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Prenatal Cocaine Exposure and Infant Cortisol Reactivity

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Abstract

This study examined the effects of prenatal cocaine exposure on infant hypothalamic-pituitary-adrenal axis activity and reactivity at 7 months of infant age. Participants were 168 caregiver-infant dyads (87 cocaine exposed, 81 not cocaine exposed; 47% boys). Maternal behavior, caregiving instability, and infant growth and behavior were assessed, and children's saliva was sampled before, during, and after standardized procedures designed to elicit emotional arousal. Results revealed cocaine-exposed infants had a high amplitude trajectory of cortisol reactivity compared to non-cocaine-exposed infants. Infant gender and caregiving instability moderated this association. The findings support a dual hazard vulnerability model and have implications for evolutionary-developmental theories of individual differences in biological sensitivity to context.

Prenatal cocaine exposure has subtle effects on developing systems responsible for reactivity in response to stress and regulation of arousal (Mayes, 2002; Sameroff & Fiese, 2000). Cocaine is known to inhibit the reuptake of monoamines at the presynaptic junction, leading to higher concentrations of norepinephrine, serotonin, and dopamine in the synaptic cleft and higher levels of activation in the catecholaminergic systems (Nassogne, Evrard, & Courtoy, 1998). Chronic prenatal cocaine exposure is also associated with reduced metabolic activity in the meso-cortical limbic system (e.g., Dow-Edwards, Freed-Malin, & Gerkin, 2001). Other animal studies have noted that prenatal cocaine exposure produces an increase in serum corticosterone and alterations in the hypothalamic-pituitary-adrenal (HPA) axis (e.g., Larson, Schrott, Bordone, & Sparber, 2001). These regions of the brain are the very centers involved in psychobiological responses to stress, context, negative affect and regulation of arousal (Mayes, 2002). Chronic cocaine exposure is also associated with chronic fetal stress due to vasoconstriction of arteries in the uterus resulting in hypoxia and increases in fetal catecholamine release in response to hypoxia. Thus, there has been growing concern that prenatal cocaine exposure may influence individual differences in biological sensitivity to context by altering HPA function and/or reactivity to stress or negative affect.

Cortisol is the primary glucocorticoid hormone produced by the HPA system in humans, and it is considered a major indicator of physiological states in response to negative affect or stress (Kirschbaum & Hellhammer, 1989). Cortisol plays an essential role in the individual's ability

to cope with stressors of daily life. Under normal, nonstressful conditions, the HPA axis shows a diurnal pattern of activity; releasing cortisol in greater concentrations in the morning hours, a steep decrease in concentration toward midday, and slowly decreasing levels throughout late afternoon into the evening hours. Studies have shown that chronically elevated levels of cortisol can have deleterious effects on the brain, including deficits in memory, learning, state-regulating capacities, and socioemotional development (Ben-Dat Fisher et al., 2007).

To date, only three human studies have examined the effects of prenatal cocaine exposure on cortisol responses to stress. The first reported depressed cortisol responses following a standard neonatal physical exam and following a heel-prick among 11 healthy preterm cocaine-exposed neonates compared to 35 non-cocaine-exposed infants, but no group differences on prestress cortisol levels (Magnano, Gardner, & Karmel, 1992). In the second study of preterm neonates, those exposed to cocaine in utero ($n = 30$) had higher urinary cortisol levels compared to those who were not exposed to cocaine (Scafidi et al., 1996). In the third study, prenatal cocaine exposure was associated with lower prestress cortisol levels ($n = 29$ infants with cocaine exposure) but not with poststress levels among 13-month-old infants tested before and after blood draw (Jacobson, Bihun, & Chiodo, 1999). Thus, the results of human studies on prenatal cocaine exposure and cortisol levels in response to stress have been few in number and have shown mixed results.

Although accumulating evidence indicates that cocaine exposure is likely to alter the HPA axis, the exact nature of the effect is difficult to ascertain. Some explanatory mechanisms would propose higher cortisol reactivity as a function of prenatal cocaine exposure, whereas others would propose the opposite. However, studies of behavioral reactivity and regulation beyond the neonatal period consistently indicate that cocaine-exposed children are more reactive and less regulated, especially in response to novel or potentially stressful situations compared to those not exposed to cocaine (Dennis, Bendersky, Ramsay, & Lewis, 2006; Schuetze, Eiden, & Coles, 2007). To the extent that behavior reflects physiological arousal, one would hypothesize that cocaine-exposed infants would exhibit higher cortisol reactivity and slower recovery compared to non-cocaine-exposed infants.

To add to this complexity, the majority of cocaine-using mothers also used other substances such as alcohol, cigarettes, and marijuana. Results from several studies, both animal and human, indicate that prenatal alcohol and cigarette exposure may have significant effects on cortisol (e.g., Haley, Handmaker, & Lowe, 2006; Jacobson et al., 1999; McDonald et al., 2006). Results are mostly consistent, indicating that prenatal alcohol and cigarette exposure are associated with greater basal levels of stress hormones, higher stress reactivity, and lower recovery to prestress levels or regulation. Thus, the association between cocaine exposure and infant cortisol measures must be examined in the context of comorbid substance use.

In addition to prenatal factors, the most relevant psychological factor in reducing stress in infancy is probably the quality of early caregiving environment (Blair, Granger, Willoughby, Kivlighan, and the Family Life Project Investigators., 2006). Cocaine-exposed infants experience higher levels of environmental risk or caregiving instability as indicated by a number of factors such as frequent separations from the primary caregiver, changes in caregiving adults, frequent changes in the living situation, and lack of a male caregiver (Brown, Bakeman, Coles, Platzman, & Lynch, 2004). Several studies have noted the importance of examining the potential effects of these differences in the caregiving situation of cocaine-exposed infants on developmental outcomes (e.g., Bendersky, Bennett, & Lewis, 2006; Brown et al., 2004). Cocaine-exposed children also experience greater risk for poor parenting. Cocaine-using mothers have been reported to be more disengaged and passive during interactions with their neonates (Molitor, Mayes, & Ward, 2003) and to display lower responsiveness and higher negative affect toward their infants (Burns, Chethik, Burns, & Clark,

1997). These aspects of parenting behavior have direct implications for infants' HPA reactivity and regulation in affect-arousing situations. Parents play a critical role in helping children manage their arousal and keep distress within tolerable limits in infancy (Feldman, Greenbaum, & Yirmiya, 1999) by reading their children's emotional signals and responding with appropriate levels of soothing or stimulation. Parents who exhibit poor sensitivity to infant cues, high negative affect, and low warmth toward their infants are more likely to have infants who have difficulty regulating arousal in affect-arousing situations.

Two theories have been used to explain the influence of caregiving experiences on stress reactivity. One is the stress buffering hypothesis that proposes that caregiving experiences characterized by high levels of nurturance serve to buffer children from stress. Research with nonhuman primates indicates that this is most evident among those who are genetically predisposed to high stress reactivity (Suomi, 2006). Studies with human infants and toddlers suggest that by the latter part of the first year, sensitive and responsive caregiving may largely buffer the HPA system from responding negatively, even under conditions that elicit marked distress and fearful behavior (Spangler & Schieche, 1998).

The second perspective is the stress inoculation hypothesis, which suggests that moderate levels of early stressful experiences increase resistance to later stressful experiences (Parker, Buckmaster, Schatzberg, & Lyons, 2004). There is empirical support for this hypothesis using nonhuman primate models for mild to moderate levels of stress experienced in infancy (Parker et al., 2004). Both these hypotheses highlight the importance of early caregiving experiences in moderating stress reactivity. However, the role of maternal behavior or the caregiving environment in predicting or moderating the association between prenatal exposure and cortisol reactivity has never been examined in studies of cocaine-exposed infants.

Boyce and Ellis (2005) have proposed that, from an evolutionary perspective, high stress reactivity could be reinterpreted as reflecting biological sensitivity to context, and this sensitivity may have certain advantages in selective contexts. When the context is stressful, high biological sensitivity to context may be adaptive by increasing vigilance to environmental threats. When the context is supportive or protective, it may result in increased ability to benefit from available resources and support. However, environmental context characterized by chronic or overwhelming stress or adversity may result in chronic activation of the stress response system with pathogenic consequences. In summary, if prenatal cocaine and comorbid substance exposure increase infant HPA reactivity, highly stressful environments may exacerbate this association, whereas protective or supportive environments may lead to more positive outcomes.

Finally, recent studies of prenatal cocaine exposure indicate that infant gender may be an important moderator of the association between exposure status and child outcomes. Evidence from animal research indicates that males are more biologically vulnerable to prenatal cocaine effects on the central nervous system, including areas involved in stress reactivity, whereas group differences as a function of prenatal exposure are less evident among females (Spear et al., 2002). Accumulating evidence from human studies also indicates greater biological vulnerability for boys exposed to cocaine across a number of different developmental outcomes (Bailey et al., 2005; Delaney-Black et al., 2004).

The purpose of this study was to examine the effects of prenatal cocaine and other substance exposure on infant cortisol at 7 months of infant age. We hypothesized that cocaine-exposed infants would have higher cortisol reactivity and slower recovery in response to affect-arousing procedures compared to those in the control group. We examined the association between cocaine exposure and pretask cortisol, but did not have any specific hypothesis regarding direction of effect based on prior literature. We expected similar associations between other

substance exposure and cortisol values. We tentatively hypothesized that boys exposed to cocaine may be at higher risk for higher cortisol reactivity and less recovery compared to those not exposed to cocaine. We also hypothesized that maternal behavior and caregiving instability would moderate the association between prenatal cocaine or other substance exposure and infant cortisol. We hypothesized that positive maternal behavior or low caregiving instability may be a protective factor for infants with prenatal substance exposure. Finally, we conducted exploratory analyses to examine the role of continued postnatal substance use in predicting the different cortisol values (pretask, reactivity, and recovery).

Method

Participants

Participants consisted of 168 mother-infant dyads participating in an ongoing longitudinal study of prenatal cocaine exposure (87 cocaine exposed, 81 not cocaine exposed). Maternal interviews and infant assessments were conducted at 7 months of infant age. By 7 months of infant age, 17 infants in the cocaine group had been removed from parental care and placed in nonparental care. Of these 17 infants, 10 (59%) were in non-kin care, with the remainder being cared for by a grandmother or maternal aunt. All 7-month assessments were conducted with the primary caregiver of the child at that time, although for ease of presentation, the term mother or maternal is used throughout the article when referring to the primary caregiver. The primary caregiver was identified as the adult who had legal guardianship of the child and accompanied the child at the 7-month appointment. An outreach worker on the project staff recruited all participants after delivery from two local area hospitals. Mothers ranged in age from 18 to 42 years ($M = 29.78$, $SD = 5.46$). The majority of mothers were African American (74%), were receiving Temporary Assistance for Needy Families (71%) at the time of their first laboratory visit (years 2001-2004), and were single (60%). Of the 168 children, 79 (47%) were male. All families were recruited from the same hospital serving a predominantly low-income population, and the two groups were matched on maternal education, maternal race/ethnicity, and infant gender. The study received approval from the institutional review boards of the hospitals as well as the primary institutions with which the authors are affiliated. Informed written consent was obtained from all recruited participants. Participants received \$50 at the 7-month assessment in the form of gift certificates, checks, and infant toys for their participation. All assessments were conducted at age corrected for prematurity.

Procedure

All mothers were screened after delivery for initial eligibility and matching criteria. Interested and eligible mothers were given detailed information about the study and asked to sign consent forms. About 2 weeks after delivery, mothers were contacted and scheduled for their first laboratory visit, which took place at the time that their infant was approximately 4-8 weeks old. A second visit was scheduled when the infant was 7 months old. All visits consisted of a combination of maternal interviews, observations of mother-infant interactions, and assessments of infant arousal and arousal regulation. In the circumstance of a change in custody arrangements, the person who had legal guardianship of the child was contacted and asked to participate. Biological mothers were interviewed at the 4-8-week assessment in order to obtain accurate information about prenatal substance use.

Once a family was recruited into the cocaine group, the closest matching non-cocaine-group family was recruited. However, a significantly higher proportion of mothers in the non-cocaine group declined participation or withdrew before formal enrollment, resulting in a smaller number of families in the control group. Of the 4,800 women screened at delivery, 340 were eligible for participation in either group. Of these 340 women, 35% either declined participation or were not enrolled in the study because they expressed initial interest but later withdrew. Of

the 220 mother-infant dyads who completed the 4- to 8-week laboratory visit, seven declined to participate in the 7-month assessment or did not show up after repeated reschedules, 10 were unable to be located, two were dropped from the study (one because of a diagnosis of fetal alcohol syndrome and another because of a diagnosis of Shaken Baby Syndrome), five were in the middle of new custody arrangements, and seven mothers no longer had custody of their children and the foster parent was uninterested in participating. Of the 189 (86%) mother-infant dyads who completed the 7-month assessment, saliva samples were collected from 168 infants. There were no significant differences between families with complete versus missing data at 7 months on any demographic or substance use variable. Approximately 60% of the infants with missing saliva samples were in the cocaine group, but this association (between missing data status and cocaine group status) was not significant. Thus, the final sample consisted of 168 families.

Assessment of Growth and Risk Status

Three measures of growth were used in this study: birth weight (in grams), birth length (in centimeters), and head circumference (in centimeters). All measurements were taken by obstetrical nurses in the delivery room and recorded in the infant's medical chart. Research staff recorded this information from the charts after recruiting the mother-infant dyad. Medical chart review at the time of recruitment also was used to complete the Obstetrical Complications Scale (OCS; Littman & Parmelee, 1978), a scale designed to assess the number of perinatal risk factors experienced by the infant. Higher numbers on this scale indicate a more optimal obstetric score. Gestational age was calculated by dates and extracted from medical records.

Identification of Substance Use

Cocaine status was determined by a combination of maternal report, chart review, and maternal hair analysis. Urine toxicologies were routinely conducted at the first prenatal visit on maternal urine and/or at delivery (for those mothers who tested positive prenatally, obtained prenatal care elsewhere, or did not receive any prenatal care) on infant and maternal urine by participating hospitals. Mothers were included in the cocaine group if self-reports were positive, regardless of urine toxicology or hair-sample results. Similarly, mothers who reported that they did not use cocaine but had positive urine toxicology or hair samples were included in the cocaine group.

Urine toxicologies consisted of standard urine screening for drug level or metabolites of cocaine, opiates, benzodiazepines, and tetrahydrocannabinol. Urine was rated positive if the quantity of drug or metabolite was >300 g/ml. Hair samples were collected from the mothers at the first laboratory visit and sent to the Psychomedics Corporation for radioimmunoanalyses (RIAH). Hair samples were screened for cocaine followed by a gas chromatography/mass spectrometry (GC/MS) confirmation for positive cocaine screens. Drugs and their metabolites are absorbed into the hair and can be extracted and measured. As hair grows at an average rate of 0.5 in per month, it can record a pattern of drug consumption related to the amount and frequency of use (see Baumgartner, Hill, & Bland, 1989). Thus, a 2-in length of hair could contain a record of approximately 4 months of use, and given adequate hair length (i.e., about 4-5 in), use per trimester may be recorded. Drugs become detectable in hair about 3-4 days after use, a time when cocaine is rendered undetectable by urinalysis. RIAH is the most well-established hair-analysis technique and has been replicated by independent laboratories across the world (see Magura, Freeman, Siddiqi, & Lipton, 1992). GC/MS confirmations of RIAH have not revealed any false positives because of testing errors (see Magura et al., 1992).

Approximately 32% of mothers in the study (55% of the mothers in the cocaine group) had positive urine toxicologies at delivery, and 25% of mothers (79% of the mothers in the cocaine group) had hair samples that tested positive for cocaine during pregnancy. The remainder of

mothers in the cocaine group admitted having used cocaine in the brief self-report screening instrument administered after delivery. Mothers in the comparison group reported not having used any illicit substances other than marijuana. Additional exclusionary criteria for all mothers were (a) maternal age younger than 18 years, (b) use of illicit substances other than cocaine or marijuana, and (c) significant medical problems for the infant (e.g., genetic disorders, major perinatal complications, baby in critical care for over 48 hr).

The Timeline Follow-Back Interview (TLFB; Sobell, Sobell, Klajmer, Pavan, & Basian, 1986) was used to assess maternal substance use before, during, and after pregnancy, and in the postnatal period. Participants were provided a calendar and asked to identify events of personal interest (i.e., holidays, birthdays, vacations, etc.) as anchor points to aid recall. This method has been established as a reliable and valid method of obtaining longitudinal data on substance-use patterns, has good test-retest reliability, and is highly correlated with other intensive self-report measures (Brown, Bakeman, Coles, Sexson, & Demi, 1998). The TLFB yielded data about the average number of days of cocaine use per week, average number of joints smoked per week, average number of cigarettes smoked per week, and average number of standard drinks per week, for each trimester of pregnancy and for the postnatal period.

Infant Cortisol

At 7 months, infant reactivity and regulation were assessed in three different behavioral paradigms designed to induce affect that were taken from the Laboratory Temperament Assessment Battery (LabTAB; Goldsmith & Rothbart, 1988). These included the arm restraint paradigm designed to elicit anger/frustration, a puppet show to elicit positive affect, and presentation of four different scary masks to elicit fear. The order of the procedures was as follows: The Time 1 or pretask saliva sample was collected after the infant arrived at the laboratory; the infant was then seated in a high chair, was hooked up to electrodes (for measurement of heart rate), and watched a Baby Einstein video for 3 min; a puppet show was presented for the next 3 min, followed by another 3 min of the video. The Time 2 saliva sample was collected at the end of these 3 min. The arm restraint procedure was presented next (consisting of two trials), followed by another 3 min of video. The infant was then unhooked from the heart rate monitor and presented with some blocks for a 3-min play episode, followed by the presentation of four masks lasting a total of 60 s. Next, the mother-infant dyad was moved to the floor with a variety of toys for 8 min of interaction. This was followed by the Time 3 saliva sample. The infant was measured and weighed, and the mother was interviewed. The Time 4 saliva sample was collected 20 min after Time 3.

Saliva samples were collected by placing an absorbent dental cotton roll in the mouth of infants. The saliva in the cotton roll was expressed into a storage vial using a 10 cc needleless syringe. Samples were immediately placed in a -80°C freezer and were shipped on dry ice to Salimetrics Laboratories (State College, PA) for assay. On the day of testing, all samples were centrifuged at $1500 \times g$ (3000 RPM) for 15 min to remove mucins. All samples were assayed for salivary cortisol using a highly sensitive enzyme immunoassay U.S. Food and Drug Administration (510 k) cleared for use as an in vitro diagnostic measure of adrenal function (Salimetrics, State College, PA). The test used 25 μ l of saliva, had a lower limit of sensitivity of .007 μ g/dl, a range of sensitivity from .007 to 3.0 μ g/dl. All samples were assayed in duplicate, average intra- and interassay coefficients of variation were less than 5% and 10%, and averaged duplicate scores were used in all statistical analyses.

Time 1 cortisol values were used to examine group differences at pretask. Although this value may not be a true baseline and may reflect some reaction to stresses associated with preparing to leave the house and the cab ride to the laboratory, it does reflect the initial cortisol level before major affective stress. Following previous studies (e.g., Ramsay & Lewis, 2003),

cortisol reactivity was indexed by the difference between the peak poststressor cortisol value (Time 3 or 4) and the pretask value. Higher values indicated higher cortisol reactivity.

Behavioral Measures of Reactivity

A series of behavioral measures was used to assess reactivity during the puppet show, the arm restraint, and the mask episodes using the guidelines of the LabTAB Manual, developed by Goldsmith and Rothbart (1988). These included peak intensity of positive affect, anger, sadness, and fear as well as latency to affect expressions for each episode. The intensity of affect expression was scored using Affex (Izard, Dougherty, & Hembree, 1983), adapted from Izard's (1983) Maximally Discriminative Facial Movement Coding System. Higher scores indicated higher arousal or reactivity. Latency scores ranged from 0 to 30, with higher scores indicating longer latencies and lower reactivity. Maternal positive vocalizations during arm restraint and the mask episodes were also coded every 5 s (a 0 vs. 1 variable for the absence or presence of positive vocalizations), and total scores per episode were computed by taking the overall frequency of the presence of positive vocalizations. Two coders blind to all information about the families coded reactivity. Interrater reliability was calculated for 14% of the tapes and ranged from 90% to 94% across the different episodes ($\kappa = .69$ and $.76$). Similarly, interrater reliability for latency to affect expressions ranged from 97% to 99% ($\kappa = .94$ and $.95$).

Maternal Depression and Parenting

Maternal depression was assessed using the depression subscale from the Brief Symptom Inventory (BSI; Derogatis, 1993). This scale is a brief form of Symptom Checklist 90-R and is a widely used mental health screening measure in a variety of clinical and research settings. The BSI subscales have high internal consistency and the measure has been used in a large number of studies, including studies of maternal cocaine use (e.g., Eiden, Foote, & Schuetz, 2007; Singer et al., 2002).

Parenting was assessed using behavioral observations during a Free Play task. Mothers were asked to interact with their infants as they normally would at home for 5 min in a room filled with toys. These interactions were coded using a collection of global 5-point rating scales developed by Clark, Musick, Scott, and Klehr (1980), with higher scores indicating more positive affect or behavior. These scales have been found to be applicable for children ranging in age from 2 months to 5 years (Clark, 1999; Clark et al., 1980). Three composite scales of maternal behavior were derived from these items: positive involvement, negative affect, and sensitivity. These scales had high internal consistencies with Cronbach's alpha ranging from .87 to .91.

Two coders rated parental behavior. Both coders were trained on the Clark scales by the first author and were unaware of group membership. The interrater reliability was conducted on a random selection of 14% ($n = 24$) of the tapes and was fairly high, ranging from intraclass correlation coefficients of $r = .86$ to $r = .92$ for the three composite scales.

Caregiving Instability

Caregiving instability was assessed using a structured caregiver interview (Platzman, Coles, Lynch, Bard, & Brown, 2001). The Structured Clinical Interview (SCI) was developed for use with high-risk infants from 6 to 24 months and was administered to the child's caregiver by a trained examiner. Following Platzman et al. (2001), individual items from the SCI were summed into a cluster called caregiving instability. The five items included in this scale were as follows: no male adult in the household, baby separated from primary caregiver for more than 48 hr, baby is fed and sleeps significantly less than average, there are custody changes, or baby does not see biological mother regularly. Thus, the total score on caregiving instability

was based on the presence or absence of the five specific risk characteristics described above (see Platzman et al., 2001; for details regarding scale development). The scale is similar to a composite risk index (e.g., Sameroff, Seifer, Baldwin, & Baldwin, 1993), based on research suggesting that the total number of risk factors may be more predictive of child outcomes than exposure to any specific risk condition (Seifer, 1995). However, unlike the traditional risk composites or environmental risk scores used in other studies, it is more narrowly focused on factors that are associated with instability in the caregiving environment, particularly among families with drug-using mothers. The caregiving instability scores ranged from 0 to 4 in the current study, with the majority of the children in the sample having a score of 0 or 1. Previous studies have reported significant positive associations between maternal drug use and higher caregiving instability (Platzman et al., 2001). The caregiving instability cluster was also associated with an acceleratory heart rate response indicating distress or arousal among cocaine-exposed infants (Bard et al., 2000) and was a predictor of child behavior problems among 2- to 5-year-old children of cocaine-using mothers (Eiden, 1999).

Data Transformations

We first examined cortisol values for extreme values. Two infants had extremely high cortisol values for all four time points (values above 4 µg/dl) that were physiologically improbable (see Jacobson et al., 1999). These infants were excluded from the data set. One infant had extreme values at three of four time points (value above 4 µg/dl), and these extreme values were set to missing, resulting in a total sample size of 166 for pretask cortisol and 165 for cortisol reactivity. Cortisol data were then examined for outliers, defined as ± 3 SD from the mean (Gunnar, Brodersen, Krueger, & Rigatuso, 1996). There were seven infants with Time 1 values, six with Time 2 values, five with Time 3 values, eight with Time 4 values that were outliers. These values were winsorized following Tukey (1977), replacing values that were 3 SD above the mean with the value of 3 SD above the mean, as in previous studies (Haley et al., 2006). As expected, the cortisol data were positively skewed and were transformed (log 10) before further analyses. There were no differences in any of the results when participants with winsorized values were included or not included in the data set. The remaining data were examined for normality and presence of outliers. The continuous measures of substance use (alcohol, cigarettes, and marijuana) were extremely skewed and were transformed using square root transformations before being used in further analyses.

Analytic Strategy

We first examined associations between cortisol values at the different time points. Association between group status and demographics, infant growth outcomes, maternal use of alcohol, cigarettes, and marijuana, and maternal behavior were examined next using analyses of variance (ANOVAs) or multivariate analyses of variance (MANOVAs). MANOVAs were used when multiple theoretically associated constructs were the dependent measures in order to control for high Type I error rate. These were followed by analyses of potential confounds using correlations or ANOVAs, as appropriate with Bonferroni corrections to control for Type I error. The degrees of freedom for the correlations ranged from 163 to 164. Confounds with significant bivariate associations with cortisol values or those of theoretical interest (e.g., other substance use) were used as covariates in all remaining analyses (if they were associated at $p < .10$). Repeated measures and univariate Analyses of covariance (ANCOVAs) with significant theoretical or empirical covariates were conducted to examine group differences on cortisol values and cortisol reactivity. ANCOVA was used to examine if infant gender moderated this association. Finally, hierarchical multiple regressions were used to examine if caregiving instability or maternal behavior variables moderated the association between prenatal cocaine and other substance exposure and infant cortisol.

Results

As with other physiological data, cortisol values are subject to the Law of Initial Value (Ramsay & Lewis, 2003). In the case of cortisol, this involves a negative association between the initial or pretask level of cortisol and the magnitude of change. Pearson correlations were computed to examine the associations among the cortisol values. Cortisol values at all time points were positively correlated with each other; there were no negative associations. The correlation between two adjacent samples was quite high, ranging from .71 (for the correlation between Time 3 and Time 4 samples) to .76 (for the correlation between Time 1 and Time 2), $p < .001$. The correlation between Time 1 and Time 3 was $r = .53$, $p < .001$, between Time 1 and Time 4 was $r = .49$, $p < .001$, and between Time 2 and Time 4 was $r = .64$, $p < .001$.

Demographics and Infant Growth Outcomes

Results from MANOVA with the demographic variables as the dependent measures and group status yielded a significant multivariate effect of group status, $F(3, 163) = 6.05$, $p < .01$. Results from univariate analyses indicated that control group mothers were younger and had lower parity and higher education compared to those in the cocaine group, although the effect sizes were generally small (see Table 1). A second MANOVA was conducted with infant birth outcomes as the dependent measures. Results indicated a significant effect of group status on infant birth outcomes, $F(5, 161) = 8.11$, $p < .001$. Univariate analyses indicated that cocaine-exposed infants had lower gestational age, birth weight, and birth length, and cocaine-using mothers had higher scores on the obstetrical complications scale compared to those in the control group (see Table 1). Eighty-six percent of the cocaine-exposed and 97% of the comparison infants were full term (>37 weeks gestational age). When these analyses were repeated after using gestational age as covariate, the differences in birth weight and length remained significant ($p < .01$).

Maternal Substance Use and Other Variables

Results from MANOVA with prenatal substance use variables as the dependent measures and group status as the independent variable yielded a significant multivariate effect of group status, $F(4, 162) = 14.32$, $p < .001$. As expected, mothers in the cocaine group were heavier users of cigarettes, alcohol, and cocaine during pregnancy (see Table 2). There was no group difference in marijuana use. Results of MANOVA with postnatal substance use measures at 7 months yielded a significant effect of group status, $F(4, 162) = 7.62$, $p < .001$. Mothers in the cocaine group continued to be heavier users of alcohol, cigarettes, and cocaine, but not marijuana, during the postnatal period (see Table 2).

Results from MANOVA with group status as the independent variable and the three maternal behavior variables as dependent measures yielded a significant multivariate effect of group status, $F(3, 163) = 4.30$, $p < .001$. Univariate analyses indicated that mothers in the cocaine group displayed lower positive involvement and higher negative affect compared to those in the control group (see Table 2). These results remained unchanged when the 17 foster care mothers were excluded from the analyses. Univariate ANOVA with the z -scored caregiving instability variable as the dependent variable indicated no significant group differences (see Table 2).

Potential Confounds

There were no significant associations between infant cortisol values (values at each time point and cortisol reactivity at alpha conservatively set at $p < .10$) and maternal education, parity, or age, with time of breakfast or time of lunch, whether the infant woke during the night, by time of day, hours of sleep the previous night, infant birth weight, gestational age, or head circumference, maternal depression, parenting behavior during free play at 7 months,

caregiving instability, foster care status, or any of the behavioral indices of infant reactivity or regulation during arm restraint or the fear episode. During the puppet show, 11 infants had a negative reaction to the puppets, and 76 infants had a neutral reaction to the puppets. When the two groups of children were combined to create a dummy coded variable of positive versus negative or neutral reaction to the puppet show, there was marginal group difference on cortisol reactivity, $F(1, 160) = 3.43, p = .07$. (Behavioral data during puppet show could not be coded for five infants due to technical difficulties during videotaping of the puppet show.) Infants who had a positive reaction to the puppet show had higher cortisol reactivity (mean change = .14, $SD = .32$) compared to infants who had a negative or neutral reaction to the puppet show (mean change = .06, $SD = .26$). Higher frequency of positive maternal vocalizations during the arm restraint was associated with lower cortisol value at Time 4, $r = -.20, p < .05$.

Given recent studies on the importance of considering medication use in cortisol studies (Hibel, Granger, Cicchetti, & Rogosch, 2007), we examined medication status as a potential confound. About 20% ($n = 33$) of the infants in the study had been administered medication within 24 hr of the laboratory visit (59% in the cocaine group, 41% in the control group). There was no association between group status and medication use. There was a significant association between use of medication and cortisol values at Time 3, $F(1, 156) = 7.67, p < .01$. Infants who were administered medication had higher Time 3 cortisol levels compared to those who were not administered medication (means = .47 and .26, $SD = .52$ and .24). There was also a significant association between medication use and the measure of cortisol reactivity, $F(1, 166) = 5.36, p < .05$. Infants who were medicated recently had higher reactivity compared to the unmedicated infants (means = .20 and .07, $SD = .36$ and .27, respectively). Medications ranged from over-the-counter cold products to asthma medication, acetaminophen, and medication for reflux.

Correlational analyses indicated significant associations between average daily ethanol intake during pregnancy and cortisol values at Time 2 ($r = .16, p < .05$) and Time 4 ($r = .25, p < .01$). Higher number of cigarettes per week during pregnancy was marginally associated with higher cortisol levels at Time 1 ($r = .14, p < .10$) and Time 4 ($r = .15, p < .10$). Finally, higher number of joints per week during pregnancy was marginally associated with higher cortisol levels at Times 3 ($r = .15, p < .10$) and 4 ($r = .15, p < .10$).

Main Analyses: Association Between Cocaine Exposure and Cortisol Levels and Reactivity

Repeated measures ANCOVAs were conducted to examine the association between group status and change in cortisol over time. Average number of cigarettes per week of pregnancy, average standard drinks per week of pregnancy, average number of joints per week during pregnancy, child use of medication in the previous 24 hr (a dummy-coded yes/no variable), and maternal positive vocalizations during arm restraint were used as covariates. Results yielded a significant Group $\times$ Time interaction, $F(3, 339) = 5.47, p < .01$, partial $\eta^2 = .08$ (see Figure 1). Simple effects analyses with alpha set at $p < .01$ (Bonferroni-type correction) indicated that cocaine-exposed infants had significantly higher cortisol values at Time 3 ($p < .01$) compared to those in the control group. There was a significant effect of time for both groups, although the linear trajectory was significant for the cocaine-exposed infants, reflecting an increase in salivary cortisol from Time 1 to Time 4, whereas the quadratic trajectory was significant for the control group infants, reflecting a decrease from Time 1 to Time 3 followed by an increase. However, neither group exhibited recovery by Time 4, presumably because the last saliva sample was taken about 30 min after the fear episode.

An examination of individual data regarding change over time indicated that there were some children who did not exhibit an increase in cortisol in response to fear (between Time 3 and Time 4). To examine this issue more closely, we created two groups. The first consisted of children who had a decrease in cortisol from Time 3 to Time 4 ($n = 44$), and the second consisted

of children who had an increase in cortisol or remained at the same level between Time 3 and Time 4 ($n = 124$). Separate MANOVAs were conducted with this cortisol grouping as the independent variable and continuous measures of maternal substance use, parenting and depression, and child behaviors during affect-arousing procedures as the dependent variables. There were no significant multivariate or univariate effects. Thus, although we had originally assumed that we would be able to assess cortisol regulation or recovery from the stress of the arm restraint paradigm, we were unable to do so, presumably because we did not allow sufficient time between the fear episode and the last cortisol measure. For the remaining analyses, we focused primarily on pretask cortisol and cortisol reactivity.

Cortisol reactivity was used as the dependent variable in the next analysis using ANCOVA with cocaine group status and child gender as the independent variables and average number of cigarettes per week of pregnancy, average standard drinks per week of pregnancy, average number of joints per week during pregnancy, child use of medication in the previous 24 hr (a dummy-coded yes/no variable), and maternal positive vocalizations during arm restraint as covariates. There was a marginal Group $\times$ Gender interaction effect, $F(1, 158) = 3.05, p < .10$, partial $\eta^2 = .02$. Analysis of simple effects indicated that among boys, those exposed to cocaine had significantly higher levels of cortisol reactivity ($p < .01$) compared to those not exposed to cocaine (see Figure 2). Among girls, there was no association between cocaine exposure and cortisol reactivity. (Results remained unchanged when a dummy-coded variable of neutral or negative reaction to the puppet show was used as an additional covariate.)

Moderators: Caregiving Instability and Maternal Behavior

We hypothesized that caregiving instability or maternal behavior may moderate cortisol values. Given the associations between other substance use and cortisol values at different time points, we computed two dummy-coded variables to reflect maternal substance use (non-substance-exposed vs. substance exposed, and non-cocaine-exposed vs. cocaine exposed) and centered the caregiving instability variable. We then computed two interaction terms reflecting interaction of caregiving instability with each of the dummy-coded substance exposure variables following Aiken and West (1991). Next, we conducted hierarchical regression analyses with child gender, medication use, and maternal positive vocalizations entered in the first step, caregiving instability and the two dummy-coded variables in the next step, and the two interaction terms between caregiving instability and substance exposure in the last step (Caregiving Instability $\times$ Substance Exposure; Caregiving Instability $\times$ Cocaine Exposure). For pretask cortisol, this analysis yielded a significant interaction of caregiving instability and substance exposure, $\beta = .44, p < .05$, and a significant interaction of caregiving instability and cocaine exposure, $\beta = .28, p < .05, R^2 \text{ change} = .04, p < .05$. As indicated in Figure 3, there was no association between prenatal substance exposure and pretask cortisol at high levels of caregiving instability. However, when caregiving instability was low, substance-exposed infants had higher pretask cortisol compared to those not exposed to any substances. The pattern was different for prenatal cocaine exposure. There was no association between caregiving instability and pretask cortisol among non-cocaine-exposed infants, but among cocaine-exposed infants, those with higher caregiving instability had higher levels of pretask cortisol (see Figure 4).

For cortisol reactivity, these analyses also yielded a significant interaction between caregiving instability and prenatal substance exposure (non-substance-exposed vs. substance-exposed dummy-coded variable), $\beta = .36, p < .05$ (see Figure 5). As depicted in the figure, there was no association between prenatal substance exposure and cortisol reactivity at lower levels of caregiving instability. However, at higher levels of caregiving instability, infants with prenatal exposure had higher levels of cortisol reactivity. Similar analyses were conducted to examine if maternal behavior variables moderated the association between prenatal substance or cocaine

exposure and infant cortisol. There were no interaction effects. Finally, correlational analyses yielded no associations between cortisol values and frequency of postnatal cocaine use, average number of joints per week, number of cigarettes per week, and average daily ethanol consumption per week.

Discussion

The first major purpose of this study was to examine the effects of prenatal cocaine exposure on infant cortisol. Results are generally supportive of an association between prenatal cocaine exposure and cortisol values across time. Cocaine-exposed infants had a different trajectory of cortisol values across the four time points compared to non-cocaine-exposed infants, and boys exposed to cocaine had higher cortisol reactivity compared to non-cocaine-exposed boys. The result regarding the association between prenatal cocaine exposure and infant cortisol is supportive of both animal and human literature regarding the effects of prenatal cocaine exposure on the functioning of the HPA axis.

Unlike previous studies (e.g., Jacobson et al., 1999), there was no association between prenatal cocaine exposure and pretask cortisol. One explanation for this discrepancy may be the difference in infant age, with 7-month-old children in the current study and 13-month-olds in the Jacobson et al. study. It is possible that the process of arriving at a strange laboratory constitutes a significantly higher stress for 13-month-old infants who generally have stranger anxiety, compared to 7-month-old infants. Thus, although the pretask cortisol measure is not a true baseline for either study, 13-month-old infants may have experienced greater stress before the pretask sample. The results from the current study regarding lack of pretask differences between the two groups are supportive of the study by Magnano et al. (1992) reporting no pretask differences between preterm cocaine-exposed and non-cocaine-exposed neonates. Future studies may better examine this issue by collecting saliva samples at various times during the day while the infant is at home.

The result regarding the association between cocaine exposure and cortisol reactivity for boys, but not girls, is similar to those reported by Haley et al. (2006) with regard to an interaction effect of child gender and alcohol group status (maternal high-frequency drinking vs. low-frequency drinking) on cortisol reactivity. Results indicated that boys exposed to higher frequency alcohol use had greater HPA reactivity compared to boys exposed to low-frequency alcohol use. Results from the current study are also supportive of a growing body of evidence from the cocaine exposure literature indicating that boys may be at higher teratological risk for higher reactivity and poor regulation (Delaney-Black et al., 2004). Although none of these studies have examined HPA responses specifically, all of these studies indicate higher biological vulnerability for cocaine-exposed boys compared to boys not exposed to cocaine. Cocaine-exposed boys exhibited higher impulsivity, hyperactivity, central processing difficulties (a combination of planning, academic, and motor skills), reactivity to the environment, and lower abstract thought compared to boys not exposed. No such associations were obtained for girls. These results from human studies are bolstered by a number of studies using animal models. For instance, Heyser, Rajachandran, Spear, and Spear (1994) reported lower basal heart rate among cocaine-exposed males compared to those not exposed, but no differences among females. Male cocaine-exposed rats were more aggressive when competing for water after water deprivation (Wood & Spear, 1998) and exhibited greater immobility after a stressor (see Spear et al., 2002) compared to males not exposed to cocaine, but there was no association for females. Thus, there is a growing body of evidence indicating that cocaine-exposed males may be more biologically vulnerable with regard to stress reactivity compared to non-cocaine-exposed males.

Higher maternal alcohol consumption during pregnancy was significantly associated with higher cortisol after puppet show (Time 2) and 20 min after the fear episode (Time 4). These results are supportive of previous studies indicating an association between heavy alcohol use and higher poststress cortisol levels (Jacobson et al., 1999) and higher cortisol reactivity (Haley et al., 2006). Thus, the effects of prenatal cocaine exposure can only be interpreted in the context of other substance use.

Because of the significant associations between alcohol exposure and cortisol values and some trends toward significance with other substance use, all of the analyses examining moderation were conducted using two dummy-coded variables as reported above. Caregiving instability was a significant moderator of the association between prenatal substance exposure and pretask cortisol and of the association between prenatal cocaine exposure and pretask cortisol. There was a significant association between prenatal substance exposure and pretask cortisol at low levels of caregiving instability, but not at higher levels of caregiving instability. Among infants with low caregiving instability, those who were exposed to substances in utero had higher pretask cortisol compared to infants not exposed. The pattern of interaction was different with regard to prenatal cocaine exposure. Cocaine-exposed infants who had experienced higher caregiving instability had higher pretask cortisol, a pattern supportive of an additive effect of cocaine and environmental risk on HPA axis functioning. We could find no previous studies examining potential interactive effects of prenatal substance exposure and caregiving on infant cortisol. Thus, these results need to be viewed with caution and replicated before further discussion. It is also important to note that the caregiving instability measure is a general or global measure similar to measures of cumulative environmental risk. Thus, these results are not informative regarding potential moderation by specific caregiving factors. However, previous studies have noted that cumulative risk measures are often more predictive of child outcomes than any single measure alone (Seifer, 1995).

Among infants experiencing higher caregiving instability, those with prenatal substance exposure had higher cortisol reactivity compared to infants not exposed and those with low caregiving instability. The results regarding the importance of the caregiving environment are generally supportive of a number of animal studies using maternal separation models indicating higher stress reactivity in response to an acute stressor after maternal separation (Lippmann, Bress, Nemeroff, Plotsky, & Monteggia, 2007; Veenema, Blume, Niederle, Buwalda, & Neumann, 2006), although these refer to a main effect of caregiving rather than an interaction with a biological risk factor. The results are also supportive of a vulnerability model (Luthar & Zelazo, 2003), indicating that in the presence of prenatal substance exposure, higher caregiving instability confers vulnerability. The theory of reactivity as biological sensitivity to context (Boyce & Ellis, 2005) indicates that the combination of higher reactivity and higher caregiving instability may be the most pathogenic for later developmental and health outcomes. Future studies with long-term follow-up of cocaine-exposed children may be able to examine whether more reactive cocaine-exposed infants with moderately supportive environments have more optimal outcomes, as suggested by this theory.

Infants who were administered medication in the 24 hr prior to the laboratory visit had higher Time 3 cortisol and higher cortisol reactivity. These results are supportive of recent studies indicating significant effects of medication use on cortisol levels (Hibel, Granger, Kivlighan, Blair, and the Family Life Project Investigators., 2006). One limitation of the current study is that we did not code specific types of medication. This may be important, as results from previous studies (see Hibel et al., 2006) indicate medication effects specific to the type of medication. For instance, infants who had been administered acetaminophen (Tylenol) had lower cortisol reactivity compared to those without acetaminophen in their system (Hibel et al., 2006). Thus, it is possible that clustering the groups into medication versus no medication may have obscured important differences within the medication group. However, at 7 months

of infant age, there were only a few infants in each medication category when medication use was categorized according to criteria suggested by Hibel et al. (2006). Future studies with larger samples of substance-exposed infants may examine this variable more carefully.

The study has several additional limitations. One major limitation was that we were unable to examine regulation or recovery from stress. Future studies with more sampling points are needed to address the issue of stress regulation among substance-exposed infants. A second limitation is that accurate assessment of substance use both prenatally and postnatally is difficult. Pregnant and postpartum women are often hesitant to divulge substance use information, particularly illicit substances such as cocaine. One strength of this study is the use of multiple methods to ascertain prenatal substance use, which partially mitigated this limitation. However, measures of postnatal substance use were based on self-report alone. A third limitation is that the measure of maternal parenting behavior was brief and limited to a single play session. However, this was an objective measure of parenting, as opposed to parent reports of their own parenting behavior. Additionally, previous studies have reported that affect synchrony is an important construct to consider as a predictor of infant reactivity and regulation (Feldman et al., 1999). It is possible that future studies that specifically assess affect synchrony during mother-infant interactions may report a different pattern of results. This is particularly critical given the lack of hypothesized associations between maternal behavior and cortisol measures. A fourth limitation is that we did not have home cortisol levels for similar time periods. Laboratory values tend to be lower than corresponding home values (for a review, see Gunnar & Donzella, 2002). Thus, it is possible that the pretask cortisol was lower than true baseline, and the increase in cortisol indicated a return to baseline rather than reactivity. Although other indices of arousal such as heart rate and behavior during the laboratory procedures indicated increases in arousal or stress (Schuetze et al., 2007) for these infants, this sympathetic arousal may not be indicative of reactivity in the HPA axis. Thus, an alternative explanation of the findings regarding reactivity may be that this represents a return to baseline levels of cortisol rather than reactivity to the affect-arousing laboratory procedures. Finally, all infant growth measures were recorded by nurses at the hospital. Growth measures by nurses are less reliable than those taken by trained examiners.

In spite of these limitations, these findings are important because they lend further empirical support to a growing body of literature documenting significant effects of prenatal cocaine and other substance exposure including alcohol on the HPA axis, especially for boys. The results regarding medication use highlight the importance of considering other influences on cortisol values. The study also advances the field because of its examination of moderating factors. Results with regard to HPA reactivity indicate that caregiving instability increases vulnerability among substance-exposed infants. These results have some significant implications for substance-exposed infants by highlighting the role of the caregiving environment as a moderator of risk. Thus, interventions focused on increasing stability of care among substance-exposed infants may serve to reduce vulnerability to stress reactivity among these infants.

Developmental science is keenly attuned to the influence of early rearing environments on individual differences in the psychobiology of the stress response as a mechanism via which social context influences child development (e.g., Gunnar & Donzella, 2002). As this knowledge base expands, we are gaining sophistication in our understanding of how nonsocial forces might also influence these biosocial models of risk and resilience. Children's exposure to environmental contaminants (e.g., cocaine, alcohol, tobacco smoke, lead) is more likely under many of the general social circumstances (e.g., parental psychopathology, unemployment, low educational attainment, and child maltreatment and neglect) studied as potentially linked to the development of children's stress vulnerability. The present findings, as well as others from our informal collaborative network (Granger et al., 2007; Gump et al., 2008) underscore that more sophisticated approaches are needed to elaborate the implications

of biological, social, and physical factors that may amplify or attenuate the possible association between early rearing environments and the psychobiology of stress.

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Figure 1.

Cocaine Group $\times$ Gender interaction effect for cortisol, controlling for other prenatal substance exposure, maternal positive vocalizations during arm restraint, and infant medication use in the past 24 hr. AR: arm restraint; 20 m: 20 min, post: after arm restraint. Forty minutes after arm restraint is about 30 min after the fear episode.

Figure 2.
Group $\times$ Gender interaction effect for cortisol reactivity.

Figure 3. Prenatal Substance Exposure $\times$ Caregiving Instability interaction effect from hierarchical linear regression analysis for pretask cortisol.

Figure 4. Prenatal Cocaine Exposure $\times$ Caregiving Instability interaction effect from hierarchical linear regression analysis for pretask cortisol.

Figure 5. Prenatal Substance Exposure $\times$ Caregiving Instability interaction effect from hierarchical linear regression analysis for cortisol reactivity.

Table 1
Group Differences in Demographic Variables and Birth Outcomes

	Exposure group				F Value	η^2
	No cocaine		Cocaine			
	M	SD	M	SD		
Demographics						
BM age	28.57	5.60	30.99	6.05	7.13**	.04
BM parity	3.28	1.66	4.25	2.27	9.93**	.06
BM years of education	12.26	1.85	11.62	1.93	4.78*	.03
Birth outcomes						
Gestational age (weeks)	39.33	1.27	38.68	1.83	6.91*	.04
Birth weight (g)	3,320.15	494.58	2,988.85	523.81	17.58**	.10
Birth length (cm)	49.83	2.90	48.29	3.12	10.81**	.06
Head circumference (cm)	35.55	1.28	33.25	2.18	1.14	.007
OCS	100.53	17.15	86.38	16.18	30.07**	.15

Note. BM = biological mother; OCS = Obstetrical complications scale score; high scores are more optimal.

*
 $p < .05$.

**
 $p < .01$.

Table 2

Group Differences in Maternal Substance Use During Pregnancy and Over the 7 Months Postnatal Period and on Parenting Variables

	Exposure group						F Value	Partial η^2
	No cocaine		Cocaine					
	M	SD	M	SD				
Pregnancy								
Cigarettes/week	9.81	21.91	36.26	42.04	35.62**	.18		
Drinks/week	0.06	0.15	5.06	13.27	20.59**	.11		
Joints/week	1.12	6.20	1.55	4.62	2.34	.01		
Days cocaine/week	0	0	0.94	1.58	28.46**	.15		
Postnatal								
Cigarettes/week	14.52	28.81	36.57	42.75	19.63**	.11		
Drinks/week	0.39	0.69	1.98	5.37	3.39	.02		
Joints/week	1.20	4.53	1.15	4.69	0.01	.00		
Days cocaine/week	0	0	0.10	0.37	11.78**	.07		
Parenting								
Positive involvement	3.90	0.88	3.53	0.96	6.25**	.04		
Negative affect	4.77	0.39	4.53	0.68	6.72**	.04		
Sensitivity	4.21	1.05	3.97	1.09	2.00	.01		
Environmental risk	3.35	1.67	3.93	1.38	6.05**	.04		
Caregiving instability	1.08	1.05	1.15	0.97	0.23	.001		

Note. High scores on negative affect indicate low negative affect.

**
 $p < .01$.