Prenatal Exposure to Maternal Depression and Cortisol Influences Infant Temperament

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ABSTRACT

Background: Accumulating evidence indicates that prenatal maternal and fetal processes can have a lasting influence on infant and child development. Results from animal models indicate that prenatal exposure to maternal stress and stress hormones has lasting consequences for development of the offspring. Few prospective studies of human pregnancy have examined the consequences of prenatal exposure to stress and stress hormones. **Method:** In this study the effects of prenatal maternal psychosocial (anxiety, depression, and perceived stress) and endocrine (cortisol) indicators of stress on infant temperament were examined in a sample of 247 full-term infants. Maternal salivary cortisol and psychological state were evaluated at 18–20, 24–26, and 30–32 weeks of gestation and at 2 months postpartum. Infant temperament was assessed with a measure of negative reactivity (the fear subscale of the Infant Temperament Questionnaire) at 2 months of age. **Results:** Elevated maternal cortisol at 30–32 weeks of gestation, but not earlier in pregnancy, was significantly associated with greater maternal report of infant negative reactivity. Prenatal maternal anxiety and depression additionally predicted infant temperament. The associations between maternal cortisol and maternal depression remained after controlling for postnatal maternal psychological state. **Conclusions:** These data suggest that prenatal exposure to maternal stress has consequences for the development of infant temperament. *J. Am. Acad. Child Adolesc. Psychiatry,* 2007;46(6):737–746. **Key Words:** pregnancy, cortisol, temperament, stress, depression.

It has become increasingly evident that the origins of many illnesses begin in fetal life and that prenatal events have beneficial and harmful effects on health (Gluckman and Hanson, 2004). The influential studies of Barker (1998, 2002) provide convincing support for the importance of human fetal experience in determining developmental patterns. Because the prenatal period is a time of enormous change, the fetus is particularly vulnerable to both organizing and disorganizing influences on the nervous system. These organizational influences on fetal development have been described as "programming" (Nathanielsz, 1999). Programming is a process by which a stimulus or insult during a critical developmental period has a long-lasting or permanent influence. Tissues develop in a specific sequence from conception to maturity. Different organs are sensitive to environmental influences at different times depending on their rate of cell division. Critical periods are defined by epochs of rapid cell division within an organ (Cameron and Demerath, 2002; Creasy and Resnik, 1994). During periods of rapid cell division, organs are especially vulnerable to perturbations such as stress (Kajantie, 2006). Thus, the timing of the stimulus during development coupled with the timetable for organogenesis determines the nature of the programming effect (Barker, 1998, 2002).

Extensive animal studies have documented lifelong effects of exposure to stress and stress hormones during prenatal development (Chapillon et al., 2002;

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Schneider, 1992; Weinstock, 2001). In humans the most well-documented consequences of prenatal stress are preterm birth and low birth weight (Hobel, 2004). Much less is known about the influence of prenatal stress on development, independent of birth outcome. Accumulating evidence suggests that the effects of prenatal maternal stress persist into the postpartum period and that consideration of the timing of exposures will be essential to our understanding of the influence of prenatal stress (Austin et al., 2005; Van den Bergh et al., 2005).

Although there are multiple pathways by which maternal stress may affect fetal development, including cardiovascular and endocrine changes in the mother, glucocorticoids (GCs) have become a primary candidate for programming the fetal brain and behavior (Owen et al., 2005). Cortisol, the primary GC in humans, is a stress-responsive hormone that is the end product of the hypothalamic-pituitary-adrenocortical (HPA) axis. Regulation of the HPA axis changes dramatically during pregnancy with the production and release of corticotropin-releasing hormone (CRH) from the placenta. In contrast to the negative control on hypothalamic CRH, cortisol stimulates the expression of CRH mRNA in the placenta. Placental CRH is released, establishing a positive feedback loop that allows for the simultaneous increase of CRH, adrenocorticotrophin hormone (ACTH), and cortisol in the maternal and fetal compartments over the course of gestation (King et al., 2001; Petraglia et al., 1996). Fetal exposure to circulating maternal cortisol is regulated by placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which oxidizes cortisol to its inactive form cortisone (Seckl and Meaney, 2004). The levels of placental 11β-HSD2 rise as gestation progresses before falling precipitously near term (Seckl and Meaney, 2004). Although placental 11β-HSD2 partially protects the fetus from the effects of maternal cortisol, 10% to 20% of active maternal cortisol passes through the placenta, and fetal cortisol levels are significantly correlated with maternal levels (Gitau et al., 1998, 2001). Thus, increases in maternal cortisol will have consequences for fetal development (Weinstock, 2005).

GCs are necessary for normal maturation including most regions of the CNS. Sustained elevations of GCs, however, can have deleterious consequences for brain structure and function (Challis et al., 2001; Hillhouse and Grammatopoulos, 2002). Results from animal models indicate that one of the primary effects of prenatal GC exposure is programming of the development of neural systems including limbic regions involved in the regulation of fear or behavioral inhibition (Owen et al., 2005). In rodent models manipulations that alter GC exposure, such as prenatal stress or GC administration, lead to increased fearful behaviors in response to challenges, such as novelty (Dickerson et al., 2005; Van den Hove et al., 2005; Weinstock, 2005). Consistent with these data, nonhuman primates exposed to manipulations that alter GC exposure, such as prenatal stress or ACTH treatment are more irritable, display increased disturbance behaviors in response to novelty (Schneider, 1992; Schneider et al., 1992), and have reduced hippocampal volume and a decrease in neurogenesis in the dentate gyrus (Coe et al., 2002). These data suggest that fetal GC exposure may have a programming influence on the fetus and that one of the primary consequences may be increased behaviorally inhibited or fearful behavior in response to novelty.

Consistent with data from animal research, accumulating evidence from human studies suggests that the effects of prenatal maternal stress persist into the postpartum period. Maternal stress, anxiety, and depression during pregnancy are associated with an increase in fear behaviors in response to novelty and an increase in behavioral and emotional problems in the offspring independent of postnatal psychological state (Davis et al., 2004a; O'Connor et al., 2002). Although there are few human studies examining the consequences of prenatal GC exposure, there is evidence that endogenous maternal stress hormones (Davis et al., 2005; Gutteling et al., 2005; Susman et al., 2001) and synthetic GCs (Davis et al., 2004b, 2006; Trautman et al., 1995) predict emotional disturbances including behavioral inhibition or fearfulness in response to novelty and impaired cortisol regulation. Recent evidence from a study with a small sample size of 17 subjects indicates that exposure to higher maternal cortisol during late pregnancy predicts increased fussiness and negative behavior in infancy (de Weerth et al., 2003). In this study maternal cortisol was measured only at the end of pregnancy and thus effects of timing of exposure were not evaluated. These studies suggest that prenatal GC exposure has consequences for the infant and child, particularly the development of behaviorally inhibited or fearful temperament.

In the present study we investigated the consequences of psychosocial indicators of prenatal maternal stress and prenatal maternal GCs for infant development using a prospective longitudinal study design. A unique component of this study is that maternal psychological state and maternal cortisol were evaluated at three points starting at the 18th week of gestation to enable evaluation of the effects of timing of exposure in a larger sample than has been studied previously. We examined first whether maternal psychological state and maternal cortisol during pregnancy had joint or independent effects on reported negative reactivity in the offspring independent of postpartum influences and, second, whether there was a sensitive period during fetal development for these effects on infant temperament.

METHOD

Participants

Participants in this study included 247 women with singleton term pregnancies who were recruited serially between 1999 and 2003 from two obstetric clinics in southern California before 18 weeks of gestation. These subjects were part of a cohort that participated in a larger study from which several papers have been published (Davis et al., 2005; Rini et al., 2006; Sandman et al., 2006), one of which (Davis et al., 2005) evaluated infant outcomes. In this study the effect of prenatal exposure to placental CRH on infant temperament was assessed. Women with evidence of smoking or drug use during pregnancy were excluded. Of the total potential participants, 63% met eligibility criteria. The most common reasons for ineligibility (in order of frequency) were non-English speaking, gestational age (GA) >18 weeks, multiple gestation, and smoking. Among the eligible women, 67.5% consented to participate and came for the initial visit. The reasons women declined participation were work or school conflict (27%), concern with an aspect of the protocol (e.g., blood draw; 25%), inability to schedule within the GA window for the first visit (19%), child care issues (11%), not interested (7.5%), transportation difficulties (1.5%), moving (1.5%), other (7.5%). Only full-term infants were included in the present study because it has been well documented that maternal stress influences length of gestation (Hobel, 2004) and that infants born prematurely or small for gestational age are at risk of a wide variety of developmental problems (Peterson et al., 2003). Thus, the 33 women who delivered prematurely (before 37 weeks of gestation) were not included in this study.

Women gave informed consent for all aspects of the protocol, which was approved by the Institutional Review Board for Protection of Human Subjects. At delivery, mothers ranged in age from 18 to 43 years (mean 30.7, SD 5.4), 70% of the women were married, and 59% were primiparous. Annual household income for this sample ranged from less than $5,000 to more than $100,000. The annual household income distribution for this sample was as follows: 19.7% between $0 and $30,000, 29.3% between $30,001 and $60,000, 28.4% between $60,001 and $100,000, and 22.6% over $100,000. Ninety-eight percent of women had graduated from high school, and 51% were college graduates. Forty-nine percent of the women were non-Hispanic white, 20% were Hispanic white, 11% were African American, and 9% were Asian. The infants of these women (118 girls and 129 boys) were assessed at 8 weeks of age (SD 2.2 weeks). All of the infants were born at term (mean GA 39.5 weeks, SD 1.1 weeks; mean weight 3,535 g, SD 509 g), and 71% were delivered vaginally. Infants in this sample were stable at the time of delivery and had a median 5-minute Apgar score of 9 (range 7–10). Twenty-one women reported any alcohol use during their pregnancy. Of these women, 16 reported drinking fewer than five drinks while they were pregnant. The remaining 5 individuals consumed between 6 and 56 drinks during their pregnancy. Removing participants who reported any alcohol use during pregnancy did not alter any of the findings described in the Results section.

Procedures

Maternal psychological state (anxiety, depression, and perceived stress) was assessed and maternal saliva samples were collected for cortisol analysis from participants at three time points during pregnancy (time 1: 19.1 ± 0.8, time 2: 24.9 ± 0.84, and time 3: 30.8 ± 1.0 weeks of gestation) and at the postpartum visit (8.0 ± 2.1 weeks; Fig. 1). Saliva samples were not obtained from three women at the second assessment and from four women at the third assessment. Infant temperament was assessed at 8 weeks postpartum from the entire sample (SD 2.1). In addition, prenatal medical history and obstetric risk were obtained through review of medical records.

Measures

Salivary Cortisol Assessment. Saliva samples were collected in the early afternoon, at least 1 hour after the participant had eaten, (mean 14:20, SD 1.5 hours) using a cotton gauze pad and placed into a syringe. Saliva samples were collected, clarified by depressing the plunger, and stored frozen at −20°C until assayed. Thawed samples were centrifuged at 3,000 rpm for 15 minutes before assay. Salivary cortisol concentrations were determined with a competitive solid-phase radioimmunoassay (Coat-A-Count; Diagnostic Products Corp.). All of the samples were assayed in duplicate and

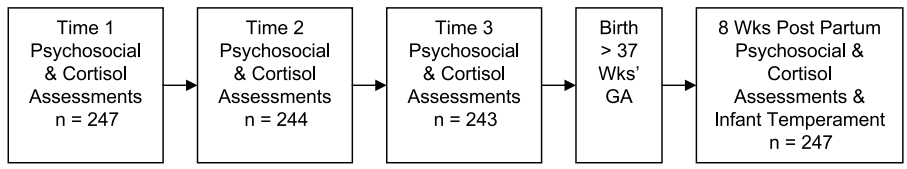

Fig. 1 Flowchart of study visits. Participants in the present study sample (N = 247) were a subset of a larger study of prenatal stress and birth outcome. Thirty-three infants who were born preterm were excluded from this study. GA = gestational age.

averaged. Intra- and interassay coefficients of variance were 5.5% and 7.6%, respectively, with a minimum detectable level of 0.02 g/dL. The cortisol values were log-transformed to normalize the distribution of the data.

Maternal Psychological Assessments. Maternal anxiety and depression were assessed at each prenatal time point and at the postpartum visit. Perceived stress was assessed at all visits with the exception of time 2. A postpartum assessment of maternal psychological state was included to control for the influence of maternal affect during the postnatal period on ratings of infant temperament, as in prior work (Davis et al., 2004a).

Maternal depression was evaluated using the short form of the Center for Epidemiological Studies Depression Inventory (CES-D; Santor and Coyne, 1997). Responses to each of the nine items in this measure were recorded on a 4-point Likert scale with a range of 0 to 3. Anchor points, in terms of days per 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 span from 0 to 27, with a higher score indicating greater impairment. This measure has been extensively used and published studies demonstrate both internal consistency (α = .84) and validity of this measure (Santor and Coyne, 1997). The CES-D is a commonly used instrument for the study of depression in the general population and has been validated in samples of pregnant women (Marcus et al., 2003). Scores on the CES-D during pregnancy are associated with birth outcome and other negative health consequences (Lundy et al., 1999).

State anxiety was measured using the 10-item State Anxiety subscale of the State-Trait Personality Inventory (STAI; Spielberger, 1979). This 10-item scale assessed the extent to which participants had experienced anxiety-related symptoms or emotions using a 4-point Likert scale ranging from 1 (not at all) to 4 (very much). State anxiety scores could range from a minimum of 10 to a maximum of 40. The STAI has been used for research purposes with both pregnant (Rini et al., 1999) and nonpregnant samples. The STAI has good internal consistency with a Cronbach's α coefficient of .92 (Spielberger, 1983).

Generalized or nonspecific stress was evaluated using the 12-item version of Cohen's Perceived Stress Scale (PSS; Cohen et al., 1983). This measure assesses how participants felt 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. Responses were made on a 5-point Likert scale ranging from 1 (never) to 5 (almost always). This measure has been used extensively with samples of nonpregnant and pregnant women (Culhane et al., 2001) and been shown to have both good internal consistency (α = .80) and validity (Cohen et al., 1983).

Medical Risk and Obstetric History. Prenatal medical history and birth outcome were determined through review of the mothers' and the infants' medical records, and a score assessing medical risk for adverse birth outcome was derived (Hobel, 1982). Factors considered included pregnancy-induced hypertension and gestational diabetes in the index pregnancy, as well as history of preterm delivery, spontaneous abortion, stillbirth, or ectopic pregnancy. In addition, parity, mode of delivery, GA at birth, and birth weight were recorded.

Infant Temperament. Infant negative reactivity was assessed using an eight-item version of the fear subscale of the Infant Behavior Questionnaire (IBQ), a standardized instrument designed to assess temperament in infancy by maternal report (Gartstein and Rothbart, 2003). The fear subscale from the IBQ assesses the extent to which infants display startle or distress in response to sudden changes in stimulation or novel or surprising stimuli (e.g., how often during the past week did the baby startle to a loud sound or sudden noise?). Mothers rated their infant on each item using a 5-point Likert scale ranging from 1 (never) to 5 (always). Responses to these items from the fear subscale of the IBQ were averaged to create a score for infant negative reactivity.

Maternal report of temperament takes advantage of a mother's ability to observe her children over a wide range of contexts. Concerns, however, have been raised about the potential for maternal bias. The IBQ was designed to reduce the influence of maternal bias by asking about concrete infant behaviors rather than asking the mother to make abstract judgments (Gartstein and Rothbart, 2003). Published reliability and validity data for the IBQ indicate that this scale has strong psychometric properties. Scores on the fear subscale are stable from 2 months to 1 year (Worobey and Blajda, 1989). Cronbach's α coefficient for this scale was .90. Furthermore, maternal report completion of the fear subscale is correlated with laboratory observational measures of infant fear (Goldsmith and Campos, 1990; Goldsmith and Reisner-Danner, 1986), and the intercorrelation between ratings given by the primary and secondary caregiver is 0.75 (Gartstein and Rothbart, 2003). To further reduce the influence of maternal reporting bias, we statistically controlled for maternal psychological state at the time of reporting on infant temperament.

RESULTS

Maternal Salivary Cortisol

As illustrated in Figure 2, levels of cortisol increased significantly from time 1: 19 weeks' GA to time 2: 25 weeks' GA (t_{244} = 3.4, p < .01) and from time 2: 25 weeks' GA to time 3: 31 weeks' GA (t_{243} = 2.5, p < .001). Furthermore, cortisol levels were higher at all pregnancy time points as compared with postpartum measures (p < .01). Although collection time was constrained, cortisol still was significantly correlated with collection time (r = −0.19 to −0.27; p < .01). Thus, time of sample collection was

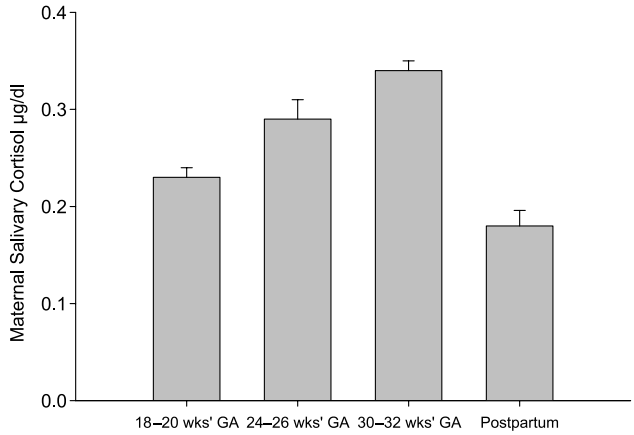

Fig. 2 Maternal salivary cortisol levels at 18–20, 24–26, and 30–32 weeks' gestation and 8 weeks' postpartum. GA = gestational age.

included as a covariate in all of the subsequent analyses. Cortisol levels among the three prenatal measurement periods were modestly correlated. Postnatal maternal cortisol was not correlated with the prenatal measures (Table 1). Neither medical risk ($r = -0.11$ to 0.05, not significant [ns]) nor household income, used as an index of socioeconomic status ($r = -0.01$ to -0.13; ns), significantly influenced maternal cortisol levels.

Maternal Psychological Assessments

The distribution of scores on the PSS, STAI, and CES-D at the pregnancy and the postpartum assessments are displayed in Table 2. Scores on the CES-D were assessed with respect to standardized cutoffs for clinically significant depression available using this measure (Santor and Coyne, 1997). During pregnancy, 12.7% to 17.8% of women were above the standardized cutoff and 13.3% of women were above this cutoff at the postpartum assessment.

Infant Temperament

Infant negative reactivity scores ranged from 1.3 to 3.4 (mean 2.0, SD 0.45). At 2 months, the mean infant negative reactivity score did not differ significantly based on the sex of the infant ($t_{247} = 0.45$; $p = .66$), mode of delivery (vaginal versus cesarean section, $t_{247} = .77$; $p = .44$), parity (primiparous versus multiparous, $t_{247} = 1.6$; $p = .13$), or feeding method (breast milk versus formula, $t_{247} = 0.24$; $p = .89$). Furthermore, neither maternal prenatal medical risk status ($r_{247} = 0.06$; $p = .36$) nor annual household income, used as an index of socioeconomic status ($r_{247} = 0.05$; $p = .31$), were correlated with infant temperament.

Maternal Cortisol and Infant Temperament

To determine whether maternal cortisol influenced infant temperament, three partial correlations were performed controlling for time of sample collection. Results indicated that maternal cortisol at 30 to 32 weeks' gestation (time 3) was significantly related to maternal report of infant negative reactivity (partial $r_{243} = 0.20$; $p < .01$), but that maternal cortisol earlier in pregnancy was not (both partial $r < 0.05$; ns). Furthermore, postpartum maternal cortisol was not significantly related to infant temperament (partial $r_{246} = 0.11$; $p = .09$). We next examined whether either cumulative exposure to maternal cortisol (sum of cortisol across the three prenatal time points) or the rate of increase in cortisol across pregnancy (time 3 cortisol–time 1 cortisol) would be associated with report of negative reactivity. Total cortisol did not predict infant temperament (partial $r_{243} = 0.03$; $p = .69$). Controlling for time of sampling, the rate of increase in cortisol significantly predicted report of infant negative reactivity (partial $r_{243} = .17$; $p < .01$). However, when the independent contribution to the prediction of infant temperament was assessed using time 3 cortisol and the rate of change, only time 3 cortisol was selected by a backward regression ($\beta = .19$, $t = 3.0$; $p < .01$).

Because there is evidence that maternal psychological state during the postpartum period influences both infant temperament and maternal perception of infant temperament (e.g., Pauli-Pott et al., 2003), it is possible that maternal psychological state could account for this association between prenatal maternal cortisol and infant temperament. Ratings of infant negative reactivity were positively associated with maternal postpartum

TABLE 1
Stability of Cortisol During Pregnancy and Postpartum

Time	Time 1 (18–20 wk GA)	Time 2 (24–26 wk GA)	Time 3 (30–32 wk GA)	Postpartum (8 wk)
1	—	0.44*	0.28*	−0.10
2	—	—	0.27*	0.04
3	—	—	—	0.01

Note: GA = gestational age.
*$p < .01$.

TABLE 2
Descriptive Information for the STAI, CES-D, and PSS During Pregnancy and Postpartum

	STAI	CES-D	PSS
Time 1 (18–20 wk GA)	Mean 20.2, SD 6.0, range 10–39	Mean 6.5, SD 4.8, range 0–22	Mean 26.8, SD 7.8, range 12–51
Time 2 (24–26 wk GA)	Mean 19.6, SD 6.0, range 10–40	Mean 6.5, SD 4.7, range 0–26	NA
Time 3 (30–32 wk GA)	Mean 19.4, SD 6.2, range 10–37	Mean 7.3, SD 5.5, range 0–27	Mean 26.7, SD 8.3, range 0–54
Postpartum (8 wk)	Mean 17.8, SD 5.3 range 10–33	Mean 5.4, SD 4.8, range 0–25	Mean 24.9, SD 7.4, range 0–45

Note: STAI = State-Trait Personality Inventory; CES-D = Center for Epidemiological Studies Depression Inventory; PSS = Perceived Stress Scale; NA = not available.

anxiety (r_{247} = 0.14; $p < .05$), depression (r_{247} = 0.10; $p = .12$), and perceived stress (r_{247} = 0.19; $p < .05$). Together these three measures of postnatal psychological state account for a significant portion of the variance in infant temperament (R^2 = 0.04; $p < .05$). To examine whether prenatal maternal cortisol significantly accounted for variance in infants' temperament independently from that accounted for by postnatal psychological state, a hierarchical regression was performed entering cortisol collection time and maternal postnatal psychological state first. Notably, maternal cortisol at time 3 still accounted for a significant portion of the variance in infant temperament after controlling for maternal postnatal psychological state (ΔR^2 = .05, β = .23, t = 3.4; $p < .01$).

Maternal Psychological Assessments and Infant Temperament

The association between the three indices of prenatal maternal psychological state and report of infant temperament is displayed in Table 3. The pattern of associations between the prenatal measures and infant outcomes was similar for the different time points. Furthermore, scores on these measures were correlated during pregnancy (STAI: r = 0.58–0.64, CES-D: r = 0.54–0.64, PSS: r = 0.50). Thus, the three prenatal measures were averaged across the three time points to create a summary measure for each of the three maternal measures (STAI, CES-D, and PSS). As shown in Table 3, average prenatal anxiety and depression but not perceived stress were significantly correlated with report of infant negative reactivity. To evaluate further whether either prenatal maternal anxiety or depression significantly accounted for variance in report of infants' temperament independent of that accounted for by postnatal psychological state, a hierarchical multiple regression was performed for average prenatal anxiety and depression entering the maternal postnatal psychological state measures first. Average prenatal depression (ΔR^2 = .02, β = .15, t = 2.1; $p < .05$) was significantly associated with report of infant temperament after controlling for postnatal psychological state.

TABLE 3
Associations Between Maternal Psychological States and Report of Infant Negative Reactivity

	STAI	CES-D	PSS
Time 1 (18–20 wk GA)	0.15**	0.18***	0.1
Time 2 (24–26 wk GA)	0.12*	0.13**	NA
Time 3 (30–32 wk GA)	0.17***	0.14**	0.1
Mean prenatal	0.17***	0.18***	0.12*
Postpartum (8 wk)	0.15**	0.10	0.19***

*$p < 0.1$; **$p < 0.05$; ***$p < 0.01$.

Influence of Maternal Psychological and Endocrine Factors on Infant Temperament

We examined the interrelationship between the psychological measures and maternal cortisol to determine whether prenatal psychosocial indicators of maternal stress and maternal cortisol had a joint or independent influence on report of infant temperament. None of the maternal measures of psychological state were significantly correlated with measures of maternal cortisol (all $r < 0.1$; ns). The two prenatal measures that significantly accounted for a portion of the variance in infant temperament after controlling for postnatal maternal psychological state were maternal cortisol at 30 to 32 weeks' GA and average prenatal maternal depression. A hierarchical regression was performed to determine whether these factors had an independent influence on report of temperament. We found that after controlling for maternal postnatal psychological state, maternal cortisol (ΔR^2 = 0.05, β = .24, t = 3.3; $p < .01$) and maternal depression (ΔR^2 = 0.02, β = .16, t = 2.2; $p < .05$) independently predicted report of infant negative reactivity. The interaction term did not significantly predict report of infant temperament (β = −.10, t = 0.40; $p = .70$). Together, prenatal maternal cortisol and prenatal depression account for a significant portion of the variance in infant temperament after controlling for postnatal maternal state (ΔR^2 = 0.07; $p < .01$).

DISCUSSION

The data reported here are among the first to examine the joint and independent effects of prenatal maternal psychosocial indices of stress and prenatal maternal cortisol levels on human infant development. Data indicate that maternal cortisol levels during the third trimester of pregnancy predict maternal report of increased negative reactivity (startle or distress in response to novel and surprising stimuli as measured by the fear subscale of the IBQ) during infancy. Furthermore, prenatal maternal psychological state had

an independent influence on report of infant temperament. The impact of prenatal exposure to maternal depression and cortisol on maternal report of increased negative reactivity is consistent with the hypothesis that the prenatal environment exerts programming effects on the fetus with consequences for infant behavior (Barker et al., 2002; Welberg and Seckl, 2001). Our findings also are consistent with findings from studies with animal models indicating that prenatal exposure to maternal stress and elevated GCs has lifelong implications for fearful behavior in the offspring (Weinstock, 2005).

There are several methodological strengths of this study that lend support to the validity of these results. First, we included a large sample of 247 mother-infant pairs. Second, only infants who were delivered full term and healthy at birth were included. This suggests that the differences in temperament are related to intrauterine influences on the fetus separate from intrauterine and postpartum complications related to preterm delivery or low birth weight. Third, we statistically controlled for the influence of postnatal maternal psychological state (anxiety, depression, and perceived stress). The effect of prenatal maternal cortisol and prenatal maternal depression on infant temperament remained significant after controlling for postpartum maternal psychological state. A fourth and unique component of this study is that maternal psychological state and maternal cortisol levels were evaluated at three gestational time points. This enabled us to assess the effects of timing of fetal exposure to maternal stress on infant outcomes.

As expected, maternal cortisol levels increased significantly during the course of pregnancy. We found that maternal cortisol levels at 30 to 32 weeks of gestation predicted infant outcomes, but that levels measured earlier in pregnancy did not. Although rate of change in maternal cortisol was additionally correlated with report of infant negative reactivity, this appeared to be driven by cortisol levels at 30 to 32 weeks of gestation. Our data that show an effect of maternal cortisol levels during the third trimester is consistent with a recent study with a small sample (n = 17) that measured maternal salivary cortisol during only the late third trimester (de Weerth et al., 2003). In this study, infant temperament was assessed longitudinally over the first 5 postnatal months. Higher third trimester maternal cortisol predicted increased fussing, crying, and negative facial expressions, particularly during the first two postnatal months. Furthermore, Yehuda and colleagues (2005) found that cortisol levels were suppressed in infants of women who developed posttraumatic stress disorder in response to the attack on the World Trade Center, if they were exposed during the third trimester, but not earlier in pregnancy. Although the study by Yehuda and colleagues did not measure prenatal maternal cortisol production, their findings provide further evidence of a vulnerability of the fetus to maternal stress during the third trimester. Thus, converging evidence increasingly suggests that fetal exposure to stress hormones influences the development of infant temperament.

The mechanism by which exposure to maternal cortisol during the prenatal period produces long-term effects is unknown. However, there is clear evidence that prenatal GC exposure influences brain development (Antonow-Schlorke et al., 2003; Uno et al., 1994). Cortisol easily passes the blood-brain barrier (Zarrow et al., 1970) and influences limbic regions, such as the amygdala, involved in the regulation of fearful behavior. For example, GCs stimulate CRH mRNA expression in the amygdala. This increase in CRH production in the amygdala is anxiogenic (Dunn and Berridge, 1990). Fetal exposure to elevated cortisol may have persisting consequences for the development and functioning of this region. Consistent with this hypothesis, it has been demonstrated that prenatal stress upregulates CRH mRNA in limbic regions (Welberg et al., 2001) and increases fearful or anxious behavior in exposed offspring (Cratty et al., 1995), an effect that can be ameliorated with CRH antagonists (Ward et al., 2000). The results of these studies with animals indicate one potential mechanism by which human fetal exposure to elevated cortisol may have long-lasting influences on the development of infant fear behaviors.

The current findings showing an association between prenatal maternal depression and anxiety and infant temperament are consistent with previous studies that have found that elevated levels of prenatal maternal depression and anxiety predict increased emotionality and behavioral inhibition in the offspring independent of postnatal maternal psychological state (Davis et al., 2004a; O'Connor et al., 2003). In the present sample, the association between depression and infant temperament remained after controlling for postnatal

psychological measures, whereas the association with anxiety dropped to the level of a trend. Perceived stress did not significantly predict infant temperament.

The mechanism by which prenatal depression influences infant temperament is not known. Consistent with previous studies (de Weerth and Buitelaar, 2005; McCool and Susman, 1994; Petraglia et al., 2001), we found that maternal psychological state was not associated with baseline maternal cortisol during pregnancy. This suggests that maternal HPA axis changes do not mediate the effects of maternal depression on the fetus.

This study provides evidence that even in a healthy population with term pregnancies, prenatal exposure to elevated maternal cortisol later in pregnancy is associated with maternal report of increased behaviorally reactive temperament in infancy. These findings support the conclusion that fetal exposures to endocrine and psychosocial indicators of maternal stress have lasting consequences for the developing infant and strongly suggest that future research should examine the persisting influences of maternal cortisol on temperament in later infancy and childhood. It is probable that examination of women who are exposed to severe sources of stress or have clinically significant psychopathology would result in more profound programming influences on developmental outcomes.

Limitations

First, infant temperament was evaluated using maternal report. Although we controlled for postnatal maternal factors (anxiety, depression, and perceived stress) that could influence maternal report of infant temperament, the possibility of maternal bias remains. Previous studies using observational measures of temperament have demonstrated stronger associations between maternal anxiety and depression and infant fearful temperament (Davis et al., 2004a). Reliance on maternal report of infant temperament may contribute to the weaker association between prenatal maternal psychological measures and infant temperament found in the present study. Second, evaluation of temperament was performed at a young age. Although the measure used in the present study is both developmentally appropriate and has been shown to predict later fearful temperament, future studies would benefit from evaluation of infants and children at older ages. Third, because this study relies on naturally occurring variations in maternal stress and stress hormones rather than experimental manipulations, the effects of stress cannot be separated from the consequences of other factors that may contribute to this association such as shared genes. These findings are, however, consistent with animal models in which random assignment is possible (e.g., Weinstock, 2001) as well as recent human studies that have evaluated the consequences for development of randomly occurring stressful events, such as natural disasters (Laplante et al., 2004). These studies provide evidence that there are consequences of exposure to prenatal stress that are not explained by genetic predispositions. Finally, this sample includes a well-educated group of pregnant women, which may limit generalizability to other samples. However, although the sample was constrained on several dimensions, the findings were not related to measures of socioeconomic status.

Clinical Implications

The increased report of negative reactivity observed in the infants of women with elevated third trimester cortisol levels may have implications for subsequent behavioral problems. The temperament measure included in this study assesses infants' reactivity to novel stimuli. Infants who are easily aroused by varied stimulation are more likely to become behaviorally inhibited as young children (Kagan et al., 1998; Pfeifer et al., 2002). Furthermore, difficulty adapting to the presentation of novel sensory stimuli in infancy is predictive of later behavioral problems such as adolescent social anxiety (Schwartz et al., 1999). Group differences in infant temperament appear to persist through adulthood. These children display greater amygdalar activation to novelty as adults (Schwartz et al., 2003). The association reported here between maternal cortisol and report of negative reactivity in infancy suggests that fetal exposure to elevated cortisol increases the risk of infant fearful temperament and perhaps the subsequent development of behavioral inhibition. This is consistent with data showing that prenatal exposure to maternal anxiety predicts childhood anxiety and depression at 10 years of age (Leech et al., 2006). Assessment of stress, anxiety, and depression during pregnancy, even at subclinical levels and using both psychological and physiological measures, may be useful in the development of interventions to control stress during pregnancy with

implications for child outcomes. Although findings from previous intervention studies aimed at reducing stress during pregnancy are not conclusive, data from the present study indicate the importance of working to further develop effective interventions for the reduction of prenatal stress.

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