal anxiety and depression scores were not associated significantly with maternal cortisol at any of the five prenatal assessments (all p 's $> .10$). Among all of the psychosocial stress measures, only perceived stress at 25 weeks gestation was associated with maternal cortisol after controlling for time of day [partial $r(115) = -.21$, $p < .05$]. This association did not remain significant after correction for multiple comparisons. Perceived stress at the other four assessment time points was not associated significantly with maternal cortisol (all p 's $> .10$).

Infant stress responses

Infant cortisol levels changed in response to the heel-stick [$F(2,114) = 10.9$, $p < .01$]. Post hoc tests revealed that infant cortisol levels were higher at 20 [$t(115) = 4.3$, $p < .01$] and 40 minutes [$t(115) = 3.3$, $p < .01$] as compared to baseline levels. Similarly, infants responded behaviorally to the heel-stick [$F(2,114) = 66.2$, $p < .01$]. Infants were in a significantly more aroused behavioral state during the heel-stick response [$t(115) = 10.2$, $p < .01$] and the recovery period [$t(115) = 2.3$, $p < .05$], as compared to baseline. Infant cortisol was not significantly associated with behavioral state scores during the baseline or response period

(p 's > .35), but was modestly associated with behavior during the recovery period. Infants who displayed a greater cortisol increase at 20 minutes and 40 minutes after the start of the heel-stick stressor also showed delayed behavioral recovery from the heel-stick stressor [$r(116) = .21$, $p < .05$ and $r(116) = .17$, $p = .06$ respectively]. Infant stress responses did not differ by sex (p 's < 0.20).

Prenatal Maternal Cortisol and Infant Stress Regulation

The trajectory of maternal cortisol across gestation was associated with the infant cortisol response to the heel-stick stressor. Growth curve analyses, including maternal and infant cortisol as continuous variables, revealed significant intercept differences between 21 and 35 gestational weeks (β 's: 0.72 to 1.11; t 's: 2.0 to 2.5, p 's < 0.05), indicating that elevated maternal cortisol was associated with a larger increase in infant cortisol in response to the heel-stick (Figure 1A). As shown in Figure 1B, high maternal cortisol between 20 to 27 gestational weeks predicted elevated infant cortisol at the recovery assessment (β 's: 0.52 to .68; t 's: 2.0 to 2.2, p 's < 0.05). The strongest association (i.e., largest β) between prenatal maternal cortisol and infant cortisol response to the heel-stick procedure was at 25 gestational weeks. This association is presented in Figure 2 illustrating that infants exposed to the highest (top quartile) or lowest (bottom quartile) maternal cortisol have the largest and smallest HPA responses respectively.

The pattern of maternal cortisol across gestation also predicted infant behavioral state during the recovery period. Mothers with high cortisol early in pregnancy had infants who displayed slower behavioral recovery from the painful stress of the heel-stick procedure. Specifically, as shown in Figure 3 elevated maternal cortisol levels at the earliest time points measured (13 to 14 gestational weeks) were associated with higher infant behavioral arousal during recovery (β 's: .54 to .65; t 's: 2.1 to 2.2, p 's < 0.05). In Figure 4 behavioral state scores are presented for infants born to mothers with high (top quartile) and low (bottom quartile) cortisol levels at 13 weeks' gestation. Infant behavioral state during the heel-stick response period (p 's > 0.3) was not significantly associated with maternal prenatal cortisol.

Prenatal Maternal Psychosocial Stress and Infant Stress Regulation

The pattern of maternal psychosocial stress across gestation also was associated with infant behavioral state during the recovery period. Elevated levels of perceived stress, anxiety and depression were associated with slower infant behavioral recovery. Infants born to mothers with elevated levels of maternal perceived stress throughout gestation maintained a higher level of behavioral arousal during recovery (from 13 to 35 weeks β 's: .09 to .10; t 's: 1.9 to 2.4, p 's < 0.05, between 36 and 38 weeks β 's: .086 to .087; t 's: 1.8 to 1.9, p 's < 0.10). Elevated behavioral state scores during recovery were additionally associated with elevated maternal state anxiety (from 26 to 38 gestational weeks; β 's: .06 to .09; t 's: 1.9 to 2.1, p 's < 0.05) and elevated maternal depression (from 24 to 38 gestational weeks; β 's: .07 to .13; t 's: 1.9 to 2.4, p 's < 0.05). Figure 5 illustrates the association between maternal perceived stress scores across gestation and infant behavioral state during recovery. Figure 6 depicts behavioral state scores for infants born to mothers with high (top quartile) and low (bottom quartile) perceived stress scores at 13 weeks' gestation. Maternal psychosocial stress measures were not associated with behavioral state during the response period or infant cortisol response or recovery (all p 's > 0.12).

Discussion

In a prospective study with multiple prenatal assessments we document that prenatal maternal cortisol and psychosocial stress program infant stress regulation. Confidence in these data is increased because associations are observed among healthy full term neonates

and thus cannot be explained by postnatal influences such as parenting style. Prenatal maternal psychosocial stress and cortisol each exert influences on infant stress regulation, independent of baseline functioning, and these influences are dependent upon the gestational period during which the fetus is exposed.

Prenatal Maternal Cortisol and Infant Stress Regulation

The results of this study indicate that the trajectory of maternal cortisol levels across gestation predicts infant behavioral and physiological responses to stress. This likely occurs through the actions of cortisol on the developing nervous system. Cortisol is important for normal maturation in most regions of the developing CNS initiating terminal maturation, remodeling axons and dendrites, myelination and cell survival (Kapoor et al., 2008; Raschke, Schmidt, Schwab, & Jirikowski, 2008; Setiawan, Jackson, MacDonald, & Matthews, 2007). Moreover receptors for cortisol are highly expressed in the developing brain (Jacobson & Sapolsky, 1991; Sanchez et al., 2000). Exposure to cortisol during gestation has widespread effects upon neuronal structure and synapse formation and the specific consequences will be dependent on the gestational interval of exposure (Seckl & Meaney, 2006).

The prenatal period of susceptibility to the programming influence of maternal cortisol differs for physiological and behavioral stress regulation. As reported here, elevations in maternal cortisol early in pregnancy affect infant behavioral stress responses, but later elevations affect infant cortisol responses. The distinct consequences of maternal cortisol based on the timing of exposure may result both from changes in placental physiology regulating fetal exposure to maternal cortisol (Brown et al., 1996) and variations in the sensitive periods of development of different fetal physiological systems (Seckl & Meaney, 2006). Between the gestational ages of 8 and 16 weeks migrating neurons form the subplate zone, awaiting connections from afferent neurons originating in the thalamus, basal forebrain, and brainstem. Once neurons reach their final destination, they arborize and branch in an attempt to establish appropriate connections (Sidman & Rakic, 1973). The connectivity between brainstem, limbic and cortical brain regions is responsible for behavioral regulation (Geva & Feldman, 2008). It is possible that early exposure to maternal cortisol affects infant behavioral regulation because of the influence of cortisol on the development of connectivity between these regions.

Exposure to maternal cortisol later in gestations may influence the development of the fetal HPA axis by modifying glucocorticoid receptor development in regions such as the paraventricular nucleus of the hypothalamus, hippocampus, and amygdala (Herman & Cullinan, 1997; Levitt, Lindsay, Holmes, & Seckl, 1996). These regions are particularly sensitive to excessive levels of glucocorticoids. Findings from animal models suggest that exposure to elevated levels of GCs alters the density of both types of cortisol receptors (mineralocorticoid and glucocorticoid) in the hippocampus and the amygdala with consequences for HPA axis feedback sensitivity (Kapoor, Dunn, Kostaki, Andrews, & Matthews, 2006). Limited information exists regarding the time course of prenatal development of cortisol receptors in humans. There is evidence that both types of cortisol receptors are present in the human hippocampus by 24 gestational weeks (Noorlander, De Graan, Middeldorp, Van Beers, & Visser, 2006) indicating that in the latter half of gestation this region is susceptible to the consequences of excess cortisol. Alterations to neurological systems at different times during fetal development resulting from prenatal exposure to GCs may determine the neonate's ability to respond behaviorally and physiologically to stressors in the postnatal environment.

Data suggesting that elevations in maternal cortisol are associated with increased behavioral stress reactivity are consistent with animal studies (Abe et al., 2007) and with studies in

humans that link maternal cortisol with increased fearful or reactive behavior during infancy (E. P. Davis et al., 2007; C. de Weerth et al., 2003). The observed association between maternal cortisol between 21 and 35 weeks and infant cortisol reactivity is partially consistent with studies which showed associations between maternal cortisol measured between 15 and 17 gestational weeks and child cortisol levels (Gutteling et al., 2004; 2005). The current investigation is the only one to evaluate timing of exposure with multiple assessments across gestation. It is plausible that differences in the type of stressor and age at assessment accounts for the variability across studies.

Maternal Psychosocial Stress and Infant Stress Regulation

Maternal psychosocial stress is additionally associated with infant behavioral stress regulation. Higher maternal perceived stress throughout gestation and higher anxiety and depression during the end of the second and the third trimester predict a slower rate of recovery of behavioral stress responses in the newborn. The positive association between prenatal maternal stress and neonatal behavioral reactivity is consistent with both experimental animal studies (Kapoor et al., 2008; Maccari & Morley-Fletcher, 2007) and human work (Bergman et al., 2007; E. P. Davis, Snidman et al., 2004) linking prenatal maternal stress with increased behavioral reactivity in the offspring in response to challenge. Prenatal psychosocial stress is not significantly correlated with neonatal cortisol regulation, suggesting that HPA functioning immediately after birth is not influenced by prenatal maternal psychosocial stress. It remains possible, however, that associations between prenatal psychosocial stress and HPA axis regulation in the offspring will emerge later in development (O'Connor et al., 2005; B. R. H. Van den Bergh et al., 2007) and that associations may be present in high risk populations in which exposures are more dramatic (Grant et al., 2009; Keenan, Gunthorpe, & Grace, 2007).

Consistent with prior work, it seems unlikely that maternal cortisol mediates the effect of maternal report of psychosocial stress on infant behavioral reactivity (Bergman, Glover, Sarkar, Abbott, & O'Connor, 2010; E. P. Davis et al., 2007; E.P. Davis & Sandman, 2010; C. de Weerth & Buitelaar, 2005). This is so because early in pregnancy (the time when maternal cortisol is associated with infant behavior) maternal cortisol and psychosocial stress are not correlated and because maternal cortisol later in gestation does not predict infant behavior. Maternal psychosocial stress exerts widespread influences on a number of stress sensitive systems other than the HPA axis including the immune and vascular systems (Dunkel Schetter & Glynn, in press). These systems are candidates for future studies examining possible pathways that might mediate effects of psychosocial stress on the developing fetus. Further, although smoking and drug and alcohol use were exclusion criteria for this study, elevated psychosocial stress is likely associated with other health related behaviors such as nutrition and physical activity (see Dunkel Schetter & Glynn, in press for review). These behavioral profiles could have contributed to associations between maternal psychosocial stress and infant behavioral regulation.

Limitations

First, because this study relied on naturally occurring variations in maternal cortisol, rather than experimental manipulations, it is difficult to separate the effects of cortisol from the consequences of other factors that might contribute to this association such as shared genes. The current findings are, however, consistent with animal models where random assignment is possible (Kapoor et al., 2008 for review) as well as human studies evaluating the consequences of a randomly occurring traumatic events, such as natural disasters (LaPlante et al., 2004; Yehuda et al., 2005). Further, recent human studies have documented developmental consequences of prenatal stress among children conceived by in vitro fertilization that were not genetically related to their mother (Rice et al., 2009). Second,

maternal cortisol is an indirect measure of fetal cortisol exposure. There is evidence with human fetuses, however, that maternal and fetal cortisol levels are significantly correlated suggesting that level of maternal cortisol is a valid index of fetal exposure (Gitau et al., 1998). Third, because of the invasive nature of a blood draw, maternal cortisol levels were assessed only on one day during each of the five assessment intervals. The growth curve modeling techniques used in this study consider cortisol trajectories enabling a more stable characterization of maternal cortisol across gestation.

Implications

The large and prolonged cortisol response and protracted behavioral responses to the stress of painful stimulation reported here may be indicative of a dysfunctional stress system. This is particularly likely given the cost associated with an inability to turn off the stress response after the stressor has passed (McEwen, 1998). Neonates who are more reactive may carry a greater risk for the development of behavioral inhibition and anxiety. There is evidence that infants and toddlers who display greater physiological arousal to challenge tend to be more fearful or anxious in response to novel situations (Buss, Davidson, Kalin, & Goldsmith, 2004; Kagan, Snidman, & Arcus, 1998; Stansbury & Gunnar, 1994). Further, poor behavioral regulation during the neonatal period is predictive of increased stress reactivity and poor attention and emotional regulation during infancy and childhood (Geva & Feldman, 2008; Lewis, Worobey, & Thomas, 1989). It is plausible that maternal cortisol influenced the development of the fetal HPA axis, including alterations to receptor development. A consequence of these changes may be the inability to respond appropriately to stressors experienced in the postnatal environment and is a potential mechanism that may underlie fetal programming of later health and development (Drake et al., 2007; Seckl & Meaney, 2006). Longitudinal follow-up of this cohort will determine the developmental implications of alterations to neonatal stress responses.

Conclusion

Prenatal maternal signals facilitate fetal adaptation to the postnatal environment. Child and adult functioning, including vulnerability to disease, appears to be determined, in part, by exposures that occur during the fetal period (Barker, 1998). Growing evidence indicates that prenatal influences play a role in the development of psychiatric disorders such as anxiety, depression and externalizing behavior problems (Bohnert & Breslau, 2008; Costello et al., 2007; Hellemans, Sliwowska, Verma, & Weinberg, 2009). It has been proposed that disruptions to HPA axis functioning may be responsible for this effect (Kapoor et al., 2008; Seckl & Meaney, 2006). The data observed here, in a large prospective study, are among the first with humans to demonstrate that prenatal maternal stress and stress hormones alter the functioning of stress regulatory systems in the offspring, independent of postpartum influences, and may be a potential mechanism for fetal programming of later psychiatric disorders. Although the observed associations are likely not explained by postnatal influences, it is highly likely that the prenatal and postnatal environment will jointly determine developmental trajectories (e.g., Bergman et al., 2008). It is our intention to continue to follow this cohort to determine the effects of prenatal and early postnatal experiences on the development of stress regulatory systems.

Key Points

- Retrospective studies have demonstrated that adult health outcomes are determined, in part, by prenatal experiences.
- Elevated maternal cortisol is associated with a larger infant cortisol response and a delayed behavioral recovery to the painful stress of a heel-stick blood draw.

- Elevated maternal psychosocial stress is associated with a prolonged behavioral response to the painful stress of a heel-stick blood draw.
- Increased physiological and behavioral reactivity is a risk factor for the development of behavioral inhibition and anxiety.
- Prenatal maternal stress and stress hormones are a potential mechanism for fetal programming of later psychiatric disorders.

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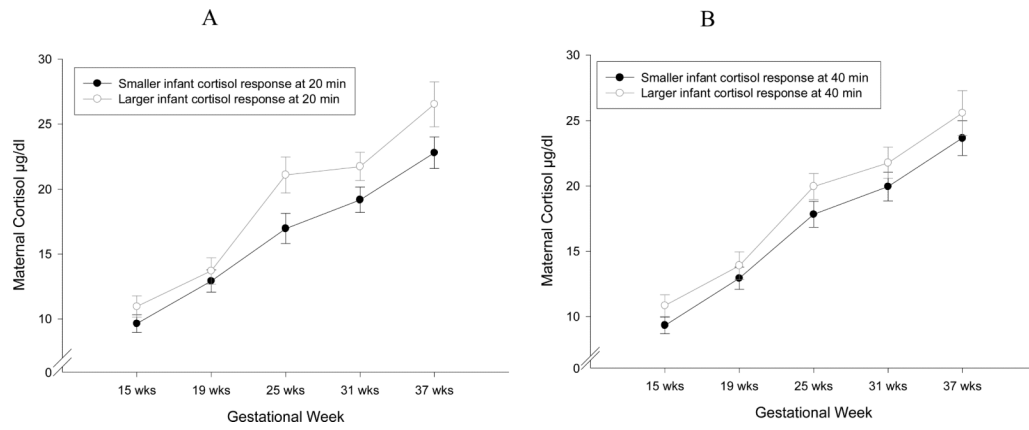


Figure 1.

Figures A and B illustrate the trajectory of maternal cortisol across gestation that is associated with the infant cortisol response to the heel-stick. Data were analyzed continuously using growth curve modeling. For illustrative purposes, prenatal maternal cortisol trajectories (with standard error bars) are displayed for mothers of infants with the highest cortisol response (top quartile) and the lowest cortisol response (bottom quartile) at 20 minutes (Figure 1A) and 40 minutes (Figure 1B) after the heel-stick.

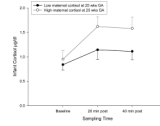


Figure 2.

This figure illustrates the infant cortisol profile that is associated with high (top quartile) and low (bottom quartile) prenatal maternal cortisol. Data are presented based on maternal cortisol at 25 weeks' gestation; the point with the strongest association between prenatal maternal cortisol and infant cortisol response to the heel-stick procedure based on growth curve models.

Note: Both maternal cortisol and infant cortisol were analyzed as continuous variables. For graphing purposes we have presented data based on the top and bottom quartile.

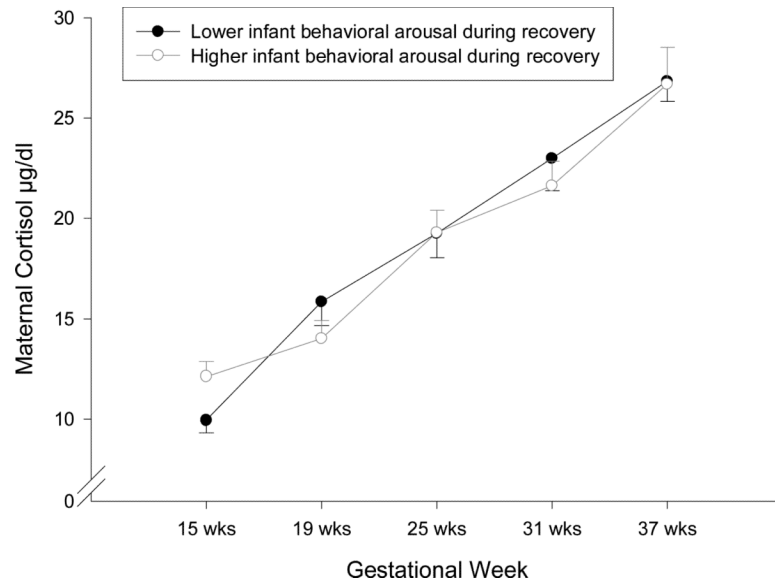


Figure 3. The trajectory of maternal cortisol across gestation that is associated with infant behavioral recovery scores is presented. Although data were analyzed continuously using growth curve modeling, for graphing purposes the trajectory of cortisol across gestation (with standard error bars) is shown for the mothers of infants with the highest behavioral recovery scores (top quartile) and the lowest behavioral recovery scores (bottom quartile).

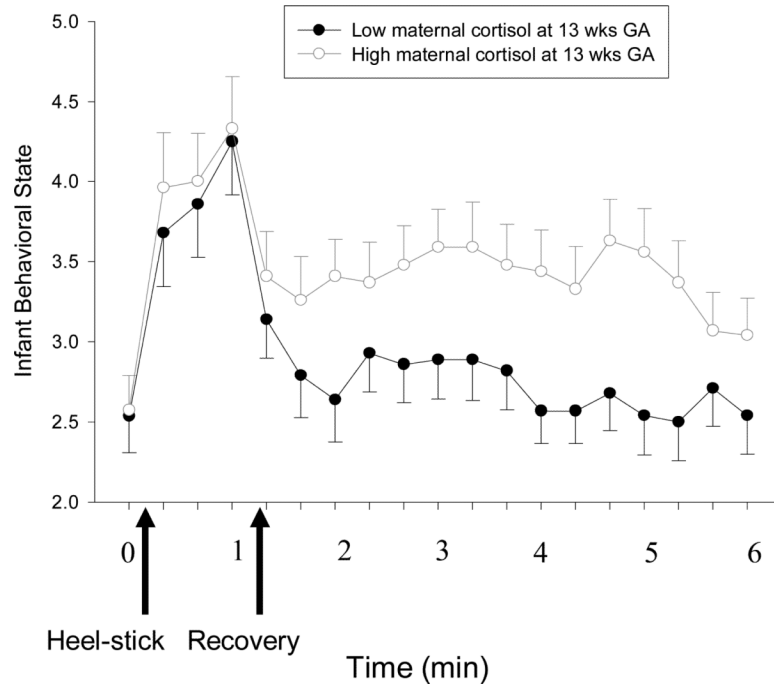


Figure 4.

This figure illustrates the pattern of behavioral responses that is associated with maternal cortisol. Behavioral state scores are presented for infants born to mothers with high (top quartile) or low (bottom quartile) cortisol levels at 13 weeks' gestation (the point with the strongest association between prenatal maternal cortisol and infant behavioral responses to the heel-stick procedure based on HLM analyses).

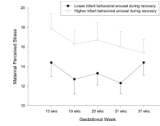


Figure 5.

The trajectory of perceived stress across gestation associated with infant behavioral recovery scores is presented. Although data were analyzed continuously using growth curve modeling, for graphing purposes the trajectory of perceived stress across gestation (with standard error bars) is presented for the mothers of infants with the highest behavioral recovery scores (top quartile) and the lowest behavioral recovery scores (bottom quartile).

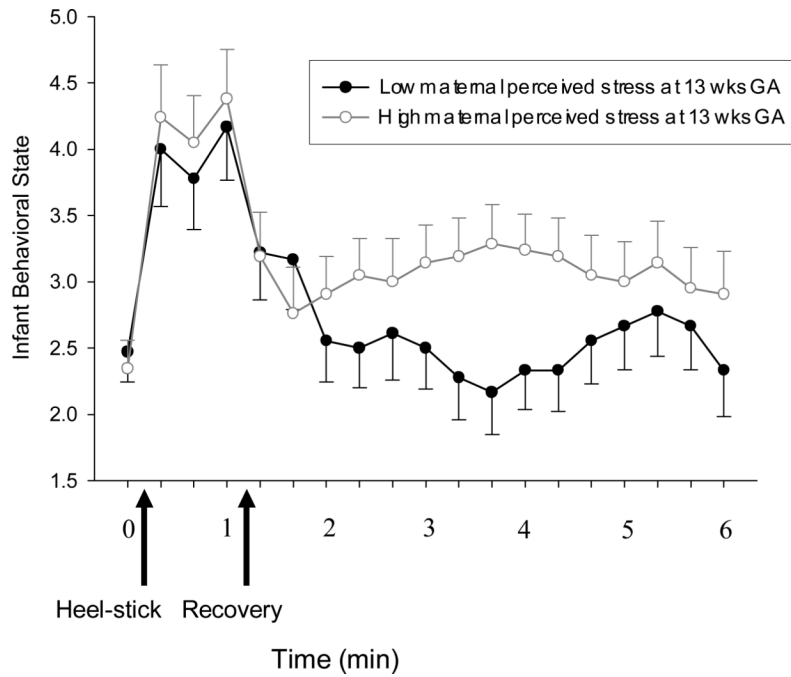


Figure 6. The pattern of behavioral responses that is associated with maternal perceived stress is shown. Behavioral state scores are presented for infants born to mothers with high (top quartile) and low (bottom quartile) perceived stress at 13 gestational weeks (the point with the strongest association between prenatal maternal perceived stress and infant behavioral responses to the heel-stick procedure based on HLM analyses).

Table 1

Demographic information for the study sample

Study Sample (N=116)	
Infant gestational age at birth (weeks)	M= 39.1, SD = 1.1
Infant birth weight (grams)	M = 3448, SD = 434
Maternal age at delivery (years)	M = 28.6, SD = 5.8
Married (%)	65
Primiparous (%)	45
Education (years)	14.5
Annual household income (%)	
\$0 to \$30,000	29
\$30,001 and \$60,000	22
\$60,001 and \$100,000	28
Over \$100,000	21
Race/Ethnicity (%)	
Non-Hispanic white	51
Hispanic	34
Asian	11

Table 2
Maternal cortisol and psychosocial stress (means and standard deviations) at the five prenatal assessment intervals

	Gestational Week				
	15	19	25	31	37
Maternal Cortisol $\mu\text{g/dl}$	10.7 (3.9)	14.6(5.4)	19.1 (6.5)	22.0 (6.4)	25.5 (7.5)
Perceived Stress	16.6(7.3)	15.0(7.7)	15.5(7.9)	14.2(7.7)	14.6(7.1)
State Anxiety	19.6(5.3)	18.7(5.8)	18.5(5.8)	18.1(5.6)	18.9(5.7)
Depression	6.7(5.3)	6.0(4.7)	6.2(5.0)	6.7(4.9)	6.7(4.9)

Table 3

Intercorrelations among prenatal maternal plasma cortisol samples

	Gestational Week			
	19	25	31	37
15 Wks GA	.33*	.32**	.24*	.30**
19 Wks GA	-	.52**	.32**	.25**
25 Wks GA	-	-	.29**	.24*
31 Wks GA	-	-	-	.33**

*
p < .05**
p < .01