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MATERNAL EMOTIONAL AVAILABILITY AT BEDTIME, QUALITY OF CO-PARENTING, INFANT TEMPERAMENT, AND INFANT DIURNAL CORTISOL AT 1 AND 3 MONTHS

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ABSTRACT

Emerging evidence within both the animal and human literatures suggests that cortisol patterning in early infancy is influenced by early experience. Specifically, recent work with human infants suggests that infant cortisol patterning is sensitive to quality of caregiving (Grant et al., 2009; Albers et al., 2008; Blair et al., 2008; Kaplan et al., 2008), as well as to environmental contextual factors related to family adversity (Saridjan et al., 2010). This study sought to examine diurnal cortisol in early infancy, as predicted by maternal emotional availability (EA) at bedtime and quality of co-parenting, a measure of the family environment. Furthermore, because infant temperament has also been associated with infant diurnal cortisol and cortisol response to stress (Kartes et al., 2009; Talge, Donzella, & Gunnar, 2008; Schmidt et al. 1997), this study sought to examine how individual differences in temperament at 3 months of age influence the development of the diurnal rhythm, as well as how infant temperament interacts with quality of caregiving and the co-parenting environment to influence the development of the diurnal rhythm. Lastly, this study sought to examine whether infant sleep quality mediates the associations of maternal EA, co-parenting quality, and infant temperament with infant diurnal cortisol. The results indicated that maternal EA, co-parenting quality, and temperament are associated with diurnal cortisol in very young infants. At 1 month, infant diurnal cortisol patterning was related to negative co-parenting in the family environment, and at 3 months it was linked to maternal EA and infant negative affectivity. In addition, maternal EA at 1 month predicted infant diurnal cortisol at 3 months. In multiple regression analyses examining the unique associations of maternal EA, co-parenting quality, and infant temperament with infant diurnal cortisol, maternal EA emerged as the most important factor influencing the development of infant diurnal cortisol patterning. The interactions of infant temperament with maternal EA and co-parenting quality were not significant, and the relationships between co-parenting quality and infant cortisol, maternal EA and infant cortisol, and infant temperament and infant cortisol were not mediated by the quality of infant sleep.

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Introduction

Emerging evidence within both the animal and human literatures suggests that cortisol patterning is influenced by early experience. Rodent models have shown that maternal caregiving behavior shapes rat pup stress reactivity, such that pups receiving less licking and grooming care from their mothers tend to show profiles of increased cortisol reactivity in response to stress (Caldji et al., 1998; Liu & Meaney, 1997). Additionally, Hofer (2006) has theorized on the basis of rodent models that "hidden regulators" of offspring functioning and development are embedded within maternal care. These hidden regulators represent individual components of maternal caregiving behavior that are associated with highly specific aspects of rat pup behavior and physiology. Furthermore, recent work with human infants suggests that infant cortisol patterning is sensitive to quality of caregiving (Grant et al., 2009; Albers, Riksen-Walraven, Sweep, & de Weerth, 2008; Blair et al., 2008; Kaplan, Evans, Monk, 2008), as well as to environmental contextual factors related to family adversity (Saridjan et al., 2010). This study sought to examine diurnal cortisol in early infancy, as predicted by quality of maternal care at bedtime and quality of co-parenting, a measure of the family environment. Furthermore, because infant temperament has also been associated with infant diurnal cortisol and cortisol response to stress (Kartes et al., 2009; Talge, Donzella, & Gunnar, 2008; Schmidt et al. 1997), this study sought to examine how individual differences in temperament at 3 months of age influence the development of a diurnal rhythm in cortisol, as well as how infant temperament interacts with quality of caregiving and the co-parenting environment to shape the development of a diurnal rhythm. Lastly, because a typical diurnal rhythm in cortisol is associated with infant sleep (de Weerth, Zijl, & Buitelaar, 2003; Gunnar & Donzella, 2002; White, Gunnar, Larson, Donzella, & Barr, 2000; Spangler, 1990), sleep quality was explored as a mediator of the associations between quality of maternal care, co-parenting quality, infant temperament, and infant diurnal cortisol.

Infant cortisol secretion, stress reactivity, and mental health

Cortisol, the predominant glucocorticoid produced by humans, is secreted by the adrenal glands as the final product of the hypothalamus-pituitary-adrenal (HPA) axis (Gunnar, Porter, Wolf, Rigatuso, & Larson, 1995). In typical individuals, cortisol secretion follows a particular pattern across the day. This diurnal rhythm consists of a spike in cortisol approximately 30 minutes after awakening in the morning, called the cortisol awakening response (CAR), followed by decreasing levels across the day and a slight rise throughout the night to moderate levels by morning (de Weerth, et al., 2003). Thus, cortisol levels are generally at their lowest at bedtime (Gunnar & Cheatham, 2003) and increase across the early morning hours. Studies cite varying estimates for when infants first establish a diurnal cortisol rhythm, but generally suggest that most infants show evidence of the typical diurnal pattern within the first 3 months of life (de Weerth et al., 2003). The emergence of the diurnal rhythm appears to be linked to the establishment of consolidated sleep (de Weerth et al., 2003; Gunnar & Donzella, 2002; White et al., 2000; Spangler, 1990). There is substantial interindividual variability in the timing of the emergence of this rhythm, as well as in its stability once established in infancy (de Weerth et al., 2003; Spangler, 1990). De Weerth et al. (2003) found the presence of a cortisol rhythm to appear and disappear across monthly samples in a subset of infants assessed from 2 to 5 months of age. An increase in cortisol secretion is also expected in response to stress. Increased cortisol mobilizes resources within the body in order to cope with a stressor. An increase in cortisol is expected within approximately 25 minutes following exposure to a stressor, and a subsequent decline back to baseline is expected within about 40 minutes. Individuals vary, however, in the amount of time it takes for this cortisol response to mount and then return back to baseline. Although an increase in cortisol is adaptive in the short-term in order to cope with stressful experiences, chronic cortisol elevation may confer vulnerability for the development of negative health and psychological outcomes, including both internalizing and externalizing problems. For example, Scher and colleagues found evidence suggesting that toddlers with greater CAR's relative to typical children show higher incidence of teacher-rated internalizing behaviors (Scher, Hall, Zaidman-Zait, & Weinberg, 2010). Thus, although a spike in cortisol is anticipated following wake-up, an exaggerated response may be indicative of behavioral or emotional dysregulation. This deviation from the typical cortisol pattern in young children is associated with increased risk for depression as they mature (Dougherty et al., 2009). Furthermore, a flattened diurnal cortisol rhythm, in which cortisol does not substantially decline across the day, has been associated with disorganized attachment in 14 month old children (Luijk et al., 2010). As disorganization in attachment has been linked to later behavior problems in childhood (Pauli-Pott & Haverkock, 2007), this association further suggests that diurnal cortisol patterning may be related to behavioral regulation. It is argued, therefore, that the establishment of a diurnal cortisol rhythm early in infancy could help to lay the foundation for behavioral and emotional regulation in childhood, and therefore decreased risk for problem behaviors. The current study seeks to examine parenting and infant temperamental factors that may contribute to the establishment of a diurnal cortisol rhythm early in infancy.

Early caregiving quality and the developing HPA axis

Work with rodent models has demonstrated that the developing HPA axis is influenced by maternal caregiving behaviors. For example, Meaney and his colleagues (1997) found evidence suggesting that naturally occurring variations in maternal caregiving behavior shape corticosterone response to stress in rat offspring. Corticosterone is the dominant glucocorticoid produced by rats, and therefore is analogous to cortisol in humans. Specifically, Liu and Meaney (1997) showed that adult rats who received high quality higher levels of maternal licking and grooming and arch-backed nursing (LG/ABN) showed reduced corticosterone responses to a stressor when compared with offspring of mothers whose caregiving was characterized by low levels of LG/ABN. Furthermore, Meaney and colleagues demonstrated that the magnitude of corticosterone response has behavioral correlates. In a separate series of experiments, they showed that rats who received low levels of LG/ABN showed signs of increased stress reactivity when compared to rodents who received high quality caregiving (Caldji et al., 1998). Increased stress reactivity manifested as increased startle responses, decreased open field exploration, and longer latency to eat food provided in a novel environment. Together, these studies indicate that stress reactivity at both the physiological and behavioral levels is shaped by the environment.

The mechanism by which early caregiving behaviors may shape the HPA axis has yet to be fully elucidated. Hofer's (2006) conceptualization of "hidden regulators," however, offers further refinement of the relationship between caregiving and the development of offspring physiology. In a series of experiments, Hofer and his colleagues separated rat pups from the dam, then introduced aspects of maternal care (i.e. warmth, food) individually to the pups, in order to isolate the rat's physiological response to each component of care. They demonstrated that specific maternal caregiving behaviors had corresponding, specific physiological and behavioral correlates in the offspring. Provision of warmth to separated pups, for example, prevented the decline in general activity level seen in separated pups that did not receive warmth. Additionally, provision of tactile stimulation prevented behavioral reactivity to novelty, and continuous infusion of milk prevented a 40% reduction in heart rate. Based on these results, Hofer suggested that highly specific "hidden regulators" of offspring behavior and physiology are embedded within maternal care. These individual components of maternal care appear to influence offspring development via separate mechanisms.

These results with rat models suggest that physiological and behavioral functioning later in life may be shaped in part by early experiences. For human infants, most early experiences are in the context of the family environment. Thus, the current study sought to explore how early caregiving and experiences within the family environment shape diurnal cortisol patterning in human infants.

Theoretical accounts of early caregiving quality

Theory supports the concept that early caregiving quality plays an important role in the development of early regulatory capacities. Greenspan (1981), for example, speculated that parents help their young infants to achieve homeostasis. He defined homeostasis as an optimal level of arousal in which infants are able to take in information from their environment. Infants maintain this state by engaging with their environment when understimulated and disengaging when overstimulated. Greenspan argued that very young infants rely on their parents to regulate stimulation from their surroundings, and that predictable routines and physical comfort in particular are important for helping infants to maintain equilibrium.

Changes in infant cortisol may underlie maintenance of homeostasis. By regulating stimulation from the environment, parents may decrease the amount of stressors their infants experience that trigger a cortisol response. High quality caregiving may also help infants return back to baseline levels of cortisol more quickly following stressful experiences. In terms of physiology, longer time spent in an equilibrium state may manifest as fewer, and less prolonged, elevations in cortisol. In a similar vein, high quality care that buffers infants from excessive stimulation may lay the foundation for early homeostatic rhythms to emerge, such as consolidated sleep-wake states and the diurnal cortisol rhythm. Over the long term, the ability to integrate stimulation across sensory modalities is critical for infants' understanding of their environment, and infants who maintain an equilibrium state are better able to integrate information from multiple sources (Hofer, 2006).

Early caregiving quality and infant stress reactivity and regulation

Consistent with theory and studies of stress reactivity in rodents, human infant cortisol reactivity and regulation appear to be influenced by early maternal caregiving experiences. Under conditions of low quality caregiving, infants tend to develop a profile of cortisol responses to stress that deviates from the typical pattern shown by their peers who receive high quality caregiving. In particular, early caregiving quality appears to be related to infant cortisol regulation following exposure to a stressor. In newborn infants, bathing has been shown to trigger an increase in cortisol secretion (Albers et al., 2008). Albers and colleagues implicated the challenges associated with thermoregulation as the cause of this response. They found that a bath evoked a cortisol increase in 3 month old infants 25 minutes after removal from bath water, and that individual differences in cortisol recovery 40 minutes after removal from the water were related to the quality of maternal behavior towards the infant across the entire bathtime. Specifically, lower quality maternal behavior, defined by lower levels of maternal sensitivity and cooperation, was associated with higher levels of infant cortisol at recovery. This suggests that infants who experienced less sensitive care during the bathtime were less able to regulate their cortisol response to the stress of removal from the bath. Reduced infant ability to recover may result from maternal caregiving that is stressful and that fails to buffer the infant sufficiently from stressful experiences within the bathtime routine. Similarly, Grant et al. (2009) found that 7 month old infants whose mothers were rated as less sensitive in a free play context showed a more prolonged cortisol recovery from the still-face paradigm. Furthermore, Blair and colleagues (2008) replicated these findings with a low-income, rural sample, as they showed that higher maternal engagement at 7 months was related to better infant cortisol recovery following emotional challenge.

The mechanisms by which maternal caregiving shapes human infant physiological functioning are not yet fully understood. It may be that the dimensions of maternal caregiving quality that are relevant to infant physiological functioning depend on context (Hane & Philbrook, in press). For example, Albers and colleagues found maternal sensitivity and cooperation to be particularly relevant to infant cortisol regulation within the context of bathtime. The most relevant dimensions of maternal caregiving to infant physiology may also be related to infant temperamental characteristics. The present study explored this idea by examining the interactions between quality of care in the context of putting the infant to sleep at night and infant temperament.

Early caregiving quality and infant diurnal cortisol

As mentioned above, one principal aim of the present study was to examine linkages between qualitative dimensions of maternal behavior at bedtime and the establishment of a diurnal cortisol rhythm in infants. A large body of literature has addressed the association between maternal sensitivity and diurnal cortisol patterning in older children in the context of maternal depression, but to our knowledge no studies have examined these associations in early infancy (e.g., 1 and 3 month old infants). For example, several studies have demonstrated that maternal depression is associated with elevated morning and baseline cortisol in children (Brennan et al., 2008; Dougherty, Klein, Olino, Dyson, & Rose, 2009; Kaplan, Evans, & Monk, 2007; Murray, Halligan, Goodyer, & Herbert, 2009). In a study examining sensitivity in mothers along a spectrum for depression, baseline cortisol was low regardless of sensitivity for infants of healthy mothers, but higher for infants receiving insensitive caregiving when their mothers were diagnosed with depression prior to the infant's birth (Kaplan et al., 2008). These results provide evidence of prenatal programming that moderates infants' responsivity to the environment, and suggest that high maternal sensitivity may buffer infants of depressed mothers from developing less adaptive patterns of cortisol across the day.

Additionally, early exposure to poorer quality caregiving may have lasting effects on cortisol patterning. In a study conducted by Murray and colleagues (2009), maternal withdrawal measured when children were 9 months old was found to be associated with elevated mean and morning cortisol when the children were adolescents. These studies suggest that early exposure to low quality caregiving is associated with increased child cortisol at several different points across the day. Child cortisol may increase not only because less sensitive mothers inadequately buffer their children from stressful events, but also because their caregiving itself is stressful.

In addition to influencing cortisol level at different points across the day, early caregiving quality may also influence the overall shape of the diurnal rhythm. For example, a recent study conducted by Luijk and colleagues (2010) suggests an association between attachment security and diurnal cortisol in 14 month old infants. The authors found infants classified as disorganized in the Strange Situation procedure to show a flattened pattern in cortisol, meaning that cortisol did not show as great a decline across the day for these infants as for infants who were not classified as disorganized. Because maternal sensitivity is theorized to be the primary determinant of attachment security in infancy (Ainsworth, Blehar, Waters, & Wall, 1978), this study further suggests that infants receiving lower quality caregiving are less likely to show the typical pattern in diurnal cortisol than their peers. Similarly, Fisher and colleagues found maternal emotional support of their preschool aged children during a challenge task to be related to the shape of the diurnal cortisol rhythm, such that children of less supportive mothers showed less of a decrease in cortisol across the day (Fisher et al., 2007). Together, these studies suggest that caregiving that is stressful or that inadequately buffers children from stressful experiences is associated with deviations from the typical diurnal rhythm. The lack of a decline across the day found in both studies may be attributable to consistent elevations in cortisol.

Family ecology and infant cortisol

In addition to quality of bedtime parenting, the present study also examined quality of co-parenting as a predictor of infant diurnal cortisol. Belsky's ecological theory of parenting (1984) proposes that parenting is influenced by a variety of social and contextual forces, including an individual's spousal support, social network, and work environment. Higher quality parenting is generally associated with greater sources of support and fewer stressors. Ecological theory also extends to children's development. Bronfenbrenner (1986) proposed that children are embedded within multiple systems of influence, some of which involve them only indirectly. Thus, children's outcomes are not only related to their relationships with each parent, but also to the relationship quality between parents themselves.

As a result of these varied contextual forces, family environments may differ in the degree to which they influence infant diurnal cortisol. Family contexts characterized by more support and less stress and conflict likely better promote the emergence of the typical diurnal pattern. Stressful family environments may decrease parents' capacity to respond sensitively to their infants, in addition to increasing the demand for infants to regulate arousal resulting from parent conflict and stress (Moore, 2010). Thus, the effect of the family environment may have both an indirect influence, via parental sensitivity, as well as a direct impact on infant cortisol levels. Recent work focusing on early family adversity supports these theoretical frameworks, though very few studies have examined linkages between ecological factors and cortisol levels in infants. Saridjan and colleagues found that infants from low income families showed higher cortisol levels across the day, as compared to infants from high income families (Saridjan et al., 2010). These infants also had a greater CAR than their counterparts in high income families. In addition, infants from families in which mothers reported higher degrees of parenting stress also showed higher daily cortisol levels. These results suggest that distal factors related to the parenting environment may be associated with infant diurnal cortisol, and furthermore, that family environments may vary in the degree to which they are supportive of infant diurnal cortisol patterning.

Co-parenting and infant cortisol

The present study examined the contextual influence of the couple relationship on infant cortisol. Specifically, parents' self-reports of co-parenting, or the ways in which partners coordinate their roles as parents, was assessed (Feinberg, 2002). Co-parenting can be understood as an element of support in Belsky's model of the determinants of parenting functioning. As a measure of marital functioning, quality of co-parenting is conceptually more proximal to parenting quality than general marital adjustment. Recent work has highlighted links between the co-parenting relationship and infant attachment security (Brown, Schoppe-Sullivan, Mangelsdorf, & Neff, 2010), as well as child behavior problems (Schoppe, Weldon, Cook, Davis, & Buckley 2009). In particular, Schoppe and colleagues (2009) demonstrated that supportive co-parenting may be related to children's behavioral regulation, as they found co-parenting to moderate the relation between effortful control at four years old and externalizing behavior one year later.

Similarly, Karreman and colleagues detected a concurrent association between co-parenting and effortful control in 3 year old children, over and above the associations between effortful control and individual parenting (Karreman, van Tuijl, van Aken, & Deković, 2008). These studies suggest that co-parenting may be particularly relevant to regulatory processes. Additionally, an intervention targeting co-parenting across the transition to parenthood was associated with increased infant behavioral regulation across the infant's first year (Feinberg, Kan, & Goslin, 2009; Feinberg & Kan, 2008). Thus, through coordinated efforts, parents may create an environment that supports the typical diurnal pattern of cortisol in the infant, particularly in the first few months of life. To our knowledge, no study to-date has examined how co-parenting may be associated with infant physiology.

Infant temperament, differential susceptibility, and cortisol

Although the present study was primarily concerned with caregiving and environmental influences on infant physiology, there may also be infant characteristics associated with the establishment of the diurnal cortisol rhythm. Previous work has addressed linkages between young children's cortisol and temperament. For example, Talge and colleagues found that greater cortisol reactivity was associated with parent report of fearful temperament in preschoolers (Talge, Donzella, & Gunnar, 2008), and Schmidt et al. (1997) found evidence suggesting that 4 year olds who were rated as wary in peer play tended to have higher morning cortisol and were behaviorally inhibited at 14 months old. Further extending these findings, Buss and colleagues (2003) observed that high basal cortisol and cortisol reactivity in 6 month old infants was associated with right frontal EEG asymmetry, which is related to fearful and withdrawal behavior. Rosen and Schulkin (1998) theorized that a temperamental inclination to respond with fear to novel or threatening situations results in larger and prolonged cortisol responses to these events, which in turn perpetuates fearful responding to these situations in the future. Extending the results of these studies to the current study, more fearful infants may also show less of a marked diurnal rhythm in cortisol in early infancy.

Infant temperament may also interact with quality of care and co-parenting quality to affect the development of the diurnal cortisol rhythm. In his differential susceptibility hypothesis, Belsky (2005) theorized that infants characterized by greater negative affectivity, including more frequent fear, sadness, distress, and negative mood, are especially sensitive to environmental influences. This temperament style, he suggested, is associated with greater reactivity to the environment in a "for better or for worse manner." Thus, he posited that infants who are characterized by greater negative affectivity tend to function exceptionally well in high quality environments, and especially poorly in low quality environments. This hypothesis is conceptually similar to Boyce and Ellis' (2005) biological sensitivity to context theory, which suggests that greater biological reactivity to stress is associated with higher sensitivity to both risk-promoting and development-enhancing environments. For infants and children, the environment mainly consists of the parents and family. Thus, numerous studies have found that young children characterized by difficult temperament, including those who are prone to distress and frustration, fearful, and less easily soothed, are particularly sensitive to quality of parenting (Poehlmann et al., 2011; Pluess & Belsky, 2010; Yaman, Mesman, van IJzendoorn, & Bakersman-Kranenburg, 2010; Bradley & Corwyn, 2008; Kochanska, Aksan, & Joy, 2007; van Aken, Junger, Verhoeven, van Aken, & Dekovic, 2007). For example, Bradley and Corwyn (2008) found that infant temperament moderated the relationship between parenting quality and teacher-rated externalizing problems in first grade, such that children who were rated as more difficult by their mothers as infants were particularly sensitive to the effects of high quality parenting. Difficult infants had more externalizing problems as first graders when they experienced low quality parenting, but fewer problems when they experienced high quality parenting. Furthermore, previous research has demonstrated that young children's cortisol reactivity is associated with both parenting quality and temperament. Kertes et al. (2009) found that higher parenting quality was related to lower cortisol elevations in response to lab stressors in extremely socially inhibited, but not nonsocially inhibited children. Social inhibition manifests as fear or shyness in social situations involving other people, whereas nonsocial inhibition is fear in situations involving novel objects or experiences. These results suggest that cortisol in young children is influenced by both temperamental characteristics (i.e. type of inhibition) and quality of parenting.

Applying the results of these studies to the current study, young infants characterized by high negative affectivity may be particularly responsive to both high and low quality parenting. Infants characterized by high degrees of negative affect who receive high quality care may be more likely to establish the typical diurnal rhythm in the first 3 months, whereas infants receiving low quality care may be less likely to show the typical pattern.

The mediational role of infant sleep quality

One indirect avenue by which quality of caregiving and co-parenting may influence infant cortisol is via sleep quality. A recent study conducted by Teti and colleagues found that infants of mothers who were rated as more emotionally available at bedtime slept better across the night, as indicated by fewer awakenings reported by parents and objectively measured via actigraphy (Teti, Kim, Mayer, & Countermine, 2010). Emotional availability (EA), also used to assess quality of care in the present study, encompasses a parent's sensitivity, structuring, nonintrusiveness, and nonhostility toward their child. Thus, EA incorporates a dimension of maternal sensitivity and is concerned with the quality of care, rather than the quantity of specific caregiving behaviors.

Furthermore, consolidated sleep is associated with a typical diurnal rhythm in cortisol. De Weerth and colleagues (2003) found that 2-5 month old infants who slept through the night showed cortisol patterning more typical of a diurnal rhythm than children who did not sleep through the night, where consolidated sleep was defined as six or more hours of consecutive sleep following the last feeding of the day. In addition, White et al. (2000) found that infants with disrupted sleep at night showed a flattened rhythm in cortisol production, such that cortisol levels showed less decline across the day. Infants with a marked rhythm, however, slept less during the daytime hours and spend more of this time in awake, non-fussy states. Infants with better nighttime sleep quality, therefore, tend to show a more typical cortisol rhythm. Thus, sensitive parenting and co-parenting that helps infants to fall asleep and stay asleep may be directly associated with infant cortisol, as well as indirectly, by way of infant sleep quality.

Sleep quality may also be related to infant temperament. Specifically, infants and young children described as having a more difficult temperament, such as those who are characterized by increased negative mood and irritability, tend to sleep less and to wake up more frequently. For example, Shaefer (1990) found that young children ranging from 6 months to 3 years of age characterized by more frequent night waking tended to be rated as more difficult by their parents. Weissbluth (1983) also found that infants described as more difficult slept less often during both the day and night, and Halpern et al. showed that infants who spent more time awake during the night at 3 weeks were rated as more irritable at 3 months old (Halpern, Anders, Garcia Coll, & Hua, 1994). Lastly, Spruyt et al. found that increased sleep was related to an easy infant temperament across the first year of life, where easy temperament was defined as high levels of approachability, rhythmicity, and adaptability, as well as low levels of distractibility (Spruyt, Aitken, So, Charlton, Adamson, & Horne, 2008). Thus, sleep quality may also mediate the relationship between temperament and infant cortisol, such that infants who are described as more difficult, including those characterized by more negative affectivity, tend to sleep more poorly, which in turn may be related to a delay in the establishment of a diurnal cortisol rhythm.

The present study

The present study examined the associations between proximal (maternal EA) and distal (quality of co-parenting) indicators of parenting quality, temperament, and infant diurnal cortisol at 1 and 3 months. Additionally, it examined how maternal EA and co-parenting quality interact with temperament to influence infant diurnal cortisol. Furthermore, it explored the extent to which infant sleep quality may mediate the relationships between maternal EA and infant cortisol, co-parenting quality and cortisol, and infant temperament and cortisol.

This study contributes to the existing literature concerning parenting, temperament, and infant cortisol in several ways. First, it examined parenting predictors of infant cortisol patterning in the first 3 months of life, when the diurnal cortisol rhythm is becoming established. Additionally, this study examined maternal EA in a unique context- bedtime. Because for many infants nighttime constitutes the longest separation of the day from their parents, it may be particularly stressful to them (Teti et al., 2010). By examining EA preceding this separation, and infant cortisol in the preceding and ensuing hours, this study seeks to assess the concurrent relationship between parenting quality and infant cortisol. To our knowledge, this study represents the first examination of the associations of maternal EA at bedtime and infant temperament with infant cortisol. Lastly, this study represents the first examination of the association between co-parenting quality and infant physiology.

Hypotheses

The following hypotheses were proposed:

Hypothesis 1: Infants of mothers who were rated as more emotionally available at bedtime were predicted to show greater evidence of a diurnal cortisol rhythm than infants of mothers who were rated as less emotionally available in this context. Thus, greater maternal EA was expected to be associated with a diurnal rhythm in cortisol when the infants were 1 and 3 months old. This is consistent with previous work relating sensitive parenting to optimal cortisol patterning in childhood (Grant et al., 2009; Albers et al., 2008; Blair et al., 2008; Kaplan et al., 2008).

Hypothesis 2: It was anticipated that infants whose parents rated their co-parenting quality more highly would show greater evidence of a diurnal cortisol rhythm than infants of parents with poorer self-reported co-parenting scores. Thus, higher quality co-parenting was expected to be associated with a diurnal rhythm in cortisol when the infants were 1 and 3 months old. Recent work highlighting the associations between higher quality co-parenting and better infant adjustment and child behavioral regulation (Brown et al., 2010; Schoppe et al., 2009; Feinberg et al., 2009; Feinberg & Kan, 2008) supports this hypothesis.

Hypothesis 3: It was hypothesized that infants whose mothers rated them as low in negative affectivity would show greater evidence of a diurnal cortisol rhythm than infants rated higher for negative affectivity by their mothers. Thus, lower negative affectivity was expected to be associated with a diurnal rhythm in cortisol when the infants were 3 months old. Temperament data was not available at 1 month. Previous studies examining the link between temperament and cortisol in young children have found that a fearful or inhibited behavioral style is associated with greater cortisol reactivity to stress and higher morning cortisol (Talge et al., 2008; Buss et al., 2003; Schmidt et al., 1997).

Hypothesis 4: Furthermore, consistent with work demonstrating that both parenting quality and temperament contribute to an individual child's cortisol reactivity (Kertes et al., 2009) and Belsky's differential susceptibility hypothesis (2005), it was hypothesized that infants who were rated as higher in negative affectivity by their mothers at 3 months would be sensitive to maternal EA and co-parenting quality in a for better or for worse manner, such that infants characterized by high levels of negative affectivity who experienced low EA and co-parenting quality would be less likely to show a diurnal rhythm in cortisol at 3 months, and infants exposed to high EA and co-parenting quality would be more likely to show the typical pattern.

Hypothesis 5: If maternal EA was found to significantly predict infant diurnal cortisol rhythm, infant sleep quality would be explored as a potential mediator of this relationship. It was anticipated that greater maternal EA would be related to better infant sleep quality, which in turn was expected to be associated with a diurnal rhythm in cortisol when the infants were 1 and 3 months old. This is consistent with previous work suggesting an association between maternal EA at bedtime and infant sleep quality (Teti et al., 2010), and between infant sleep quality and infant diurnal cortisol (Scher et al., 2010; White et al., 2000). If the differential susceptibility hypothesis was supported when infants were 3 months, the mediational model would be conducted only for infants with the highest scores for negative affectivity. This is because the differential susceptibility hypothesis would suggest that the infants characterized by negative affectivity were driving the effects of maternal EA on diurnal cortisol, whereas less negative infants were not as sensitive to the effects of quality of care.

Hypothesis 6: If co-parenting quality was found to significantly predict infant diurnal cortisol rhythm, infant sleep quality would also be explored as a potential mediator of this association. It was anticipated that higher co-parenting quality would be associated with better infant sleep quality, which in turn was expected to be related to a diurnal rhythm in cortisol when the infants were 1 and 3 months old. Increased parental conflict resulting from lower co-parenting quality may increase infant arousal, making it more difficult for infants to fall asleep and stay asleep (Moore, 2010). Poorer sleep quality may then be associated with a less marked infant diurnal cortisol rhythm (Scher et al., 2010; White et al., 2000). Thus, co-parenting quality may be associated with infant sleep quality, which in turn is associated with infant diurnal cortisol. Here again, if evidence for differential susceptibility was found at 3 months, this mediational model would be conducted only for infants with the highest scores for negative affectivity.

Hypothesis 7: If infant temperament was found to significantly predict infant diurnal cortisol rhythm, infant sleep quality would be explored a potential mediator of this association. It was expected that lower negative affectivity would be associated with better infant sleep quality, which in turn was anticipated to be related to a diurnal rhythm in cortisol when the infants were 3 months old. There is some research suggesting that infants characterized by less negativity sleep better and for longer periods of time than infants who are rated as more negative (Spruyt et al., 2008; Halpern et al., 1994; Shaefer, 1990; Weissbluth, 1983).

Method

Participants

Participants in the study were part of Project SIESTA (Study of Infants' Emergent Sleep TrAjectories), a NIH-funded, ongoing, longitudinal study of parenting, infant sleep, and infant development (R01HD052809). Mothers were approached by project staff at local hospitals when their infants were born and given information regarding the study. Two weeks later, mothers who expressed interest in the study were called and recruited into the project. Informed consent was obtained from parents during the first visit to the home when their infant was 1 month old. Data for 160 one-month-old infants (48% male) and their families was available to be analyzed for this study. Their mean age was 1.21 months (*SD* = .16) at the time data collection began. Data for 147 of these infants were available at 3 months old. Their mean age at this time point was 3.10 months (*SD* = .31). Fifty-seven infants were firstborns, and 103 were later born. Composite maternal EA was available for 102 infants at 1 month old and 81 infants at 3 months old. EA could not be coded for the remaining sample because there was an insufficient amount of video-recorded mother-infant interaction (less than 1 minute). Complete cortisol data were available for 113 infants at 1 month, and 87 at 3 months. Complete co-parenting data were available for 144 mothers and fathers at 1 month, and 133 mothers and fathers at 3 months. Complete sleep quality data were available for 141 infants at 1 month, and 127 at 3 months. Temperament data were available for 138 infants at 3 months. Temperament was not assessed at 1 month.

When their infants were 1 month old, 82% of mothers were married and living with their infant's father. Mothers' average age was 29.5 years old (*SD* = 5.2), and they ranged in age from 18 to 43 years old. Fathers' average age was 32.2 years old (*SD* = 5.8), and they ranged in age from 21 to 49 years old. Eighty-three percent of mothers were White, 4% were Asian American, 4% were African American, 6% were Latino, and 4% identified themselves as "Other." Eighty-four percent of fathers were White, 4% were Asian American, 5% were Latino, 3% were African American, and 3% identified themselves as "Other." Eighty-seven percent of mothers completed some post-secondary education, and 63% were employed outside the home. Eighty-six percent of fathers completed some post-secondary education, and 95% were employed outside the home. Average yearly family income was $70,000 (*SD* = $48,000) and ranged from $1500 to $300,000.

Overall procedure

Families were visited at three time points across seven days of data collection at both 1 and 3 months of infant age. At the first visit, parents were given a variety of questionnaires to complete, including one measure regarding their co-parenting experience and 7 sleep diaries to record the times the infant spent sleeping across each of the days. At 3 months, mothers were also given a questionnaire to complete regarding their infant's temperament. In addition, parents were given an actiwatch for their infant to wear continuously across the seven days in order to measure activity across both the waking and sleeping hours. Infants wore the watch on their ankle. On the 6th day of data collection, project staff set up video and audio recording equipment within each family's home in order to capture parents' interactions with their infants at bedtime, as well as across the night. This same day, parents were given materials with which to collect and store their infant's saliva for cortisol assay. Project staff returned on the following day in order to collect the questionnaires, actiwatches, equipment, and samples.

Questionnaire measures

Quality of co-parenting

Both parents' ratings of the quality of their co-parenting were assessed at 1 and 3 months using the Co-parenting Relationship Scale questionnaire (CRS; Feinberg, Brown, & Kan, 2012). The CRS consists of thirty-five items that tap into seven dimensions. These dimensions encompass each parent's rating of agreement, closeness, support and cooperation, division of labor, and competition and undermining in their relationship with their partner within the context of parenting, as well as the extent to which their child is exposed to conflict between them and their endorsement of their partner's parenting. Twenty-seven of the items require parents to rate the degree to which a particular statement describes their parenting relationship with their partner on a 7-point Likert-type scale (i.e. My partner appreciates how hard I work at being a good parent; 1 = Not true of us, 7 = Very true of us). Eight of the items concerning their children's exposure to conflict ask parents to indicate the number of times within a typical week their child is present for both positive and negative exchanges between them (i.e. Say something positive about or praise your partner to this child; 1 = Never, 7 = Very Often). Internal consistency for the CRS has been found to be high, with an average Cronbach's alpha of .97 across a sample of mothers and fathers (M. Feinberg, personal communication, 2011). The factor loadings for each of the scales have been shown to be acceptable, with loadings ranging from .58-.91 across of sample of mothers and fathers assessed at two time points (M. Feinberg, personal communication, 2011).

Infant temperament

When their infants were 3 months old, mothers completed the Infant Behavior Questionnaire-Revised (IBQ-R; Gartstein & Rothbart, 2003), designed for use with infants between 3 and 12 months old. The IBQ-R consists of 191 items that tap into three broad dimensions: surgency/extraversion, negative affectivity, and orienting/regulation. For each item, mothers rate how often their infant displayed a particular behavior in the past week on a 7-point Likert-type scale (i.e. When face was washed, how often did the baby fuss or cry?; 1 = Never, 7 = Always). The present study was particularly concerned with negative affectivity, which encompasses sadness, distress to limitations, and fear. Internal consistency for the negative affectivity scale of the IBQ-R has been demonstrated to be high, with a Cronbach's alpha of .91, and the factor loadings are acceptable, ranging from .31-.79 (Gartstein & Rothbart, 2003).

Infant sleep quality

For each of the seven days of data collection, parents completed a sleep diary, adapted from Burnham et al. (2002), in which they indicated the times when their infants fell asleep and woke up during both the day and night. From this data, a summary score across the seven days was obtained that indicated average frequency of infant night waking.

Bedtime Videos

Video collection

Project staff set up video and audio recording equipment within each family's home on the 6th day of data collection at both 1 and 3 months, consulting with parents about the locations in the home where the infant was taken to prepare for bed, where the infant slept, and where the infant was taken upon awakening during the night. In most homes, 2-4 cameras were required to capture the majority of parent-infant interaction across bedtime and nighttime. Video and audio recordings were made by a Bosch Divar XF 8-Channel Digital Versatile Recorder. Video was captured using infrared security cameras made by ARM Electronics (Model No. C420BCVFIR), and audio information was collected via Channel Vision microphones (Model No. 5104-MIC). Parents and project staff were able to view the recordings on an Audiovox Portable LCD Monitor and DVD Player (Model No. D9000). If the infant's bedtime and nighttime routines took place across different rooms, up to two cameras were set up wirelessly. For all families, one camera was placed above the crib or bed where the infant slept. Up to three additional cameras were placed such that they provided a view of a chair where the infant was fed or rocked, the infant's changing table, or an overview of the room, depending on what the parents communicated to project staff as the infant's bedtime and nighttime routine. Cameras were positioned using boom stands, gorilla pods, or foam pieces such that they were as unobtrusive to the families as possible. All equipment was connected to one power strip that staff asked parents to turn on one hour prior to bedtime to ensure that all of the bedtime routine was captured on camera. Parents were asked to turn off the cameras in the morning, after their infant was awake and out of bed for the day.

Maternal emotional availability coding

Maternal emotional availability during the infant's bedtime was scored using the Emotional Availability Scales (EAS; Biringen, Robinson, & Emde, 1998). The EAS is designed to measure the emotional quality of parents' interactions with their children and consists of four scales: sensitivity, structuring, nonintrusiveness, and nonhostility. Two additional scales assessing children's emotional availability to the parent were not scored in this study because of the very young age of the infants. In previous studies, maternal EA in the context of interaction with infants has been shown to be negatively associated with maternal depression (Vliegen, Luyten, & Birigen, 2009) and positively associated with infant attachment (Ziv, Aviezer, Gini, Sagi, & Koren-Karie, 2000) and infant sleep quality (Teti et al., 2010). All coding of maternal EA was conducted by a reliable coder, who was certified on EAS 2007.

This present study was concerned with maternal EA at bedtime, rather than during night wakings, because the longest bouts of mother-infant interaction took place at this time. Similarly, this study concentrated on maternal rather than paternal EA because of the limited number of tapes with sufficient father-infant interaction at bedtime to code for EA. Coders coded bedtime from when the infant first appeared on camera until the end of five consecutive minutes of infant sleep, where sleep was determined by closed eyes and the absence of gross motor movement. Because EA is typically coded across parent-infant play contexts, maternal EA in this study was coded using the same adaptations to the bedtime context described by Teti et al., 2010. Briefly, highly sensitive mothers were rated as such when they showed awareness of infant cues, as well as an accurate interpretation and response to these signals. If mothers did not respond to distressed infant vocalizations following one minute of crying, they were scored lower for sensitivity. Mothers of infants who slept in a separate bedroom could still receive high scores for sensitivity if they did not respond to nondistressed infant vocalizations after their infants were placed in their cribs. Mothers received high scores for structuring when they used quiet, soothing routines that guided their infants toward sleep. Mothers scored highly on nonintrusiveness by keeping the volume of their voices low and quiet when talking to their infants or other family members, and reducing the number of initiated interactions with their infants as they came closer to sleep. Mothers were rated highly for nonhostility when they showed no overt or covert irritability or anger towards their infants during bedtime. Interrater reliability was established between two coders on the four EA dimensions, based on 12 tapes when infants were 1 and 3 months old. Intercorrelations (IC) for absolute agreement on maternal sensitivity, structuring, nonintrusiveness, and nonhostility were .94, .82, .99, and .96 respectively. A composite maternal EA score was created by converting the scores for each of the four dimensions to *z* scores, then summing the converted scores across the standardized dimensions. Internal reliability for composite EA was .99. Interrater reliability based on absolute agreement was .97.

Actigraphy

In addition to maternal report via infant sleep diaries, infant sleep quality was objectively assessed via actigraph recordings using the Mini-Mitter Actigraphy wristwatches (Model AW-64). Following data collection, the information from the watches was downloaded onto a laboratory computer by project staff. These recordings provide information about infant activity level. Using actiwatch software, infant activity was summarized across the night and attributed to either wakefulness or sleep. High activity was attributed to wakefulness, and low infant activity was attributed to sleep. Using the software, the number of infant wake bouts across nighttime was calculated, then averaged across the seven days to create a summary score of average frequency of infant night waking. Actigraphy has been shown to a valid measure of sleep and wakefulness in infants (So, Michael Adamson, & Horne, 2007).

Cortisol Collection and Assay

Cortisol sampling

Infant cortisol was assessed via saliva samples taken the same night as the bedtime video when infants were 1 and 3 months old. Parents were provided with four sets of filter paper to place on their infant's tongue for 2 minutes at four time points across the evening and following morning. The first sample was taken in the late afternoon, preceding dinnertime, the second sample was taken just before the infant fell asleep at bedtime, the third sample when the infant awoke in the morning, and the fourth sample 30 minutes following awakening. These times were selected in order to model the change in infant diurnal cortisol across the evening and nighttime hours. Parents placed each sample in labeled plastic tubes following collection and stored the tubes in their freezer. The tubes were transported in cooler bags and stored in a freezer in the laboratory until cortisol assay by project staff. The filter paper method has been shown to be a valid measure for collecting saliva and assessing cortisol levels in very young infants (Neu, Goldstein, Gao, & Laudenslager, 2007).

Cortisol assay

Two procedures were completed with the saliva tubes prior to assay. First, the tubes were removed from the laboratory freezer and placed in racks for 10-15 minutes at room temperature to thaw. Next, 1500 μL of diluted (10:1) buffer solution was added to each tube.

Tubes were then placed on a test tube rotator for 24 hours in order to extract the saliva samples from the filter paper. Following rotation, tubes were refrozen. On a separate day, the tubes were vortexed for 15 seconds in order to remix the cortisol and wash buffer solution. Next, 600 μL of each sample were pipetted into correspondingly labeled Eppendorf tubes. These Eppendorf tubes were placed in a SpeedVac machine for 4-10 hours in order to concentrate the samples.

On a separate day, the samples were assayed using the Salimetrics, LLC High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (catalog No. 1-3002). Prior to assay, samples were placed in racks at room temperature until thawed, then centrifuged for 15 minutes at 4500 rpm. Assays were carried out by project staff, following the procedures included with the kit.

Results

Preliminary analyses

In order to reduce data, the 5 positive co-parenting sub-scales (agreement, increased closeness, support and cooperation, endorsement of partner's parenting, division of labor) within the Co-parenting Relationship Scale were averaged to create a positive co-parenting dimension, and the 2 negative co-parenting subscales (child's exposure to conflict between parents, competition and undermining) were averaged to create a negative co-parenting dimension. Separate positive and negative co-parenting variables were created for mothers and fathers, and for 1 and 3 month data. Cronbach's alphas for these scales generally supported combining the data in this way; alphas ranged from .47 (maternal negative co-parenting, 1 month) to.84 (maternal positive co-parenting, 1 month), and the mean alpha was .67.

The infant cortisol values were positively skewed and therefore were log transformed prior to analysis. Three contrast variables were then created that represented the change in cortisol between each of the 4 time points: from T1 to T2 (afternoon cortisol change), T2 to T3 (overnight cortisol change), and T3 to T4 (morning cortisol change, or the CAR). Contrast variables were created separately for 1 and 3 month data. These were the primary dependent variables of interest in the current study. A decline in cortisol from late afternoon to bedtime, a gradual increase overnight, and sharp increase in the morning post-awakening were theorized to mark the presence of a diurnal rhythm in infant cortisol. Cortisol level at bedtime at both age points was also examined as a dependent variable because of the current study's focus on parenting quality at bedtime.

Correlations between predictor variables

At 1 month, maternal and paternal reports of co-parenting quality were moderately associated. The positive and negative co-parenting dimensions, as rated by both parents, were inversely associated. None of the co-parenting variables were related to maternal EA. These correlations are reported in Table 1.

At 3 months, maternal and paternal reports of co-parenting quality were moderately associated. The positive and negative co-parenting dimensions, as rated by both parents, were inversely associated. Infant negative affectivity, as reported by mothers via the IBQ-R, was negatively associated with paternal report of positive co-parenting and positively associated with maternal report of negative co-parenting. Additionally, maternal report of negative co-parenting was negatively associated with maternal EA. These correlations are reported in Table 2.

Covariates

Because family income has been shown to be related to infant cortisol (Saridjan et al., 2010), it was entered as a covariate in all analyses. Family income was reported by mothers via questionnaire when infants were 1 and 3 months old. One hundred forty eight mothers reported their family income at 1 month; 124 mothers reported family income at 3 months. Correlational analyses revealed that family income was positively associated with infant CAR at 1 month, $r(111) = .33$, $p < .001$. Family income was not significantly correlated with afternoon cortisol change, cortisol at bedtime, or overnight cortisol change at 1 month, or any of the cortisol variables at 3 months.

Primary Analyses

To test study hypotheses, partial correlation analyses were first run in order to examine the associations between maternal EA, co-parenting quality, infant temperament, and each of the cortisol variables, controlling for family income. At 1 month, maternal EA and co-parenting quality were correlated with the infant cortisol variables; at 3 months, maternal EA, co-parenting quality, and infant negative affectivity were correlated with the infant cortisol variables. Separate partial correlation analyses were run for each predictor variable. Analyses for the 1 month variables were conducted first, followed by the 3 month analyses. Furthermore, partial correlation analyses were subsequently conducted that examined the associations between maternal EA and the co-parenting quality variables at 1 month and the infant cortisol variables at 3 months.

Next, a set of regression analyses were conducted such that the predictor variables were entered into the same multiple regression models at each time point in order to examine each predictor's unique association with the cortisol variables, while controlling for the other predictors. The unique longitudinal associations between the predictor variables at 1 month and the cortisol variables at 3 months were also examined in a multiple regression model. Family income was entered into all the regressions as a covariate. Because there were no a priori predictions about the ordering of the predictor variables, they were entered simultaneously into the multiple regression models.

Maternal EA, co-parenting quality, and infant diurnal cortisol at 1 month

The first set of partial correlation analyses tested the first and second study hypotheses at 1 month. The first hypothesis predicted that infants of mothers who were rated as more emotionally available at bedtime would show greater evidence of a diurnal cortisol rhythm than infants of mothers who were rated as less emotionally available in this context. The second hypothesis predicted that infants of parents who rated their co-parenting quality more highly would show greater evidence of a diurnal cortisol rhythm than infants of parents who rated their co-parenting as lower quality. Thus, composite maternal EA as well as mothers' and fathers' ratings of co-parenting quality were associated with the cortisol change variables and infant cortisol at bedtime, controlling for family income. Separate partial correlation analyses were run for each predictor variable.

1 month maternal EA

At 1 month, maternal EA was not significantly associated with any of the infant cortisol variables.

1 month maternal co-parenting quality

At 1 month, maternal ratings of negative co-parenting were associated with infant CAR, such that a greater CAR was related to less negative co-parenting, $r(99) = -.22$, $p < .05$. Maternal ratings of positive co-parenting quality were not significantly associated with infant cortisol variables.

1 month paternal co-parenting quality

None of the paternal ratings of co-parenting quality were significantly associated with the infant cortisol variables at 1 month.

The 1 month partial correlation results are included in Table 3.

Maternal EA, co-parenting quality, infant temperament, and infant diurnal cortisol at 3 months

The next set of partial correlation analyses tested the first, second, and third study hypotheses at 3 months. The first and second hypotheses concerned the predicted associations of maternal EA and co-parenting quality with the infant cortisol variables. The third hypothesis predicted that infants who were rated as lower in negative affectivity by their mothers would show greater evidence of a diurnal cortisol rhythm than infants who were rated as higher in negative affectivity. Thus, composite maternal EA, mothers' and fathers' ratings of co-parenting quality, and infant negative affectivity were associated with the cortisol change variables and infant cortisol at bedtime, controlling for family income. Separate partial correlations were run for each predictor variable.

3 month maternal EA

At 3 months, maternal EA was significantly associated with infant afternoon cortisol change, such that greater EA was related to a greater decrease in infant afternoon cortisol, $r(44) = -.42$, $p < .01$. Greater maternal EA at bedtime was also associated with a greater increase in cortisol overnight, $r(44) = .32$, $p < .05$. Maternal EA was not significantly associated with infant cortisol at bedtime and infant CAR.

3 month maternal co-parenting quality

At 3 months, none of the maternal ratings of co-parenting quality were significantly associated with the infant cortisol variables.

3 month paternal co-parenting quality

None of the paternal ratings of co-parenting quality were significantly associated with the infant cortisol variables at 3 months.

3 month infant negative affectivity

Infant negative affectivity was significantly associated with infant afternoon cortisol change, such that infants rated lower in negative affectivity showed a greater decrease in afternoon cortisol, $r(67) = .32$, $p < .01$. Infant negative affectivity was not significantly associated with infant overnight cortisol change, infant cortisol at bedtime, or infant CAR at 3 months.

The 3 month partial correlation results can be found in Table 4.

Longitudinal Associations

Next, the longitudinal associations between maternal EA and the co-parenting quality variables at 1 month and the infant cortisol variables at 3 months were examined in separate partial correlation analyses.

Maternal EA longitudinal associations

Maternal EA at 1 month was not significantly associated with any of the infant cortisol variables at 3 months.

Maternal co-parenting quality longitudinal associations

Neither of the maternal co-parenting quality variables at 1 month were significantly associated with the infant cortisol variables at 3 months.

Paternal co-parenting quality longitudinal associations

Neither of the paternal co-parenting quality variables at 1 month were significantly associated with the infant cortisol variables at 3 months.

Maternal EA, co-parenting quality, and infant negative affectivity as simultaneous predictors

The previous analyses examined the associations between each predictor variable and the infant cortisol variables in separate partial correlation analyses controlling for family income. In the next set of analyses, the predictor variables were entered into the same simultaneous multiple regression models in order to examine each predictor's unique association with the cortisol variables, while controlling for the other predictors. Family income was entered as a covariate in all analyses. Because the results did not differ depending on whether they included maternal or paternal report of co-parenting, only maternal ratings of co-parenting quality were included in the models reported here.

1 month

At 1 month, the model predicting infant CAR, which included family income, maternal EA, and maternal ratings of positive and negative co-parenting, was significant, $R^2 = .15$, $p < .05$. Family income significantly predicted infant CAR, such that the CAR was greater for infants from families with higher incomes, $\beta = .38$, $t(65) = 3.15$, $p < .01$. None of the other predictors were significant. The models predicting infant afternoon cortisol change, cortisol at bedtime, and overnight cortisol change were not significant.

The results for these models are included in Table 5.

3 months

At 3 months, several models were significant. First, the model predicting infant afternoon cortisol change, which included family income, maternal EA, and infant negative affectivity as well as maternal report of positive and negative co-parenting, was significant, $R^2 = .28$, $p < .05$. Maternal EA significantly predicted infant afternoon cortisol change, such that infants of mothers who were more emotionally available showed a greater decrease in cortisol across the afternoon, $\beta = -.47$, $t(39) = -3.09$, $p < .01$. None of the other predictors were significant.

Although the model predicting infant cortisol at bedtime, which included family income, maternal EA, infant negative affectivity, and maternal report of co-parenting quality, was only marginally significant, $R^2 = .21$, $p < .10$, maternal EA significantly predicted infant bedtime cortisol. Greater EA was associated with lower cortisol at bedtime, $\beta = -.39$, $t(41) = -2.60$, $p < .05$. None of the other predictors were significant.

Additionally, although the model predicting overnight cortisol change, which included family income, maternal EA, infant negative affectivity, and maternal report of co-parenting quality, was not significant, $R^2 = .16$, $p > .05$, maternal EA significantly predicted infant overnight cortisol change. Infants of more emotionally available mothers showed a greater increase in overnight cortisol, $\beta = .41$, $t(41) = 2.64$, $p < .05$.

The model predicting infant CAR was not significant.

The results for these models are presented in Table 6.

Longitudinal analyses

Lastly, the 1 month maternal EA and co-parenting predictor variables were entered into the same simultaneous multiple regression models in order to examine each predictor's unique association with the 3 month cortisol variables, while controlling for the other predictors.

Although the model predicting infant cortisol at bedtime, which included family income, maternal EA, and maternal ratings of co-parenting quality, was not significant, $R^2 = .14$, $p > .05$, maternal EA at 1 month significantly predicted infant bedtime cortisol at 3 months. Greater maternal EA was associated with lower cortisol at bedtime, $\beta = -.31$, $t(42) = -2.01$, $p = .05$. None of the other predictor variables were significant.

The models predicting infant afternoon cortisol change, overnight cortisol change, and CAR at 3 months using the 1 month predictor variables was not significant, nor were any of the coefficients in these models.

These results are summarized in Table 7.

Tests of differential susceptibility

Analyses were then conducted to test the fourth hypothesis, which proposed that the differential susceptibility hypothesis would be supported at 3 months. Thus, it was predicted that infants who were rated as higher in negative affectivity by their mothers at 3 months would be more sensitive to maternal EA and co-parenting quality, for better or for worse, such that infants characterized by high levels of negative affectivity who experienced low EA and co-parenting quality would be less likely to show a diurnal rhythm in cortisol at 3 months, and infants exposed to high EA and co-parenting quality would be more likely to show the typical pattern. Variables representing the interaction between negative affectivity and maternal EA, negative affectivity and positive co-parenting, and negative affectivity and negative co-parenting were created by centering each variable and multiplying them together. These interaction variables were then examined as predictors of the infant cortisol variables at 3 months. None of these interactions were significant.

Tests of mediation

Lastly, infant sleep quality was explored as a mediator of the relationships between maternal EA and infant cortisol, co-parenting quality and infant cortisol, and infant temperament and infant cortisol. The fifth and sixth hypotheses predicted that infants of mothers who were rated higher for EA and parents who rated themselves higher for co-parenting quality would have better sleep quality, which in turn would predict greater evidence of diurnal cortisol patterning. The seventh hypothesis predicted that infants who were rated lower in negative affectivity would have better sleep quality, which in turn would predict greater evidence of diurnal cortisol patterning. If the tests of the interaction between negative affectivity and maternal EA, negative affectivity and positive co-parenting, or negative affectivity and negative co-parenting had been significant when the infants were 3 months old, supporting the differential susceptibility hypothesis, then the mediational models would be run only for infants that received the highest scores for negative affectivity. Tests of the differential susceptibility hypothesis, however, demonstrated that these interactions were not significant, and thus mediational analyses were conducted on the full sample.

Sleep quality was measured as mean infant night waking across a seven day period, as indicated by actigraph recordings and maternal report via infant sleep diaries. Though each gives information regarding the frequency of infant night wakings, actigraph recordings and infant sleep diaries represent different sources of information. Actigraphy determines infant night waking on the basis of movement, which may or may not be noticed by parents, whereas sleep diaries reflect parent report of infant night waking. Thus, actigraphy data and data reported by mothers were analyzed separately.

In order to satisfy Baron and Kenny’s (1986) preconditions for mediation, there must be a significant relationship between the predictor and outcome variables, the predictor and the mediator, and the mediator and the outcome variables. Thus, each of the predictor and outcome variables at 1 and 3 months were correlated with infant sleep quality at these time points.

The partial correlation analyses indicated that maternal EA at 3 months old was negatively associated with infant afternoon cortisol change and positively associated with infant overnight cortisol change. Only infant cortisol change overnight was associated with infant sleep quality at 3 months. Infants who showed a greater cortisol increase overnight were reported as waking up more frequently by their mothers, $r(82) = .23$, $p < .05$. Because maternal EA was not significantly associated with infant sleep quality, however, Baron and Kenny's (1986) pre-requisites for testing for mediation were not fully satisfied. Thus, the model examining infant sleep quality as a mediator of the relationship between maternal EA and infant overnight cortisol change was not tested.

Additionally, the previous analyses demonstrated that maternal ratings of negative co-parenting were negatively associated with infant CAR at 1 month. Infant CAR at 1 month was not significantly associated with infant sleep quality, however.

Infant negative affectivity was shown to be positively associated with infant afternoon cortisol change when infants were 3 months old. Infant afternoon cortisol change was not associated with infant sleep quality, however, so a mediational model was not tested.

Discussion

The findings from the current study contribute to the existing literature concerning parenting, temperament, and infant cortisol in several ways. First, they demonstrate that maternal EA, co-parenting quality, and infant negative affectivity are related to measures of infant diurnal cortisol in the first 3 months of life, when the diurnal rhythm is becoming established. They suggest that at 1 month, infants may be particularly attuned to the broader co-parenting environment, while parenting quality directed toward the infant and infant temperament are relevant to infant diurnal cortisol at 3 months. Furthermore, the results presented here suggest that maternal EA at 1 month is related to infant diurnal cortisol patterning at 3 months. Lastly, these findings suggest that while co-parenting and infant temperament are related to infant diurnal cortisol, these associations are not as robust as the relationship between maternal EA and infant cortisol. The results did not support the hypothesis of differential susceptibility, nor did the results provide any evidence for mediation of these relationships by sleep quality.

Co-parenting and infant CAR at 1 month

At 1 month, mothers' ratings of negative co-parenting quality predicted a smaller infant CAR, above and beyond the effects of family income alone. These results suggest that very young infants living in more stressful family ecologies, as indexed by negative co-parenting quality, may be less likely to show the typical morning burst in cortisol following awakening. Because cortisol is expected to increase sharply following awakening in the morning, these results suggest that young infants from stressed parenting environments showed less evidence of a diurnal rhythm in their cortisol patterning. These findings extend recent work demonstrating the associations between higher quality co-parenting and better infant adjustment and child behavioral regulation (Brown et al., 2010; Schoppe et al., 2009; Feinberg et al., 2009; Feinberg & Kan, 2008) to diurnal cortisol in the very early infancy period.

Generally, this finding suggests that at 1 month, infant diurnal cortisol patterning may be particularly attuned to the quality of the broader caregiving environment. This result supports Moore's (2010) theory that stressful family environments increase the demand for infants to regulate arousal resulting from parent conflict and stress. At 1 month, increased negative co-parenting appears to be related to less effective infant regulation of physiological arousal. Furthermore, the covariate analyses revealed that income, a variable that has been used to index the quality of the environment (Saridjan et al., 2010), was related to measures of infant diurnal cortisol at 1 month, but not at 3 months. One month old infants from higher income families showed a greater CAR. This result extends Saridjan and colleagues' findings with 12-20 month olds by demonstrating an association between family income and infant CAR in 1 month old infants. Thus, the associations of both co-parenting quality and income with cortisol generally suggest that very young infants from less stressed environments tend to show cortisol patterns that are more typical of the diurnal rhythm. Interestingly, maternal but not paternal report of negative co-parenting was related to infant diurnal cortisol at 1 month. This indicates that maternal perceptions of co-parenting issues may be more relevant to the infant than are paternal perceptions. Mothers may have also been more accurate reporters of parental conflict and competition and undermining.

Maternal EA, co-parenting quality, infant temperament, and infant diurnal cortisol at 3 months

When infants were 3 months old, higher maternal EA was related to a greater decrease in cortisol across the afternoon and a greater change in cortisol overnight, above and beyond the effects of family income. These results are consistent with the typical diurnal rhythm, in which cortisol declines across the afternoon and evening and then rises slowly overnight, as well as previous work relating sensitive parenting to both cortisol stress reactivity and diurnal cortisol in childhood (Grant et al., 2009; Albers et al., 2008; Blair et al., 2008; Kaplan et al., 2008).

Furthermore, maternal report of infant negative affectivity was associated with less of a decline in cortisol across the afternoon. Previous work has suggested that a fearful or inhibited temperament in infancy and early childhood is associated with greater cortisol reactivity to stress and higher morning cortisol (Talge et al., 2008; Buss et al., 2003; Schmidt et al., 1997). The current study expands on this by demonstrating that infants rated as higher in negative affectivity in early infancy show less typical cortisol patterning in the afternoon. This finding suggests that an individual's predisposition for establishing a diurnal rhythm in cortisol early in infancy may be related to individual differences in temperament.

Unique associations

In a final set of regression analyses, the unique associations between maternal EA, co-parenting quality, and infant negative affectivity and the infant diurnal cortisol variables were examined. At 1 month, higher family income was found to significantly predict a greater infant CAR. None of the maternal EA and the co-parenting quality predictors were significant. At 3 months, several results were significant. First, greater maternal EA continued to significantly predict a greater decline in infant cortisol across the afternoon. Infant negative affectivity, which significantly predicted less decline in afternoon cortisol in the partial correlation analyses, was not a significant predictor of infant afternoon cortisol change. Furthermore, greater maternal EA significantly predicted lower infant cortisol at bedtime. Lastly, maternal EA continued to significantly predict greater increase in cortisol overnight.

The unique associations between maternal EA and infant diurnal cortisol and co-parenting quality and infant cortisol were also examined in the longitudinal analyses. Here too, maternal EA emerged as the most important factor influencing infant diurnal cortisol, as greater maternal EA at 1 month significantly predicted lower cortisol at bedtime at 3 months. This finding suggests that the quality of care experienced very early in life may influence the development of the diurnal cortisol rhythm over time.

Summary

Altogether, these results suggest that as the diurnal cortisol rhythm develops during the first 3 months of life, it is sensitive to the quality of the family environment, parenting quality, and infant temperament. At 1 month, infant diurnal cortisol seems particularly affected by negative co-parenting, whereas variation in positive co-parenting is less relevant. At 3 months, however, parenting quality and infant temperament seem to be important for shaping infant diurnal cortisol patterns. Thus, these results suggest that very young infants may be aware of particularly negative stimuli related to the broader parenting environment at 1 month, but may not be attuned to variation in maternal caregiving quality at bedtime until 3 months. Bedtimes at 1 month may also be quite variable as families establish an evening routine, which could make it difficult to examine stable parenting patterns this early in life. Furthermore, while co-parenting quality and temperament appear to be related to infant diurnal cortisol in early infancy, maternal EA seems to be of particular importance to the developing diurnal cortisol rhythm. Lastly, the results provide some evidence that maternal EA very early in infancy may be associated with the development of the diurnal cortisol rhythm over the ensuing months.

The findings from the current study suggest that bedtime is a particularly important time for examining the associations between parenting processes and child physiology. Infants with lower cortisol at bedtime tended to have mothers who were rated higher for EA at bedtime. These findings suggest that bedtime is less physiologically arousing for infants whose mothers establish quiet, soothing routines and are responsive to their infants' distress. Because for many infants bedtime marks the beginning of the longest separation of the day from their parents, it may be especially stressful to them (Teti et al., 2010). The results of the current study suggest that the sensitivity with which mothers' handle this impending separation is related to infant cortisol patterning during this potential stressor.

These results have implications for infants' later developmental outcomes, as deviations from the typical diurnal rhythm in childhood are associated with behavioral and emotional dysregulation. Scher et al. (2010) found that an exaggerated CAR was associated with internalizing behaviors and negative emotionality in toddlers, and Luijk et al. (2010) showed that a flattened rhythm in cortisol across the day was associated with disorganized attachment in infants. Thus, the establishment of a diurnal cortisol rhythm early in infancy may help to lay the foundation for behavioral and emotional regulation in childhood, and therefore decreased risk for problem behaviors. The results of the current study suggest that the quality of the parenting environment, the quality of maternal care experienced, and infant temperamental differences may all contribute to development of the typical diurnal rhythm in early infancy.

The hypothesis of differential susceptibility, which suggests that infants who were rated as higher in negative affectivity by their mothers at 3 months would be sensitive to maternal EA and co-parenting quality in a for better or for worse manner, was not supported with these data. This null result could be attributable to the young age of the sample. Typically, temperament is not assessed via questionnaire until 3-4 months of age, and the current sample is at the very minimum of this age range. Over time, individual differences in temperament tend to become more stable, potentially allowing more subtle differential susceptibility effects to be detected.

Furthermore, the current study did not find support for the premise that infant sleep quality mediates the associations between maternal EA and infant cortisol, co-parenting quality and infant cortisol, or infant temperament and infant cortisol. These results suggest that the quality of the parenting environment and maternal EA at bedtime may be directly related to infant diurnal cortisol. In fact, the current study found only one significant correlation between a measure of sleep quality and diurnal cortisol in very young infants. These findings contrast with previous work, which has detected linkages between consolidated sleep and a diurnal rhythm in cortisol in infants as young as 2 months old (de Weerth et al., 2003). Perhaps because most infants at this young age in the current sample were not sleeping through night, it might have been difficult to detect reliable linkages between sleep quality and cortisol. As infants grow older and begin to develop consolidated sleep, the association between these constructs may become clearer. De Weerth et al. also found the presence of a diurnal cortisol rhythm to appear and disappear from 2-5 months of age, which may mean that the relation between sleep quality and infant cortisol is variable as well.

Limitations and future directions

There are several limitations of the current study that must be noted. First, samples were only taken across one night at each time point. Because there is substantial intraindividual variability in diurnal cortisol across days in infancy (De Weerth et al., 2003), it would be valuable to have data for multiple days at each age. This data could provide more information concerning intra- and interindividual variability in cortisol patterning as infants develop a diurnal rhythm across the first few months of life.

It would also be valuable to sample infant diurnal cortisol across the day, in addition to examining cortisol levels across the night as in the current study, in order to facilitate comparison to previous work. Samples across the day also allow for the calculation of the Area Under the Curve (AUC), a measure of diurnal cortisol that taps into the amount of cortisol secreted by an individual across the day. This allows for interindividual comparisons of daily cortisol secretion.

Furthermore, it would be particularly useful to continue to examine diurnal cortisol as infants grow older. De Weerth et al. (2003) found the presence of a cortisol rhythm to appear and disappear across monthly samples in a subset of infants assessed from 2 to 5 months of age. Thus, a diurnal pattern in cortisol may be detectable in the first 3 months of life, but a clear, established pattern may not develop until later infancy. Co-parenting quality, maternal EA, and infant negative affectivity may all continue to influence the development of this pattern into later infancy. It would be particularly interesting to examine whether maternal EA at bedtime continues to be of the particular relevance to diurnal cortisol as infants grow older. As noted earlier, it may also be useful to examine the interaction between infant temperament and these parenting and environmental factors as infants grow older and interindividual temperamental differences became more stable. Additionally, future work is needed that examines the potential role of sleep quality as a mediator of the relationship between these variables and diurnal cortisol as infants begin to develop consolidated sleep in later infancy. Replication work that examines these relationships in a larger sample may also prove valuable, as the current study was limited by a relatively small sample size, particularly for the longitudinal analyses.

It may also be useful to incorporate multiple measures of both maternal and paternal EA across different contexts in future work examining the development of the infant diurnal cortisol rhythm. The current study demonstrated that maternal EA at bedtime is of particular relevance to infant cortisol, but parental EA in other caregiving contexts (i.e. changing, bathtime, feeding) may also be related to the infant's developing physiology.

Conclusion

In sum, the current study extends the literature concerning the development of a diurnal cortisol rhythm in infancy in several ways. First, it demonstrates that co-parenting quality, maternal EA, and temperament are associated with diurnal cortisol in very young infants. At 1 month, infant diurnal cortisol patterning appears to be influenced by negative co-parenting in the family environment, and at 3 months it is affected by the quality of care the infant receives, as well as infant temperament. In addition, maternal EA at 1 month is associated with infant diurnal cortisol at 3 months. In multiple regression analyses examining the unique associations between maternal EA, co-parenting quality, infant negative affectivity, and infant diurnal cortisol, maternal EA emerged as the most important factor influencing the development of infant diurnal cortisol patterning. The results did not provide evidence supporting the differential susceptibility hypotheses, and the relationships between co-parenting quality and infant cortisol, maternal EA and infant cortisol, and infant temperament and infant cortisol were not mediated by the quality of infant sleep. Future work that examines the influences of these factors over time may prove valuable for examining the contributions of the family environment, parenting quality, and infant temperament to the development of an established diurnal cortisol rhythm in infancy.

APPENDIX

TABLES 1-7

Table 1

Correlations between predictor variables, 1 month

	Mother positive co-parenting	Mother negative co-parenting	Father positive co-parenting	Father negative co-parenting	Maternal EA
Mother positive co-parenting	1	-.48**	.45**	-.46**	-.08
Mother negative co-parenting	--	1	-.32**	.51**	-.17
Father positive co-parenting	--	--	1	-.66**	-.04
Father negative co-parenting	--	--	--	1	.03
Maternal EA	--	--	--	--	1

Notes: ** Significant at $p < .01$.

Table 2
Correlations between predictor variables, 3 months

	Mother positive co-parenting	Mother negative co-parenting	Father positive co-parenting	Father negative co-parenting	Maternal EA	Infant negative affectivity
Mother positive co-parenting	1	-.61**	.61**	-.43**	.00	-.15
Mother negative co-parenting	--	1	-.41**	.45**	-.25*	.26**
Father positive co-parenting	--	--	1	-.60**	.04	-.19*
Father negative co-parenting	--	--	--	1	-.01	.13
Maternal EA	--	--	--	--	1	-.21
Infant negative affectivity	--	--	--	--	--	1

Notes: ** Significant at $p < .01$.
* Significant at $p < .05$.

Table 3

Partial correlations between parenting variables and infant diurnal cortisol, 1 month

	Infant afternoon cortisol change	Infant cortisol at bedtime	Infant overnight cortisol change	Infant CAR
Maternal EA	.04	-.19	-.06	-.10
Mother positive co-parenting	-.03	-.05	.08	.06
Mother negative co-parenting	.11	.08	-.06	-.22*
Father positive co-parenting	.01	-.05	.04	.10
Father negative co-parenting	.07	.06	-.10	-.08

Notes: * Significant at $p < .05$.
Controlled for family income.

Table 4

Partial correlations between parenting and temperament variables and infant diurnal cortisol, 3 months

	Infant afternoon cortisol change	Infant cortisol at bedtime	Infant overnight cortisol change	Infant CAR
Maternal EA	-.42**	-.13	.32*	.04
Mother positive co-parenting	.10	.09	-.23	-.04
Mother negative co-parenting	-.04	-.02	.21	-.01
Father positive co-parenting	-.08	.07	-.02	.00
Father negative co-parenting	.04	-.08	.13	-.03
Infant negative affectivity	.32**	.21	-.10	-.02

Notes: ** Significant at $p < .01$.
* Significant at $p < .05$.
Controlled for family income.

Table 5

Concurrent multiple regression results, 1 month

	Infant afternoon cortisol change			Infant cortisol at bedtime			Infant overnight cortisol change			Infant CAR		
	β	R^2	F	β	R^2	F	β	R^2	F	β	R^2	F
		.02	.30		.03	.56		.03	.57		.15	2.94*
Family income	.03			.13			-.16			.38**		
Maternal EA	.08			-.14			-.05			-.12		
Mother positive co-parenting	-.02			.07			.05			-.14		
Mother negative co-parenting	.10			.03			-.05			-.19		

Notes: ** Significant at $p < .01$.
* Significant at $p < .05$.

Table 6
Concurrent multiple regression results, 3 months

	Infant afternoon cortisol change			Infant cortisol at bedtime			Infant overnight cortisol change			Infant CAR		
	β	R^2	*F*	*β*	R^2	*F*	*β*	R^2	*F*	*β*	R^2	*F*
		.28	3.16*		.21	2.22		.16	1.60		.06	.58
Family income	.24			.24			-.10			-.02		
Maternal EA	-.47**			-.39*			.41*			.00		
Mother positive co-parenting	.13			.19			.00			-.26		
Mother negative co-parenting	-.20			-.06			.22			-.03		
Infant negative affectivity	.27			.14			-.12			-.09		

Notes: ** Significant at $p < .01$.
* Significant at $p < .05$.

Table 7
Longitudinal multiple regression results

	Infant afternoon cortisol change, 3 months			Infant cortisol at bedtime, 3 months			Infant overnight cortisol change, 3 months			Infant CAR, 3 months		
	β	R^2	F	β	R^2	F	β	R^2	F	β	R^2	F
		.18	2.21		.14	1.73		.12	1.50		.05	.60
Family income, 1 month	.29			.19			-.10			.03		
Maternal EA, 1 month	-.30			-.31*			.28			-.15		
Mother positive co-parenting, 1 month	.07			.19			-.18			-.22		
Mother negative co-parenting, 1 month	-.20			-.01			.07			-.07		

Notes: * Significant at $p \leq .05$.

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