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Maternal Stress and Behavior Modulate Relationships between Neonatal Stress, Attention, and Basal Cortisol at 8 Months in Preterm Infants

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Abstract

There is evidence that the developmental trajectory of cortisol secretion in preterm infants is altered, with elevated basal cortisol levels observed postnatally through at least 18 months corrected age (CA). This alteration is possibly due to neonatal pain-related stress. High cortisol levels might contribute to greater risk of impaired neurodevelopment. Since maternal factors are important for the regulation of infant stress responses, we investigated relationships between infant (neonatal pain-related stress, attention, cortisol) and maternal (stress, interactive behaviors) factors at age 8 months CA. We found that interactive maternal behaviors buffered the relationship between high neonatal pain-related stress exposure and poorer focused attention in mothers who self-reported low concurrent stress. Furthermore, in preterm infants exposed to high concurrent maternal stress and overwhelming interactive maternal behaviors, higher basal cortisol levels were associated with poor focused attention. Overall, these findings suggest that maternal factors can influence the cognitive resilience at 8 months of preterm infants exposed to early life stress.

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Keywords

premature birth; pain; stress; maternal stress; maternal behaviors; infant attention

INTRODUCTION

Preterm infants born at a very low gestational age (VLGA, ≤ 32 weeks) are exposed to stresses of the extra-uterine environment in the neonatal intensive unit (NICU) during a time of increased central nervous system vulnerability, immature regulation of the blood–brain barrier, and rapid development of the neuronal cytoarchitecture (Volpe, 2001). Repeated routine medical procedures administered in the NICU over a prolonged period induce early life stress in VLGA infants, which might contribute to vulnerability to negative developmental outcomes.

The majority of preterm infants show decreased attention, learning, and memory compared to full-term infants (e.g., Haley, Weinberg, & Grunau, 2006; Rose, Feldman, & Jankowski, 2001, 2002; Rose, Feldman, Jankowski, & Van Rossem, 2005; Ruff, 1986), and these deficits persist through childhood and adolescence (e.g., Anderson, Doyle, & the Victorian Infant Collaborative Study Group, 2004; Bhutta, Cleves, Casey, Craddock, & Anand, 2002; Grunau, Whitfield, & Davis, 2002; Grunau, Whitfield, & Fay, 2004b; Hack et al., 1994; Saigal, Hoult, Streiner, Stoskopf, & Rosenbaum, 2000). These poorer developmental outcomes may at least partially reflect alterations in the development of stress-sensitive systems. In children born extremely premature, we found heightened behavioral stress responses to cognitive challenge involving novelty both in the preschool years (Grunau, 2003) and in later childhood (Whitfield, Grunau, & Holsti, 1997), as well as a higher prevalence of anxiety-related behavior in adolescence (Grunau et al., 2004b; Levy-Shiff, Einat, Mogilner, Lerman, & Krikler, 1994), compared to full-term controls of comparable socio-demographic background. These observations of heightened stress and anxiety behavior in preterm populations are consistent with animal studies showing poor stress regulation in adult rats exposed to early life stress (Meaney, 2001). That is, in experimental animal models, exposure to early life stress is associated with enhanced hypothalamic-pituitary-adrenal (HPA) responsiveness to stressors, and reduced cognitive abilities in adulthood (for a review, see Meaney, 2001). These effects have been linked to reduced synaptogenesis and expression of hippocampal glucocorticoid receptor mRNA levels in juvenile and adult rats (Meaney, 2001). It remains unclear whether stress-related alterations in the HPA axis and cognitive function are detectable at an earlier developmental stage, that is, in infancy. Importantly, enrichment of the rearing environment of juvenile rats partially compensates for early life stress (Bredy, Humpartzoomian, Cain, & Meaney, 2003; Francis, Diorio, Plotsky, & Meaney, 2002) related to both hormonal and behavioral responsiveness. This raises the question of whether early caregiving environments in humans during and after the neonatal period may also buffer developmental difficulties induced by early stress exposure.

To our knowledge, studies in human infants born preterm have not examined whether or not stress during the neonatal period adversely affects cognitive function through altered HPA activity. Repeated pain exposure in neonatal rat pups has not resulted in heightened HPA activity at later ages (Anand, Coskun, Thirivikraman, Nemeroff, & Plotsky, 1999; Walker, Kudreikis, Sherrard, & Johnston, 2003). However, an increase in nurturing maternal behaviors (licking and grooming) following pup exposure to repeated neonatal pain appeared to prevent later elevation in HPA activity (Walker et al., 2003).

There is evidence that during early childhood, exposure to maternal distress concurrently or during infancy, or to stress-related situations such as low socioeconomic status is related to elevated cortisol levels in children born full-term (Essex, Klein, Cho, & Kalin, 2002; Lupien,

King, Meaney, & McEwen, 2000, 2001). These high cortisol levels were associated with later externalizing and internalizing behavior problems (Essex et al., 2002), and poorer cognitive performance (Lupien et al., 2000, 2001). Moreover, maternal stress within the months following delivery has been found to predict more negative maternal attitudes and behaviors during interactive play with their infants (Crnic, Ragozin, Greenberg, Robinson, & Basham, 1983), and reduced maternal sensitivity and increased controlling maternal behavior during interactions with their 6-month old preterm infants (Muller-Nix et al., 2004). Since high quality of caregiving can buffer HPA secretion in children (reviewed by Gunnar, 1998), taken together, these findings suggest that maternal stress during infancy may affect HPA secretion in children through interference with the quality of interactive maternal behavior.

Most studies examining the HPA function in preterm infants during the neonatal period showed evidence of HPA downregulation, for example, lower basal cortisol levels (e.g., Grunau et al., 2005; Hingre, Gross, Hingre, Mayes, & Richman, 1994; Huysman, Hokken-Koelega, De Ridder, & Sauer, 2000; Lee, Rajagopalan, Berg, & Moshang, 1989; Ng et al., 2004; Watterberg, Gerdes, & Cook, 2001). These lower levels appear to persist to the early infancy period (Haley, Grunau, Weinberg, & Whitfield, 2004; Haley et al., 2006). In contrast, at 8 and 18 months corrected age (CA) for prematurity (CA), cortisol levels in the tiniest preterm infants were found to be higher than in full-term infants, suggesting a resetting of the HPA axis associated with extreme prematurity (Grunau, Weinberg, & Whitfield, 2004a; Grunau, Haley, Whitfield, & Weinberg, in press). These elevated levels were found to be linked to cumulative exposure to neonatal stress indexed by the number of skin-breaking procedures from birth to term, adjusted for initial illness severity and neonatal intravenous morphine exposure.

In the adult population, basal cortisol modulates cognition through an inverted U-shape curve. Specifically, too little or too much circulating cortisol levels can impair memory function. Very little is known about the relationship between cortisol and cognition in infants and young children. To our knowledge, only one study has investigated such relationship in young infants. At 3 months CA, moderate basal and reactive cortisol levels were associated with better immediate and delayed learning in both VLGA and full-term infants (Haley et al., 2006). Given the evidence that in preterm infants, the HPA axis might be downregulated during the neonatal and early infancy, and then, upregulated at 8 and 18 months CA, it is possible that the direction of the relationship between cognition and cortisol at 8 months CA would be different from that reported by Haley et al. (2006) at 3 months CA.

Infant attention is frequently used as a marker of cognition, since attentional processes predict cognitive outcomes in early childhood (e.g., Mirsky, 1996; Posner & Petersen, 1990; Posner & Raichle, 1994; Ruff & Rothbart, 1996). Focused attention during toy exploration in the middle of the first year of life is sensitive to differences between high-risk preterm and full-term infants (Ruff, 1988; Ruff, Lawson, Parrinello, & Weissberg, 1990; Ruff, McCarton, Kurtzberg, & Vaughan, 1984), and can predict cognitive abilities in 2, 3, and 4/5 years (Lawson & Ruff, 2004).

In the present study, we investigated the relationships among neonatal pain-related stress, cognitive indices (focused attention), and basal cortisol levels in VLGA infants at 8 months CA. Further, we investigated the potential role of maternal factors (parenting stress and interactive behaviors) in ameliorating possible adverse relationships among stress, cortisol, and focused attention. We hypothesized that maternal factors will modulate: (1) the relationship between neonatal pain-related stress and focused attention and (2) the relationship between cortisol and infant focused attention, at 8 months CA. In addition, we included a sample of full-term infants as a reference group for infant focused attention and maternal stress and behavior.

METHODS

Participants

One hundred three infants born at VLGA ≤ 32 weeks and 55 healthy full-term infants born at ≥ 38 weeks gestation participated in this study. Preterm infants were recruited from the neonatal intensive care unit (NICU) at Children's and Women's Health Centre of British Columbia (C&W), which is the major tertiary neonatal unit in British Columbia. Full-term infants were recruited from the full-term nursery at the same institution. All infants were recruited between 2001 and 2004 by one neonatal research nurse and were assessed in a laboratory setting at the Child and Family Research Institute at 8 months CA. The participants in this study are part of a larger cohort involved in a longitudinal study (Grunau et al., under review). Mother and infant demographic characteristics are presented in Table 1.

Procedures

Informed consent was obtained from each mother at recruitment when the child was in early infancy, and again prior to participation in the visit at 8 months CA, in accordance with the requirements of the Clinical Research Ethics Board of University of British Columbia (UBC) and the Research Review Committee at C&W. Upon arrival at the laboratory, the mother–infant dyad was given several minutes to settle and get acquainted with the testing environment. They were then presented with the following tasks: 6-min toy exploration play session for assessment of the quality of focused attention, followed by a 5-min interactive free play session during which maternal behaviors were assessed using criteria set by Crnic et al. (1983). Saliva samples for basal cortisol assay were collected shortly after arrival in the lab while the infant was seated on his/her mother's lap. Since feeding and blood contamination due to teething can interfere with the accuracy of salivary cortisol assays (Kivlighan, Granger, Schwartz, Nelson, & Curran, 2004; Schwartz, Granger, Susman, Gunnar, & Laird, 1998), saliva samples were collected at least 25 min after feeding, and we verified that there had been no traces of blood from teething in 24 hr prior to the start of experimental session. The HPA axis is known to follow a circadian rhythm that is apparent as early as the 3rd month of life under normal conditions (Antonini, Jorge, & Moreira, 2000; de Weerth, Zijl, & Buitelaar, 2003; Gunnar, 1992; Larson, White, Cochran, Donzella, & Gunnar, 1998). Therefore, as much as possible, experimental sessions took place in the morning, between 8:30 a.m. and 1:30 p.m., which is an appropriate time of day for optimal infant cognitive performance. Five preterm and three full-term infants were seen between 1:30 p.m. and 3:00 p.m. Since cortisol can decrease transiently during naptime (Watanura, Sebanc, & Gunnar, 2002), the time of saliva collection and time since waking were included as covariates in data analyses of infant cortisol.

Measures

Infant Measures

Medical chart review: A neonatal research nurse conducted medical and nursing chart review that included, but was not limited to, birthweight, gestational age, illness severity (Score for Neonatal Acute Physiology II, SNAP-II, on postnatal Days 1) and infant medications. The total cumulative number of skin-breaking procedures from birth to term (39 weeks and 6 days) was used as an index of neonatal pain-related stress. These procedures included, but were not restricted to, heel lance, intramuscular injections, chest tube insertion, and central line insertion. Cumulative neonatal morphine exposure was indexed as the average daily dose of intravenous morphine adjusted for daily weight, multiplied by the number of days of intravenous morphine administration. Knowing that exposure to postnatal glucocorticoids can affect the HPA axis of juvenile offspring (Burlet et al., 2005), we also recorded the number of days of exposure to postnatal glucocorticoids for each preterm infant. Overall, six infants received glucocorticoids postnatally.

Focused attention: The quality of focused attention during toy exploration was scored from videotape following the methods of Lawson and Ruff (2001). The toy exploration session comprised presenting four different toys consecutively for 90 s per toy. Toys were chosen to be age-appropriate, made of washable plastic, varied in color and texture, and were presented to the infant in a standard order. Quality of focused attention is defined as the global quality of visual examination while the infant was not banging or mouthing the toys, using a five-point rating scale, ranging from 1 (little or no investment of energy in object exploration) to 5 (high levels of sustained and concentration attention). This global rating of focused attention during toy exploration in preterm infants has been shown to predict cognitive outcome later in childhood (Lawson & Ruff, 2004). With this paradigm, two blinded coders independently rated the global quality of focused attention in all infants. If the two coders did not agree, they re-rated the video independently and if agreement was reached, then this second rating was retained. If disagreement remained and the difference between scores was less than one point, then the mean score was used. Differences beyond one point were resolved by a third independent coder (from personal communications with Dr. Lawson). The quality of infant focused attention was averaged across the four toys to yield one single score of focused attention.

Maternal Measures

Demographic characteristics: Maternal characteristics at delivery, including the mother's age and years of education, were collected through a questionnaire. Antenatal glucocorticoids, which are given to mothers as a preventive measure against premature delivery may affect the infant's basal (Ashwood et al., 2006; Kajantie et al., 2004) and reactive (Glover, Miles, Matta, Modi, & Stevenson, 2005) HPA secretion in early infancy (before 4 months of age). Very little is known about long-term changes. Therefore, we have also collected information regarding maternal exposure to antenatal glucocorticoids ($n = 8$).

Interactive behaviors: Interactive maternal behaviors during the play session were recorded on videotape, and later scored by blinded coders on a scale from 1 (poor) to 5 (high) for Gratification, Affect, Sensitivity, and Organization, according to the criteria of Crnic et al. (1983). Inter-rater reliability was carried out on 25% of infants. Weighted kappa using agreement within one scale point was .95, 1.0, .79, and .94 for Gratification, Affect, Sensitivity, and Organization, respectively. To reduce the number of variables, we combined the four subscales of maternal behaviors (Gratification, Affect, Sensitivity, and Organization) into a single factor score using principal component analysis.

Parenting stress: Mothers completed the long-form Parenting Stress Index (PSI), a 101-item questionnaire (Abidin, 1995) which provides parenting stress scores on the Parent Domain, Child Domain, Total Stress, and Defensive Responding. Low scores (<24) on Defensive Responding are viewed as under-reporting (Abidin, 1995), therefore infants whose mothers scored below 24 were excluded from analysis, leaving 84 preterm (43 boys, 46 girls) and 37 full-term (20 boys and 17 girls) mother–infant dyads available for study. Using median split of the Total PSI scores (median evaluated at 220), mother–infant dyads were divided a posteriori into low (PRETERM-LOW, $n = 40$; 20 boys, 20 girls; FULL-TERM LOW, $n = 19$, 10 boys, 9 girls) and high (PRETERM-HIGH, $n = 44$; 20 boys, 24 girls; FULL-TERM HIGH, $n = 18$, 11 boys, 7 girls) parenting stress groups.

That sample size, knowing that 10 participants per predictor variable are required to perform linear regression analysis, was appropriate to examine the relationship between the quality of focused attention (dependent variable) and neonatal pain-related stress (adjusted for cumulative intravenous morphine exposure and initial illness severity) and maternal interactive

behavior (independent variables) within each of the PRETERM-LOW and PRETERM-HIGH parenting stress groups.

Infant Cortisol Analysis

Saliva was collected using cotton dental rolls, extracted by needleless syringes into vials, and stored at -20°C until assayed in Dr. Weinberg's laboratory at the UBC. Salivary cortisol was assayed using the Salimetrics High Sensitivity Salivary Cortisol Enzyme Immunoassay kit (Salimetrics LLC, Philadelphia, PA). Intra and interassay coefficients of variability were 3.04 and 5.65%, respectively.

Statistical Analysis

The cohort of full-term infants was used as a reference group for both infant (quality of focused attention) and maternal (parenting stress, interactive behaviors) characteristics. Given that preterm boys have poorer developmental outcomes than preterm girls (e.g., Hindmarsh, O'Callaghan, Mohay, & Rogers, 2000), we have included sex of the infant as a grouping variable in our analyses. Comparisons of effects of sex of the infant, prematurity status, and parenting stress group on infant and maternal characteristics were performed using multivariate analysis of variance (MANOVA). All measures are presented in Table 1, as mean \pm standard deviation (SD).

To address our hypotheses, we used hierarchical regression analyses to examine whether neonatal-pain related stress (number of skin-breaking procedures from birth to term, adjusted for initial illness severity and cumulative i.v. morphine exposure from birth to term: entered in block 1) and maternal behaviors score (entered in block 2) predicted the quality of focused attention (dependent variable), in PRETERM-LOW and PRETERM-HIGH parenting stress groups separately. In addition, we performed another set of analyses to investigate whether basal cortisol (covaried for time of testing and time since waking) was associated with the quality of focused attention in the PRETERM-LOW and PRETERM-HIGH parenting stress groups separately using linear regression analyses. For all analyses, statistical significance was set at $p < .05$.

Only a small number of mother-infant dyads received either antenatal ($n = 8$) or postnatal glucocorticoids ($n = 6$). None received glucocorticoids both ante- and postnatally. Due to the small sample size, rather than adding their user status as a grouping variable, we compared statistics obtained with and without including these mother-infant dyads in the analyses.

RESULTS

Demographic Characteristics

Infant Measures—As expected, MANOVA showed that birthweight and gestational age were lower in preterm than in full-term infants [$F(1,120) = 525.24$; $p < .001$ and $F(1,120) = 553.54$, $p < .001$, respectively]. Regardless of prematurity status, boys had greater birthweight than girls, $F(1,120) = 3.99$, $p = .048$. There was no main effect of parenting stress group, or interaction effects of sex of the infant, prematurity status, or parenting stress group on birthweight or gestational age. Importantly, neonatal illness severity and i.v. morphine exposure were significantly greater in the PRETERM-HIGH parenting stress compared to the PRETERM-LOW parenting stress group [$F(1,79) = 6.44$, $p = .013$ and $F(1,79) = 7.01$, $p = .010$, respectively]. No other main or interaction effects involving sex of the infant or parenting stress were observed for neonatal illness severity, morphine exposure. Infant characteristics are presented in Table 1.

Repeated measures ANOVAs performed across all four toys used in toy exploration showed that overall, there was a trend in lower quality of focused attention in preterm infants compared to full-term infants [$F(1,173) = 3.60, p = .060$; Preterm: $2.25 \pm .08, 2.82 \pm .10, 2.49 \pm .09$, and $2.58 \pm .10$ and Full-term: $2.36 \pm .11, 3.13 \pm .13, 3.01 \pm .12$, and $2.84 \pm .13$ for Toys 1, 2, 3, and 4, respectively]. No other main or interaction effects involving sex of the infant, prematurity status parenting, and stress group were observed.

We did not find any significant main or interaction effects of sex of the infant, prematurity status or parenting stress group on basal cortisol concentrations.

Maternal Measures—Main effect of prematurity status on years of maternal education, $F(1,141) = 13.32; p < .001$. There was no main effect of prematurity status on maternal age, however, among mothers of preterm infants, there was an overall effect of parenting stress groups on maternal age [$F(1,129) = 6.13, p = .015$]. Mothers of the PRETERM-HIGH parenting stress group were younger (by about 3 years) than mothers of the PRETERM-LOW parenting stress group ($p = .022$) and of the full-term groups ($p = .004$). Similarly, years of maternal education also differed among the groups [$F(1,129) = 8.23, p = .005$], with the lowest education in the PRETERM-HIGH parenting stress group ($p < .001$) compared to both the PRETERM-LOW and FULL-TERM parenting stress groups. There were significant differences in maternal age and years of education due to sex of the infant.

Principal component factor analysis (PCA) was used to reduce the four subscales of interactive maternal behaviors (Gratification, Affect, Sensitivity, and Organization) to one score. The contribution of these components was .857, .817, .623, and .615, respectively. Neither individual interactive maternal behavior components (Gratification, Affect, Sensitivity, or Organization) nor the overall score generated by PCA differed due to sex of the infant, parenting stress group, or prematurity status.

Mothers of preterm infants had higher scores on the Total PSI [$F(1,120) = 13.13, p < .001$], the Child Domain [$F(1,120) = 7.60, p = .007$], and the Parent Domain [$F(1,120) = 6.30, p = .013$], than mothers of full-term infants. However, within each group parenting stress, that is, lower median and upper median, scores in mothers of preterm infants did not differ from scores in mothers of full-term infants. No main or interaction effects of sex of the infant were noted for scores on the Child Domain, Parent Domain, and Total PSI. Maternal characteristics are presented in Table 1.

Because only birthweight was affected by sex of the infant, in the following set of analyses, boys and girls will be pooled together to be considered as one group.

Maternal Factors and Parenting Stress

Given the lower maternal age and education observed in PRETERM-HIGH compared to PRETERM-LOW parenting stress groups, analyses were conducted to examine potential associations between these demographic variables and PSI scores. Pearson correlations revealed no statistically significant association between maternal demographic characteristics and the Child Domain, Parent Domain, or Total PSI score.

Neonatal Pain-Related Stress and Basal Cortisol Concentrations

Neonatal pain-related stress, indexed by the number of skin-breaking procedures, did not correlate with basal cortisol concentrations in either the PRETERM-LOW or PRETERM-HIGH parenting stress groups.

Neonatal Pain-Related Stress and Interactive Maternal Behavior in Relation to Infant Focused Attention

In the PRETERM-LOW parenting stress group, after controlling for illness severity and i.v. morphine exposure, greater neonatal pain-related stress was associated with poorer focused attention ($\beta = -.39, p = .047$, Fig. 1), and greater interactive maternal behavior was associated with better focused attention ($\beta = .35, p = .017$, see Fig. 2). This model explained 19% of the variance observed. In the PRETERM-HIGH parenting stress group, though neonatal stress was also associated with poorer focused attention ($\beta = -.35, p = .024$, Fig. 3), there was no buffering effect of maternal behavior on infant focused attention. This model explained 15% of the variance observed.

Infant Cortisol in Relation to Quality of Focused Attention

Linear regression analyses showed no significant association between infant basal cortisol (adjusted for time of testing and time since waking) and quality of focused attention in either the PRETERM-LOW or PRETERM-HIGH parenting stress groups. However, when the participants were subsequently divided into low and high interactive maternal behaviors (using median split), infants from the PRETERM-HIGH parenting stress group whose mothers displayed high interactive maternal behavior showed a negative association between basal cortisol and the quality of focused attention ($\beta = -.34, p = .04$, 23% variance explained, Fig. 4). High infant basal cortisol was associated with poorer attention in this subgroup. No significant correlations were seen for full-term infants.

Use of Antenatal and Postnatal Glucocorticoids

Analyses performed on a sample of mother–infant dyads unexposed to antenatal or postnatal glucocorticoids did not reveal any differences in the direction and significance of findings presented above. For both PRETERM-LOW and PRETERM-HIGH parenting stress group, larger number of painful procedures remained associated with poorer quality of focused attention and higher maternal interactive behavior score was associated with better attention in the PRETERM-LOW parenting stress group only (PRETERM-LOW: number of painful procedures, $\beta = -.39, p = .05$, maternal interactive behavior $\beta = .33, p = .027$, 18% variance explained; PRETERM-HIGH: number of painful procedures $\beta = -.58, p = .017$, 21% variance explained). In PRETERM-HIGH parenting stress group exposed to overwhelming maternal interactive behavior, higher basal cortisol concentrations were associated with poor quality of focused attention, $\beta = -.34, p = .045$, 23% variance explained.

DISCUSSION

To our knowledge, this is the first study to examine the contribution of maternal factors on the relationship between neonatal pain-related stress, cognitive indices, and basal cortisol in the preterm infant population. Simultaneously, we investigated the influence of maternal factors such as parenting stress and interactive behavior in relation to cognition and cortisol. We found that greater neonatal pain-related stress was associated with poorer quality of focused attention at 8 months CA and that maternal behavior can buffer this relationship in mothers reporting low parenting stress. Moreover, in infants whose mothers reported high parenting stress and displayed high interactive maternal behavior, higher basal cortisol was associated with poorer quality of focused attention in infants.

Children and adolescents born prematurely have a range of problems related to cognition, attention learning, and behavior (e.g., Grunau et al., 2004b). Little is known, however, about the etiology of these difficulties. Furthermore, although prolonged neonatal pain-related stress, at a time of rapid brain development, has been proposed as one possible contributing factor that may affect cognition and behavior (Anand, 2000; Grunau, 2002), this theory has received

little direct empirical investigation. In this study, we used the quality of infant focused attention as a measure of cognition since it predicts later cognitive outcome (Lawson & Ruff, 2004). In agreement with previous work, we found that the quality of focused attention was poorer in preterm compared to full-term infants (Ruff, 1986, 1988; Ruff et al., 1990). Importantly, we show for the first time that neonatal pain-related stress, indexed by the total number of skin-breaking procedures from birth to term, is associated with poorer quality of focused attention in preterm infants. Low tactile threshold and immaturity of descending modulatory systems (Fitzgerald, 2005) underlie greater sensitivity to pain in VLGA preterm infants. Due to immaturity of the biological systems, sensitization occurs such that even inherently noninvasive routine nursing procedures induce stress responses (Holsti, Grunau, Oberlander, & Whitfield, 2005). Thus, the number of skin-breaking procedures is a marker of overall neonatal stress associated with tactile interventions. Other sources of stress, such as lighting and noise, are well-controlled in current NICU practice with the implementation of covered incubators and periods of no intervention as part of developmentally oriented care (Als et al., 1994). However, pain-related stress is highly correlated with number of days on mechanical ventilation, a situation that is inherently stressful. Further, since morphine treatment to reduce stress is associated with respiratory depression and lengthens time on ventilation (Bhandari, Berqvist, Kronsberg, Barton, & Anand, 2005), pain and stress inherent to NICU care is difficult to manage or eliminate. Therefore, we cannot evaluate a direct causal relationship between early pain and poorer cognition; rather we propose that pain-related stress, as a marker of neonatal stress, is likely a contributing factor. This is consistent with the animal literature on the adverse impact of early *stress*, especially in the case of prolonged maternal separation. Animal studies of neonatal pain have focused on long-term effects on pain systems, and to our knowledge, none has examined later cognition. Experimental animal models addressing whether neonatal pain affects later cognition are needed.

Importantly, we found that maternal parenting stress overrode the buffering effect of interactive maternal behaviors on the relationship between the high number of skin-breaking procedures in the NICU and poor quality of focused attention in preterm infants. Thus, VLGA infants exposed to neonatal pain-related stress appeared to benefit from positive interactive maternal behavior, but only if their mothers self-reported low concurrent stress. These findings support and extend previous reports that mother–infant interactions can mediate the trajectories of mental development in the at-risk infant population (Poehlmann & Fiese, 2001). Maternal parenting stress may act indirectly through interactive maternal behavior or may affect infant cognition directly. In infants born close to term (below 38 weeks gestation), maternal stress did not affect quality of interactive maternal behavior during a free play session (Crnic, Greenberg, Robinson, & Ragozin, 1984). However, in preterm infants born below 34 weeks gestation, interaction at 6-months was affected more by maternal traumatic stress since birth than perinatal risk factors (Muller-Nix et al., 2004). Maternal worries at the time of delivery also predict maternal stress during infancy (Combs-Orme, Cain, & Wilson, 2004). Consistent with these studies, maternal stress appears to have greater effects in mothers of infants born very preterm, such as in our study.

Our finding that the consequences of neonatal pain-related stress on cognitive outcomes might be partially compensated for by sensitive and balanced quality of interactive maternal behaviors, is congruent with the animal literature. Such literature has demonstrated that adverse effects of early stress can be partially ameliorated through enriched rearing environments (Bredy et al., 2003; Francis et al., 2002), and suggests possibilities for remedial intervention. Recent preliminary evidence in macaques suggests that maternal perinatal stress and maternal behavior are associated with infant behavior during development (Bardi & Huffman, 2006). We speculate that in the current study, maternal parenting stress interfered with interactive maternal behavior, resulting in poorer quality of caregiver “support” in promoting cognitive development, expressed as quality of focused infant attention. Most studies addressing the

contribution of interactive maternal behavior to infant cognitive outcomes use “sensitivity to infant cues” as the key component of maternal behavior. Interestingly, in reducing the four components of interactive maternal behavior, that is, Gratification, Affect, Sensitivity, and Organization derived from Crnic et al. (1983), to a single factor, we found that Gratification and Affect might play a role that is at least as important as Sensitivity in the quality of interactive maternal behavior. Previously, maternal stress had been suggested to impede maternal sensitivity to infant cues (Ragozin, Basham, Crnic, Greenberg, & Robinson, 1982). Here, we report that other components of interactive maternal behavior, such as Affect, might interact with high maternal stress and possibly overwhelm the infant (Field, 1994). Given that communication styles between a mother and her preterm infant can change significantly over the first 2 years of life (Muller-Nix et al., 2004), it is important to extend this question beyond age 8 months. Additional work is needed to test this mediating role of maternal stress further.

Maternal behavior has long been known to be a strong regulator of an infant’s biological vulnerability (e.g., Gunnar, 1998; Hofer, 1994; Spangler, Schieche, Ilg, Maier, & Ackermann, 1994; Thompson et al., 1994) and has been suggested to mediate the developmental trajectories in at-risk populations (Magill-Evans & Harrison, 1999; Poehlmann & Fiese, 2001). Moreover, associations linking maternal stress and depressive symptoms, with elevated child cortisol levels and cognitive and behavioral outcomes have been reported in recent years (Essex et al., 2002; Lupien et al., 2000, 2001). Interestingly, Walker et al. (2003) suggested that increased maternal behavior buffered HPA responsiveness to stress in two week-old rat pups exposed to repeated neonatal pain. We extend these findings by suggesting that in infants born very preterm, maternal factors might mediate the vulnerability of preterm infants to early pain-related stress. It is important to keep in mind that in rodents, maternal care is essential for basic functions such as thermoregulation, feeding, and excretion. Conversely, in humans, the role of maternal behavior as a regulator of infant biological and cognitive development appears to act at a much higher and likely more complex cortical level. Protective factors play a greater role in individuals who are at higher risk of poor self-regulation. Further work is needed to elucidate the contribution of various aspects of maternal behavior on HPA buffering in the preterm and full-term infant population.

Previous studies have shown that the tiniest preterm infants born at extremely low gestational age (ELGA, <29 weeks GA) had greater cortisol levels at 8 months CA, compared to preterm infants born with more maturity and full-term infants (Grunau et al., 2004a). In this study, at a similar age, we did not find higher basal cortisol concentrations in preterm infants born with greater gestational age (<32 weeks gestation), when compared to full-term infants. It is possible that in the current study, the inclusion of preterm infants born with greater maturity at birth might have prevented us from findings such group differences in basal cortisol concentrations. For similar reasons, we failed to detect any significant correlations between the number of skin-breaking procedures and basal cortisol concentrations, even though this association has already been reported previously in ELGA infants by our own research group (Grunau et al., 2004a).

In this study, we found that high cortisol was *negatively* associated with quality of focused attention only in preterm infants exposed to both high maternal parenting stress and high maternal interactive behavior. At first sight, this finding could seem unexpected given that a *positive* relationship between basal cortisol and cognition in preterm infants at 3 months CA has been recently described (Haley et al., 2006). However, at 3 months CA, VPGA infants are in a state of downregulation, when low cortisol levels are predominant (Haley et al., 2006). In contrast, at 8 months CA, ELGA infants, are in a state of upregulation, that is, levels are higher compared to full-term infants (Grunau et al., 2004a; Grunau et al., under review). When comparing our cortisol values collected at 8 months CA, to those presented in Haley et al. (2006), we found that in full-term infants, from 3 months CA to 8 months CA, basal cortisol

levels decreased (mean .32 µg/dL vs. .25 µg/dL, respectively), while they remained similar for VLGA infants (.2 µg/dL at both time points). This shift from downregulation to upregulation state could explain the opposite direction of association we are reporting in this study.

It is encouraging that at 8 months CA, only a small proportion of preterm infants, that is, those exposed to both high parenting stress and high interactive behavior, presented poor cognition in association with higher basal cortisol concentrations. Further studies are needed to determine the exact reasons for this finding. Some stress-related events experienced by mothers have been linked to higher basal cortisol levels and poorer cognition in children (Essex et al., 2002; Lupien et al., 2000, 2001). Because we have reported that high maternal parenting stress may override the beneficial contribution of maternal interactive behavior on cognition in preterm infants exposed to neonatal pain-related stress, it is possible that both maternal parenting stress and interactive behavior could mediate the association between infant cognition and basal cortisol levels. In our search to understand these findings, we contemplate the hypothesis of “overwhelming” maternal influences on the preterm infant. We speculate that this maternal-induced “overwhelming” of the preterm infant could lead to a more rapid *emergence from the HPA hyporesponsive* state normally observed during the first year of life. This emergence to a *responsive* state would enable the HPA axis to present negative correlations with the quality of focused attention. This is in accordance with the hypothesis that cortisol levels during the early human development are regulated by social factors derived from the parents during the first years of life, and from interacting with the peers at the daycare and beyond (Gunnar & Donzella, 2002). Importantly, in our study, full-term infants did not readily show any association between cortisol and infant attention. Thus, it appears that based on our maternal measures, this maternal-induced “overwhelming” might be more easily detected in mothers of infants displaying poor self-regulation. Additional work is needed to investigate this matter further.

Cortisol levels collected in laboratory settings have been consistently reported to be lower than those measured in the same infants, in a home setting, at comparable times of day (Gunnar, Mangelsdorf, Larson, & Hertsgaard, 1989; Larson, Gunnar, & Hertsgaard, 1991). Although this could be an obstacle to the generalization of our findings to daily life situations, high correlations between baseline cortisol levels collected prior to laboratory-induced stressors and basal levels sampled at similar times at home suggest that observations made in the laboratory reflect the infant’s phenotype, without the need to collect home samples as reference levels (Goldberg et al., 2003). Therefore, not only would our findings regarding cortisol correlates represent a realistic phenotype of daily life in preterm infants, but also it is possible that the relationship might be even more pronounced in daily life situations.

Our findings were not affected by excluding mother–infant dyads exposed to antenatal or postnatal glucocorticoids from our analyses. However, because of the small sample size of infants exposed, we cannot entirely rule out the possibility that glucocorticoids exposure, whether ante- or postnatally, did not affect our findings. In contrast to the animal literature, only a limited number of human studies have investigated the long-term consequences of antenatal and postnatal exposure to glucocorticoids on the HPA in neonates (for a review, see Matthews, 2001; Purdy & Wiley, 2004). Findings suggest that despite showing greater behavior problems during childhood, infants exposed to antenatal and postnatal glucocorticoids might not show any detectable side effects impeding neurodevelopment until later in life (Purdy & Wiley, 2004). With animal work hypothesizing the presence of sex-specific long-term consequences of antenatal glucocorticoids exposure favoring females (Matthews, 2001), more work in the human population would be needed to better understand these interactions.

While examining the demographic characteristics of mother–infant dyads, we found that compared to the FULL-TERM group, mothers of the PRETERM-LOW parenting stress group

had similar ages, years of education and Total, Child, and Parent Domains of the PSI. Conversely, PRETERM-HIGH parenting stress mothers were younger and had fewer years of education. Lower education has been linked to reduced coping and protective resources in the face of stressful events (for a review, see Kristenson, Eriksen, Sluiter, Starke, & Ursin, 2004). However, we did not find any significant association between maternal parenting stress and interactive behavior, and maternal education or age. Mothers in the PRETERM-HIGH parenting stress group were younger in age and had fewer years of education (average age 31.0 years and 14.2 years of education) compared to mothers in the PRETERM-LOW parenting stress group and mothers of full-term infants (average age 34.0 and 34.4 years, 15.8 and 17.2 years of education, respectively). However, given that maternal age and education were relatively high in all our groups, it is unlikely that these variables played a significant role in our findings. Importantly, although infants in the PRETERM-HIGH parenting stress group had higher illness severity on the first postnatal day, they did not differ from the PRE-TERM-LOW parenting stress group in birthweight, gestational age, days on mechanical ventilation, or amount of neonatal pain-related stress. Furthermore, we found that greater illness severity on Day 1 had a lasting impression on the parents only in the PRETERM-LOW parenting stress group. During early infancy, greater neonatal physiological vulnerability is known to be associated with greater maternal distress during infancy (Combs-Orme et al., 2004; Singer et al., 1999) and poorer developmental outcome (Miceli et al., 2000). However, this association appears to be more apparent during early infancy since over time, the impact of the early social environment appears to exceed the influence of neonatal factors as the infant reaches preschool age (Miceli et al., 2000).

Sex differences in developmental outcomes of preterm born children have been favoring girls over boys. Preterm male neonates seem to require more neonatal care than female preterm neonates (Elsmen, Hansen Pupp, & Hellstrom-Westas, 2004). Despite medical advances in recent years, boys are yet to show less severe neonatal brain injury and illness, and lower infant and perinatal mortality (Bhaumik et al., 2004; Nunez & McCarthy, 2003; Vatten & Skjarven, 2004). Despite this evidence of sex dichotomy in preterm outcome, we did not find any significant sex differences in the outcomes that we have measured in this study. Unlike child temperament and indices of developmental psychopathology, where sex differences are apparent at a relatively early age, very few have reported clear sex differences for cognitive abilities in preterm infants (Hindmarsh et al., 2000). It is possible that sex differences in the areas investigated in our study, that is, maternal stress, maternal interactive behavior, infant cortisol, and infant focused attention may require some time to unfold. Importantly, due to bias related to participation to research studies like ours, it is also possible that some of the boys enrolled in our study might be among those who fared better after hospital discharge.

Although mothers of preterm infants had greater parenting stress scores than mothers of full-term infants, within each of the LOW and HIGH maternal parenting stress groups, there was no difference in the Total PSI scores due to prematurity status. The higher Total PSI score found in mothers of a preterm infant (higher by 16 points) might be driven by slightly higher scores in the PRETERM-HIGH group, than in the FULL-TERM HIGH parenting stress group (mean of 242.6 vs. 229.3, respectively). Previous studies have shown that maternal factors contribute to a larger variance in developmental outcomes in the preterm population compared to the full-term infant population (e.g., Greenberg & Crnic, 1988; Hofer, 1994; Poehlmann & Fiese, 2001). Indeed, our analyses showed that at 8 months, full-term infants might not be as influenced by maternal factors as the preterm population. Protective factors such as maternal behavior come into greater play when the infant is at risk for poorer self-regulation. Thus, compared to an infant born very prematurely, a healthy full-term infant, with lower risk for poor self-regulation, might not rely on the maternal protective influence as much. It is important to remind our readers that our findings by no means imply that full-term infants will not be affected by poor quality of maternal interactive behavior. Our measures might simply not be

sensitive enough to detect the contribution of maternal factors in full-term infants. Furthermore, our median of 220 on the Total PSI observed in mothers of preterm infants should be interpreted with caution. The PSI is a self-reported inventory that has limited clinical significance when used in a context of research such as the current study. The 50th percentile norms for parents of healthy infant at age 12 months are listed at approximately 200, and a suggestive cut-off score for clinical referral for excessive parenting stress suggested at 260 (Abidin, 1995), which none of our participants has reached, our findings indicate that mothers were only slightly into the upper range of normative scores. Therefore, in our study, PSI scores in mothers of preterm infants did not need to reach the excessive stress level that could interfere with infant outcomes. Finally, it is important to note that we have excluded mothers who portrayed a “defensive” profile, self-reporting abnormally low scores. These abnormally low scores have been believed to under-report underlying higher parenting stress profile. However, given the relevance of stressful events during the neonatal and infancy period, for parents of very preterm infants, they may self-report less stress once their infant’s health has stabilized after NICU discharge. Thus, excluding mothers with defensive profile from the analyses prevented us from including under-reporting in our analyses; it is also likely that we have excluded mothers who truly reported low parenting stress.

In our study, high scores in both the Child and Parent Domains contributed to the high total PSI score. The fact that high stress mothers reported higher scores on Domains indicates that interventions targeted to alleviate parenting stress attributed to parental characteristics might only partially reduce the level of parenting stress. These findings are in agreement with previous observations that maternal concerns during delivery might predict the extent of maternal stress during infancy (Combs-Orme et al., 2004). Moreover, the PSI is a self-report scale and we acknowledge that further studies using a more objective marker of stress, such as cortisol, would provide valuable information in conjunction with PSI scores, especially since anticipation of adverse events is known to be among the psychological determinants of cortisol secretion (e.g., Gaab, Rohleder, Nater, & Ehler, 2005; Mason, 1968; Smyth et al., 1998).

Prenatal maternal anxiety and stress are predictors of premature delivery, poor neurodevelopment, and more disrupted HPA function in the infant (Austin & Leader, 2000; DiPietro, Novak, Costigan, Atella, & Reusing, 2006; Glover, 1997; King & Laplante, 2005; Laplante et al., 2004; Wadhwa, Sandman, & Garite, 2001). In our study, recruitment took place after delivery. To account for both prenatal and postnatal risks factors of poorer neurodevelopment and stress-regulation, a much larger sample of mother–infant dyads than in the current study would be needed. Therefore, it is not possible to verify whether presence of antenatal maternal stress and anxiety would contribute independently to poorer infant outcomes, in combination with high postnatal maternal stress.

Infants born very prematurely are vulnerable because they enter the world with immature physiological and central nervous systems. During this critical period, as part of routine neonatal care, they are exposed to considerable pain and stress, which may contribute to “resetting” basal cortisol levels (Grunau et al., 2004a; Grunau et al., under review). Importantly, little is known about maternal distress after hospital discharge, even though maternal worries during the perinatal period can predict maternal distress later during infancy (Combs-Orme et al., 2004). In this study, we have identified maternal factors that may ameliorate adverse effects of neonatal pain exposure on infant cognition and affect the vulnerability and resilience of psychobiological function of at-risk infants. Our findings will contribute to current knowledge regarding postnatal influences after hospital discharge, and suggest the importance of developing targeted interventions to alleviate stress in the mothers of preterm infants.

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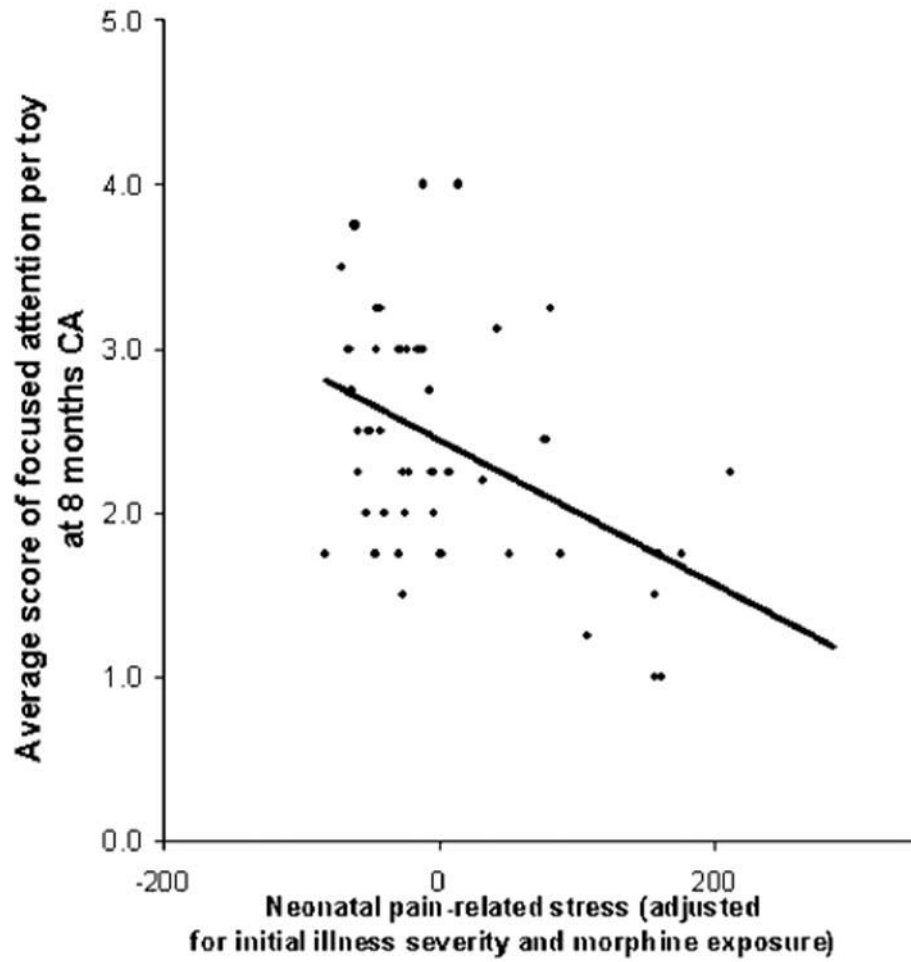


FIGURE 1. Relationship between the average score of focused attention per toy and cumulative neonatal pain-related stress (adjusted for initial illness severity and morphine exposure) in 8-month-old CA preterm infants exposed to low maternal parenting stress.

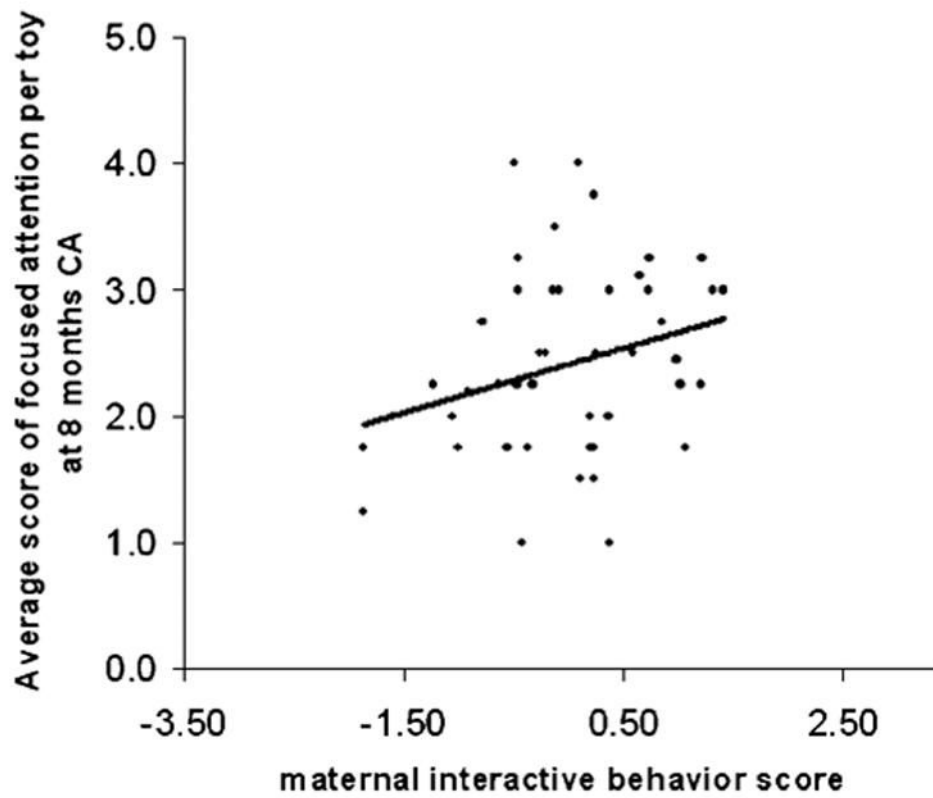


FIGURE 2. Relationship between the average score of focused attention per toy and interactive maternal behavior score in 8-month-old CA preterm infants exposed to low maternal parenting stress.

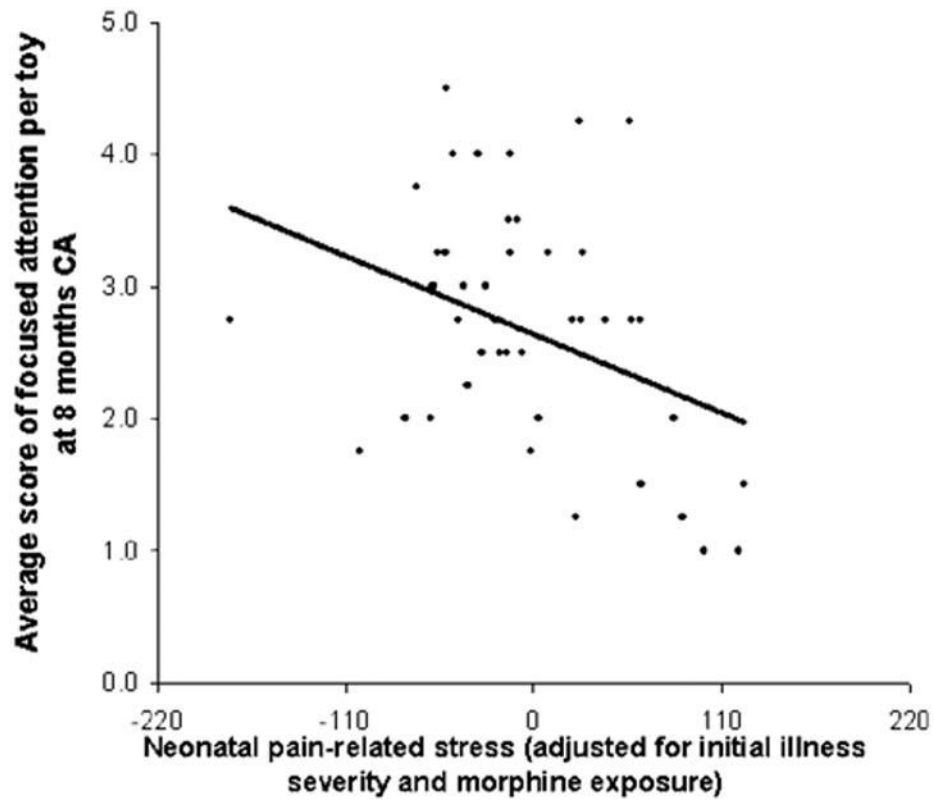


FIGURE 3. Relationship between the average score of focused attention per toy and cumulative neonatal pain-related stress (adjusted for initial illness severity and morphine exposure) in 8-month-old CA preterm infants exposed to high maternal parenting stress.

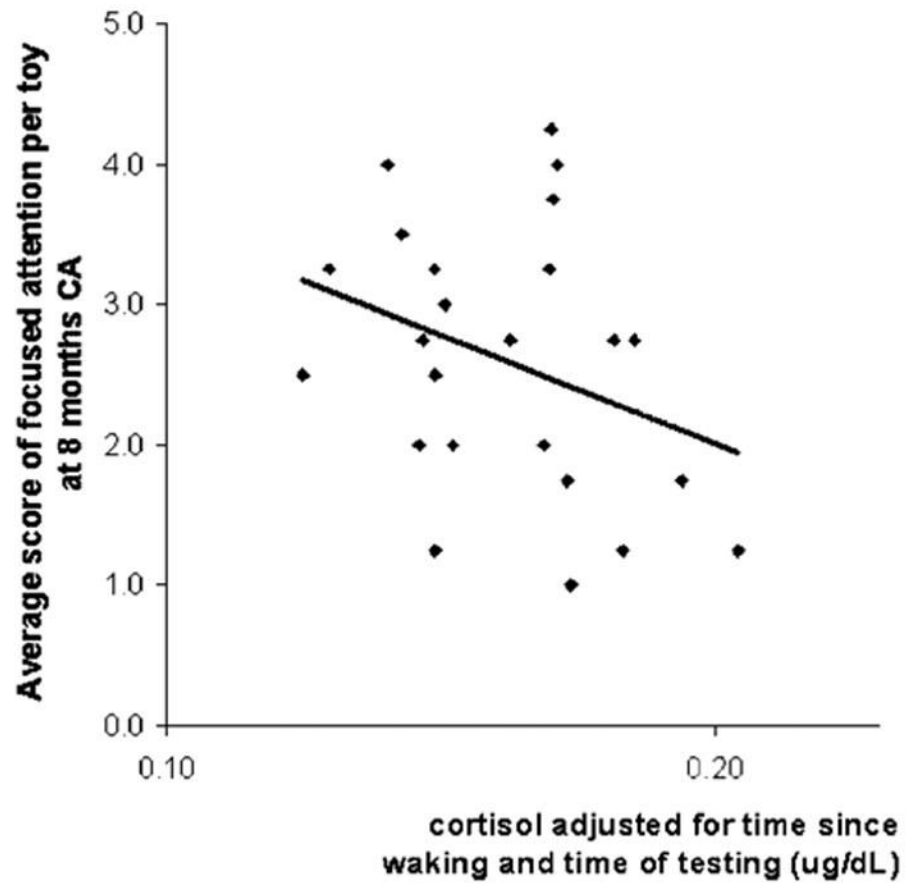


FIGURE 4. Relationship between basal cortisol (adjusted for time since waking and time of testing) and the average score of focused attention per toy in 8-month-old CA preterm infants of mothers who reported high parenting stress and overwhelming interactive behaviors.

Table 1
Demographic Characteristics (Mean \pm SD)

	Preterm	Full-Term	<i>p</i> -Value
Birthweight (g)			
LOW parenting stress	1,369.8 (445.1)	3,590.8 (590.1)	.389
HIGH parenting stress	1,205.3 (503.7)	3,599.2 (533.9)	
Overall	1,282.7 (481.4)	3,594.9 (555.6)	.001 ^a
Gestational age (weeks)			
LOW parenting stress	29.6 (2.6)	40.1 (1.0)	.528
HIGH parenting stress	29.0 (2.7)	40.1 (1.0)	
Overall	29.3 (2.7)	40.1 (1.0)	.001 ^a
Number of skin-breaking procedures			
LOW parenting stress	110.5 (99.2)	NA	.455
HIGH parenting stress	129.0 (92.2)	NA	
Overall	120.1 (95.5)	NA	NA
Illness severity (SNAP-II Day 1)			
LOW parenting stress	9.2 (10.3)	NA	.025 ^b
HIGH parenting stress	15.8 (11.9)	NA	
Overall	12.6 (11.5)	NA	NA
Days of mechanical ventilation			
LOW parenting stress	10.3 (16.7)	NA	.291
HIGH parenting stress	15.1 (21.2)	NA	
Overall	12.8 (19.2)	NA	NA
Days on postnatal glucocorticoids			
LOW parenting stress (<i>n</i> = 1)	11.0 (.0)	NA	.06
HIGH parenting stress (<i>n</i> = 5)	3.6 (2.5)	NA	
Overall (<i>n</i> = 6)	4.8 (3.8)	NA	NA
i.v. Morphine (mg/kg \times days)			
LOW parenting stress	.4 (1.1)	NA	.006 ^b
HIGH parenting stress	3.1 (6.3)	NA	
Overall	1.8 (4.8)	NA	NA
Total PSI score			
LOW parenting stress	194.1 (11.9)	179.7 (19.2)	.001 ^b
HIGH parenting stress	242.6 (23.8)	229.3 (12.0)	
Overall	219.7 (30.9)	203.9 (29.7)	.001 ^c
Child domain PSI score			
LOW parenting stress	84.8 (10.0)	77.4 (11.5)	<.001 ^b
HIGH parenting stress	108.3 (13.6)	101.7 (9.8)	
Overall	97.2 (16.8)	89.2 (16.2)	.007 ^c
Parent Domain PSI score			
LOW parenting stress	109.3 (10.5)	103.3 (9.74)	<.001 ^b
HIGH parenting stress	134.3 (15.2)	127.7 (11.8)	
Overall	122.5 (18.1)	115.2 (16.3)	.013 ^c
Maternal behavior			
LOW parenting stress	.2 (.9)	-.2 (1.0)	.505
HIGH parenting stress	.03 (1.1)	-.3 (1.0)	
Overall	.1 (1.0)	-.2 (1.0)	.109
Maternal age (years)			
LOW parenting stress	34.0 (4.4)	35.1 (4.5)	.032 ^d
HIGH parenting stress	31.1 (6.0)	33.7 (3.4)	
Overall	32.5 (5.5)	34.4 (4.0)	.064 ^a
Maternal education (years)			
LOW parenting stress	15.9 (2.8)	17.9 (3.20)	.006 ^d
HIGH parenting stress	14.3 (2.7)	16.5 (2.7)	
Overall	15.0 (3.0)	17.2 (3.0)	<.001 ^a
Attention			
LOW parenting stress	9.9 (3.3)	11.8 (3.2)	.426
HIGH parenting stress	10.6 (3.7)	9.8 (3.5)	
Overall	10.3 (3.5)	10.8 (3.5)	.398
Basal cortisol concentrations (mg/dL)			
LOW parenting stress	.2 (.2)	.3 (.3)	.258
HIGH parenting stress	.2 (.2)	.2 (.1)	
Overall	.2 (.2)	.2 (.2)	.722
Time of testing (min since midnight)			
LOW parenting stress	641.3 (86.0)	637.6 (105.3)	.820
HIGH parenting stress	624.3 (103.8)	658.8 (113.7)	
Overall	632.3 (95.7)	647.9 (108.5)	.550
Time since last wake (min since midnight)			
LOW parenting stress	115.1 (134.6)	102.4 (59.9)	.280
HIGH parenting stress	105.2 (116.0)	69.0 (49.7)	
Overall	109.8 (124.4)	86.2 (57.0)	.297

Data are presented for preterm and full-term infants in the LOW and HIGH parenting stress groups, and overall. There were $n = 40$ PRETERM-LOW, $n = 44$ PRETERM-HIGH, $n = 19$ FULL-TERM LOW, and $n = 18$ FULL-TERM HIGH mother–infants dyads, unless specified otherwise. p -values reflect differences between low and high stress groups (first row) and between preterm and full-term infants (second row). No interaction effects were observed.

^aPreterm < full-term.

^bLOW parenting stress < HIGH parenting stress.

^cPreterm > full-term.

^dLOW parenting stress > HIGH parenting stress.