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Saliva Cortisol and Heart Rate Variability as Biomarkers in Understanding Emotional Reaction and Regulation of Young Children—A Review[*]

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Cortisol and heart rate variability (HRV) are good indicators for the non-invasive assessment of the hypothalamic-pituitary-adrenal (HPA) and autonomic nervous system (ANS) activity in response to psychophysiological stress respectively. Emerging evidence from previous studies suggests a link between cortisol and HRV response to stress and social experiences during early development. However, research in this area has been constrained by a number of conceptual and methodological challenges. Time is a crucial variable that needs to be taken into account in study designs since stress-sensitive physiological systems change over time in response to changing intrinsic and extrinsic states. In this review, our focus is on the HPA axis and HRV responses as an allostatic system with young children's individual differences in temperament, social regulation, and environmental sources of influence taken into account. The conclusions include: 1) cortisol levels are related to various time courses, ranging from moment-to-moment changes to changes occurring over the course of days, months, and years in consideration of individual differences in state and trait emotions; 2) it is necessary to take individual characteristics, multi-faceted constructs related to early development, and developmental changes into account in studies of reactivity and regulation patterns of the cortisol and HRV in young children; and 3) prospective examination is needed on the long-term outcomes of various individual characteristics and environmental influences (e.g., attachment quality, family and daycare environment, and environmental control of the child) in early experience that are related to reactivity differences in HRV and atypical cortisol patterns.

Keywords: Cortisol; HRV; Children; Emotion

Introduction

Measuring the activity of the hypothalamic-pituitary-adrenal (HPA) axis is a method that is used to monitor and understand the stress response. When the HPA axis is activated due to exposure to a threat, circulating cortisotroid concentration raises in about 15 - 30 min and returns to un-activated levels some time after the stressor disappears or has been removed (deKloet, Sibug, Helmerhorst, & Schmidt, 2005). The HPA system is one of the biological mechanisms that activate the attention and energy responses needed to face threats (Gunnar, 2001; Dickerson & Kemeny, 2004; Segerstrom & Miller, 2004). Reactivity and regulation of the HPA system has been implicated in the etiology of physical illness, substance abuse, and serious psychiatric conditions such as depression, anxiety disorder, and posttraumatic stress disorder (Goeders, 2003; Gold & Chrousos, 2002; Luby et al., 2002; Mathew et al., 2003; Roma, Champoux, & Suomi, 2006).

Although cortisol (the major hormone produced by the HPA system) provides only a partial understanding of the activity of this neuroendocrine system, its regulation may bear importance for human development (Gunnar & Donzella, 2002). The release of cortisol can be reliably measured in saliva (Kirschbaum & Hellhammer, 1989) and its measure has made possible the assessment of the immediate biological impact of the environment. In the last two decades, a considerable amount of research on the regulation and dysregulation of the stress system in young children has relied on salivary cortisol measures.

Studies on rodents and primates suggest that activity and regulation of the stress system later in life may be shaped by experiences during early development; stressful events trigger immediate changes in the stress system that may permanently alter brain functions and behavior (deKloet, Sibug, Helmerhorst, & Schmidt, 2005; Gunnar & Donzella, 2002). Research with human infants and young children show an association between cortisol reactivity and learning or memory for voice/object correspondence (Thompson & Trevathan, 2008) and intervention effectiveness in improving the HPA axis regulation of individuals with early separation from caregivers (Dozier, Peloso, Lewis, Laurenceau, & Levine, 2008).

Elevations in cortisol are typically the focus of research on HPA axis dysregulation; however, it is important to note that the system can respond bi-directionally. A wealth of research

demonstrates that elevations in cortisol and dysregulation of the HPA axis are related to physical illness, substance abuse, and serious psychiatric conditions such as depression, anxiety disorder, and posttraumatic stress disorder (Goeders, 2003; Gold & Chrousos, 2002; Luby et al., 2002; Mathew et al., 2003; Vanitallie, 2002). Nevertheless, studies also found that cortisol levels of young children under conditions of neglectful and abusive care are reduced rather than increased (Gunnar & Donzella, 2002). Relations between hypoarousal of the stress system and antisocial, aggressive, and criminal behaviors were shown in adults (Raine, 2002; Susman & Pajer, 2004; Susman, 2006). Therefore, chronic elevation and depression of cortisol are considered possible influences of long-term behavioral and developmental outcomes.

The parasympathetic branch of ANS as measured by vagal control has been the primary focus of research on individual differences in behavioral or temperament-based responding to the environmental stimuli, while the sympathetic branch of ANS has been the focus of normative changes in response to the environment (Stifter & Jain, 1996). Circadian vagal tone is an index of the functional status of the parasympathetic nervous system that has been considered as a psychophysiological marker of emotion regulation and arousal (Porges, 1995; Porges, 2001). Parasympathetic nervous system functioning, measured by high frequency variability in heart period, is related to the control of attention, emotion, and behavior. The high frequency power in heart period is mainly a result of respiratory influences (respiratory sinus arrhythmia). Porges (1995) has developed methods for quantification of power in this frequency band and has named it vagal tone.

Recently, measurement of HRV (also a non-invasive technique) has been widely used to investigate the functioning of the ANS, especially the balance between sympathetic and vagal activity. It has been proven to be very useful for both research and clinical studies concerned with hypertension, psychiatric and psychological disorders, cardiovascular disease, and diabetic autonomic dysfunction. Over the past decade, HRV has been used increasingly in the analysis of changes in sympathetic-vagal balance related to individual differences (e.g., temperament and emotion regulation strategies) and psychological/environmental stressors (Santucci, Silk, Shaw, Gentzler, Fox, & Cohn, 2008; von Borell et al., 2007).

Examination of the physiological underpinnings of social-emotional regulation in childhood is valuable for understanding individual emotional responses, self-regulation behaviors, and social regulations under stress. To more accurately assess internal emotional states and interpret environmental influences, physiological measures of young children's stress system are needed. It is difficult to distinguish between two individuals who behaviorally respond to a stimulus in a similar manner. Similar situations may elicit different levels of emotional arousal from different individuals. Also, due to individual differences in the intensity of emotional reactivity, individuals may exhibit similar levels of distress behaviorally but show evidence of different levels of physiological arousal. Behavioral displays maybe mislabeled or misinterpreted without physiological measures to provide supplementary information about emotional responses.

HRV is especially suitable for studying social-emotional regulation, since it allows a much more detailed and continuous determination of the regulatory characteristic of the ANS activity in response to psychophysiological stress. Although cortisol change has been used to investigate within-person differences in response to an acute stressor or momentary (state) emotion (Dickerson & Kemeny, 2004; Adam, 2010), it is not easy to collect saliva sample over and across the study time period. Moreover, saliva cortisol levels take about 20 - 25 minutes post stressor to peak, and take up to an hour to recover to pre-stress baseline levels. Although some studies have linked momentary negative mood states, such as anger, worry, and sadness to acute increases in cortisol, most studies suggest that situations that pose threats are the most consistent and powerful acute activators of the HPA axis (Dickerson & Kemeny, 2004; Adam, 2012).

In the following sections, we will provide: 1) an outline of the inheritability of and individual differences in cortisol; 2) a brief summary of environmental influences on cortisol levels of young children that are related to environmental controllability, social interaction in the family, and childcare upbringing; and 3) discussions about the measurement and methodological challenges in salivary cortisol and HRV studies of young children with a conclusion of possible directions for further research.

Inheritability and Individual Differences

Although psychologists agree that infant emotionality (presumed to be temperamental in origin) is rooted in biology, empirical studies of the stability of infant emotionality have revealed that sensitive and appropriately responsive parenting in infancy is related to more optimal patterns of behavioral and physiological reactivity and regulation. With environmental experiences taken into account, findings in molecular genetics research suggest that specific genes are related to infant emotionality and later problems with depression, impulse control problems, and externalizing/antisocial behaviors, especially when paired with insensitive parenting or other adverse family environments (Propper & Moore, 2006).

Variations in Inheritability

HPA axis polymorphisms have been linked to risk for development of depression and posttraumatic stress disorder (Gillespie, Phifer, Bradley, & Ressler, 2009) and individual differences in reactivity to laboratory-based stressors (Thode et al., 2008). Moreover, the interactions between HPA axis polymorphisms and measures of early life adversity best predict stress reactivity depression, and PTSD (Binder et al., 2008; Gillespie et al., 2009; Tyrka et al., 2009). Besides differences in gene sequence, additional genetic approaches have focused on epigenetic changes. Epigenetic changes are experience driven alterations to portions of the DNA that can serve to turn up or turn down the expression of particular genes. Recent research supports the possibility of experience-driven epigenetic programming in humans.

Possible gene-environment interplay was found in estimating the contributions of genes and environment to cortisol response to stress in young children (Ouellet-Morin et al., 2008). Based on 130 identical and 216 fraternal 19-month-old twins, their study reveals that the genetic environmental bases of hormonal response to stress depend on the context in which a child was brought up. Patterns of differing genetic and environmental contributions in cortisol reactivity to stress are found to be contingent on familial adversity as high familial adversity may

have a developmental effect that programs cortisol reactivity. For children from a favorable family environment, genetics account for 40% of the individual differences in cortisol response to unfamiliar situations. However, for those growing up in different family circumstances, the environment completely overrides the genetic effect as if it had established a programed hormonal conditioning to stress.

Individual Differences—Temperament as Predictors of Emotion Reactivity and Regulation

Individual differences in emotion reactivity (i.e., response to stimuli reflected in changes in the somatic, endocrine, and autonomic nervous system) and emotion regulation (i.e., processes that adjust reactivity through approach, avoidance, or attention mechanisms) have been considered genetic in origin and stable over time and across contexts (Rothbart & Bates, 1998). Temperamentally vulnerable children (e.g., fearful, anxious, internalizing, and easily angered or frustrated) are more likely to exhibit elevations in cortisol under conditions of less than optimal care. A number of personal characteristics were shown to influence cortisol activity level, including being a boy, more socially fearful (Crockenberg, 2003), and emotionally negative and having less self-control (Dettling, Parker, Lane, Sebanc, & Gunnar, 2000). Responses of children to child care also display individual variations, partly depending on how closely individual needs are met (Greenspan, 2003).

Traditionally, mid-morning and mid-afternoon levels of cortisol have been used as an indicator of the effect of childcare. Results of previous studies suggest that cortisol levels increase or remain "flat" in young children across the day in school. However, when an analysis of the childcare effect was done with individual differences in temperament taken into account, an interaction effect was found among groups of children with different internalizing levels. Results revealed that the children least internalized showed a significant decrease in cortisol levels from morning to noon and after nap while cortisol levels of the other groups across the day fit the pattern of the upward curve (Li & Shen, 2008).

Moreover, individual differences and maturation of the central nervous system (CNS) and autonomic nervous system (ANS) are considered as the foundation for emotional and behavioral regulation. Individual differences in arousal and reactivity present early in live have been suggested to be part of an individual's temperament related to development of emotional experience and behavioral control (Fox & Calkins, 2003). Theories focusing on the underlying physiological arousal and reactivity of temperament highlight the maturation of the CNS and ANS as the foundation for emotional and behavioral regulation (Santucci et. al., 2008). The ANS is considered primarily responsible for the physiological arousal related to emotional experiences, resulting from input of both the excitatory sympathetic nervous system (SNS) and inhibitory peripheral nervous system (PNS). Two current theories regarding autonomic reflexivity and emotional responding are Neurovisceral integration theory (Thayer & Lane, 2000, 2009; Thayer & Ahs, 2012; Fredrikson, Sollers III, & Wager, 2012) and Porges' Polyvagal theory. The PNS, usually measured by vagal control of the heart, has been the primary focus of research on individual differences in temperament-based responses to the environment (Calkins & Swingler, 2012).

Environmental Sources of Influence on Cortisol and HRV Levels

As mentioned earlier, individual differences in emotional reactivity and emotion regulation exist with genetic bases. However, research results also support the instability of emotion, suggesting possible influences of the environment (Pettit & Bates, 1984; Wilson & Matheny, 1986). Although the contribution of environmental factors to emotion reactivity and regulation is not well established, research has consistently found the parent-infant relationship to be important to the development of young children's behavioral regulation, especially in early childhood (e.g., Crockenberg & Leerkes, 2004; Rosenblum, Mc-Donough, Muzik, Miller, & Sameroff, 2002).

Social Interaction and Social-Emotional Regulation in the Family

Recent research has studied how parenting influences the underlying physiology and genetics of infant emotionality (for a review, see Propper & Moore, 2006). Caregivers seem to play important roles in regulating reactivity of the HPA system during environment. In rodents, licking and grooming by the dam and the delivery of milk into the gut maintain the adrenal hyporesponsive period, a period between postnatal days 4 and 14 when it is difficult to elevate glucocorticoid levels (Suchecki, Rosenfeld, & Levine, 1993). In non-human primates, the presence of the mother serves to buffer activity of the HPA axis, allowing the infant to behaviorally express distress to help elicit maternal care without producing concomitant elevations in cortisol (Bayart, Hayashi, Faull, Barchas, & Levine, 1990). In humans, the attachment relationship between a caregiver and child impacts cortisol reactivity (Gunnar, Larson, Hertsgaard, Harris, & Brodersen, 1992; Lamb, 1998; Sims, Guilfoyle, & Parry, 2006; Gunnar & Donzella, 2002). Cortisol levels of children with secure relationships tend to return to basal levels more quickly after the threat has been removed (Gunnar & White, 2001; Sims, Guilfoyle, & Parry, 2006). Moreover, having secure relationships impede the risk for increases in cortisol (Gunnar et al., 1992; Gunnar & White, 2001). In general, empirical research supports the theory that sensitive parenting in infancy is related to more optimal patterns of physiological reactivity and regulation.

Social support reduces stress levels in both animals and humans. In contrast, adverse experiences early in life may predispose individuals to affective pathology through their effect on the activity of the HPA system (Graham, Heim, Goodman, Miller, & Nemeroff, 1999; Heim, Owen, Plotsky, & Nemeroff, 1997). A number of retrospective studies suggest that adults who suffered emotional loss (e.g., loss of a parent), maladaptive relationships with attachment figures, or maltreatment during childhood exhibit heightened levels of corticotropin-releasing hormone (CRH) and/or evidence of dysregulation of the HPA axis (Gunnar & Donzella, 2002).

Family is a fundamental environment in which the relationships determine the child's development. Differing genetic and environmental contributions according to familial adversity was found in a recent study by Ouellet-Morin and colleagues (2008), suggesting that high familial adversity may have a developmental effect that programs cortisol reactivity. In conditions of high familial adversity, both shared and unique environmental factors (but not genetic factors) accounted for the variance in

cortisol reactivity. The familial risk factors that affect the variations in heritability of cortisol reactivity include tobacco use during pregnancy, low family income, low education level, single parenthood, very early parenthood, low birth weight, and maternal hostility toward the child (Ouellet-Morin et al., 2008). However, human development is by nature a reciprocal interaction. Therefore, the social relationships that are established within the family environment may bear importance on the link between cortisol response to stress and familial difficulties during early development.

Environmental Controllability and Social Competence

The development of emotionality is also a result of changes of social-emotional experience in cognition and information processing (Propper & Moore, 2006). Environmental controllability of individual experience can be an important influence on behavior, personality, and response to live events (e.g., novelty). Based on varied research and theory, it was proposed that animals and humans are naturally motivated to produce change in their environment in order to build "competence" and that, given the cumulative nature of "mastery" motivation, early childhood is the most fertile ground for its development (White, 1959; Roma, Champous, & Suomi, 2006).

Controllable or contingent stimulation in infancy facilitates positive developmental outcomes including cognitive development, exploration and learning motivation, and positive emotional states (Gunnar, 1980a). Experiments based on the creation of an environment that develops "competence" (not just a lack of "helplessness") provide powerful endorsements of controllability during infancy. Results showed that infants from the master group were more exploratory in a novel environment and less reactive during fear tests or stressful, novel situations (Gunnar, 1980b; Mineka, Gunnar, & Champoux, 1986; Clarke-Stewart, 1973; Joffe, Rawson, & Mulick, 1973; Roma, Champous, & Suomi, 2006). In a recent study, Gunnar and colleagues examined a group of preschoolers' increases in salivary cortisol from midmorning to midafternoon in full-time home-based day care. Increases were found in the majority of children (63%) at day care, with 40% classified as a stress response. Observations at day care also revealed that intrusive, over-controlling care was associated with the cortisol rise (Gunnar, Kryzer, Van Ryzin, & Phillips, 2010).

Moreover, social competence is proposed to be a different factor on coping than appetitive or inanimate controllability and to have increasing salience in early childhood development (Gunnar, 1980a; Roma, Champous, & Suomi, 2006). According to a study of rhesus monkeys, the coping advantages gained by appetitive control were limited to the context congruent with the individual mastery experiences and did not transfer to social group situations (Roma, Champous, & Suomi, 2006). In studies comparing human children's HPA axis activity across home and childcare settings, it was found that the amount and complexity of play with peers and teacher-reported social fearfulness are related to the mid-afternoon increases in cortisol when sampled at childcare, especially for toddlers (Watamura, Donzella, Alwin, & Gunnar, 2003). In future studies, aspects of peer interaction (such as the amount of social control) could be mapped to young children's cortisol in daycare settings in order to further understand the impact of social competence.

Childcare Environment

The HPA axis activation pattern is dependent on social context. Based on the comparisons of children's cortisol levels across home and childcare settings, studies have shown mid-morning to mid-afternoon increases in some children at childcare but normal decreases at home during the same testing time (Dettling, Gunnar, & Donzella, 1999; Dettling et al., 2000). Moreover, response of children to childcare was found to be dependent on: 1) the relationship between parent and child and the child's sense of psychological separation from parent (Jarvis & Creasey, 1991) and 2) the temperament characteristics (such as internalizing) of the child (Li & Shen, 2008).

Children's individual differences in physiological and behavioral reactions to stress also play a crucial role in social-emotional regulation in the childcare environment. Young children who are either under- or over-reactive to stimuli are especially vulnerable in childcare settings that do not tailor to the child's needs (Greenspan, 2003). Those who are highly reactive to stimuli may become too distressed to elicit helpful regulation processes (Coplan, Rubin, Fox, Calkins, & Stewart, 1994), and frequently distressed children may be more likely to elicit negative responses from caregivers. Early physiological reaction also contributes to later social competence at four years of age (Calkins & Fox, 2002). Furthermore, children with less-developed social skills were found to exhibit higher cortisol levels and greater increases in cortisol in group care environments across the day period (Watamura, Donzella, Alwin, & Gunnar, 2003).

Measurement and Methodological Challenges

Although pharmacological methods (e.g., dexamethasone suppression test) may provide important information about the activity of the HPA system, pharmacological tests have rarely been used with young children. Most of the research on the stress system in young children involves salivary measures of cortisol since it is an easy and non-intrusive way of gathering biological data and the release of cortisol can be reliably measured in saliva (Kirschbaum & Hellhammer, 1989). However, the reliance on salivary cortisol measures also imposes limitations related to measurement and methodological issues.

Developmental Changes, Dynamics of Cortisol Secretion, and Timing of Sample Collection

Children's cortisol levels are slightly lower than those of adults and are characterized by great individual variability (Sims, Guilfoyle, & Parry, 2006). A common phenomenon in past studies signifying individual variations of cortisol secretion is that standard deviations of salivary cortisol are large, close to or even bigger than the mean values of salivary cortisol most of the time (e.g., Davis, Donzella, Krueger, & Gunnar, 1999). In addition to individual differences, a significant cortisol secretion in young individuals is significantly affected from one day to another. Mean cortisol levels vary significantly from one day to another, even for a fixed sampling time, indicating significant environmental influences on cortisol levels at similar time points of different days (Li, Chiou, & Shen, 2007). Within subject cortisol levels differed significantly in the early morning, early afternoon, and late afternoon cortisol data, and the significance of the variation was related to the magnitude of the correlation between cortisol and internalizing disposition. Mid-afternoon cortisol levels showed the most significant day effect

and the highest correlation with internalizing disposition.

Moreover, developmental changes existing in cortisol response and basal concentrations need to be taken into account in study designs. The hypo-responsive period of cortisol reactivity, a period when the reactivity dampens, was found between 4 and 14 postnatal days for rodents and at about the first year for human infants (Suchecki, Rosenfeld, & Levine, 1993; Gunnar & Donzella, 2002). However, little is known about 1) how environmental influences, such as caregiver sensitivity and responsiveness, influence the maintenance of the hypo-responsive period and 2) how other developmental changes affect cortisol reactivity and basal concentrations of cortisol during infancy or over the course of early development.

Novelty has been believed to increase cortisol levels. However, new activities that engage attention may produce a decrease instead of an increase in cortisol levels for young children. New activities or novel events such as a car trip (Larson et al., 1991), swimming lessons (Hertsgaard, Gunnar, Larson, Brodersen, & Lehman, 1992), taking part in a play group (Legendre & Trudel, 1996), and attending childcare settings (Dettling et al., 1999) were shown to decrease cortisol in infants and preschoolers (compared to their home baseline), especially when the exposure to novelty occurred in the mother's presence and the novelty elicited generally positive affect. Preschool-aged children attending a half-day nursery program, regardless of morning or afternoon sessions, show lower cortisol levels as compared to the home baselines (Gunnar et al., 1997). However, the effects of novelty described above were no longer seen in children five years and older (Dettling et al., 1999; Gunnar & Donzella, 2002).

Play with peers is fun, but the difficulty in learning to make friends and play nicely with peers is challenging varies with age. For most young children, social competence will improve with age over the early childhood years. However, the development of skilled social interaction with peers is more challenging for children with internalizing disposition and may be related to cortisol increase over the day in childcare settings (Gunnar & Donzella, 2002; Li & Shen, 2007). Young children between 21 and 40 months of age, when children become highly motivated to make friends and play with peers, were found to show greater increase in cortisol over the day than children of other ages. Gunnar and Donzella (2002) suggest that the rise in cortisol over the childcare day emerges at about the age when peer relations become a focus of young children in group-care settings and that the increase in cortisol diminishes with the increase in social competence. However, the hypothesized links need to be examined prospectively in order to better understand the long-term consequences of early experiences.

Thus, in order to detect the interplay of environmental influences and individual characteristics and to avoid under-representation of the correlations between personality traits and cortisol responses, one could use a data aggregation method and select the optimal sampling time. However, as there are clear day effects, in order to better understand the dynamics of cortisol levels, further research must observe and recode the activities of the individual child, peers, and caregivers/teachers in the classroom to investigate possible sources of variation and explore situational effects with HRV data.

HRV Data as Indicators of Temperament and Emotion Regulation

Cardiac vagal tone has been considered as a psychophysi-

ological marker of emotion regulation and arousal (Porges, 1995). Over the past decades, HRV has been used increasingly to analyze changes in sympathovagal balance related to individual characteristics such as temperament and coping strategies. HRV has been successfully used as a measure of autonomic regulation of cardiac activity in human and animal studies to assess stress and well-being under various conditions and to characterize and understand individual traits such as temperament and coping strategies, in both human and animal studies (von Borell et al., 2007). von Borell and colleagues (von Borell et al., 2007) claim that the same psychophysiological principles can be applied to humans and non-human mammals based on a thorough review of related studies.

Baseline resting levels of vagal tone has been found to be related to individual differences in reactivity and soothability of young children (e.g., Calkins, 1997; Calkins & Fox, 2002; Stifter & Fox, 1990). Moreover, low resting vagal tone was found to be generally related to negative affectivity (Beauchaine, 2001), while high vagal tone was found to be associated with approach to strangers, high activity level, lower levels of aggression, and regulated distress in frustrating situations for toddlers (Calkins & Dedmon, 2000; Porges, Doussard-Roosevelt, Portales, & Greenspan, 1996; Stifter & Jain, 1996) and greater empathy, social competence, and subjective feelings of sympathy, and sociability and emotion regulation of young boys (Eisenberg, Fabes, Murphy, Maszk, Smith, & Karbon, 1995; Fabes, Eisenberg, & Eisenbud, 1993; Fabes, Eisenberg, Karbon, Troyer, & Switzer, 1994). On the other hand, suppressed vagal tone during a challenging task was claimed to be related to regulation of attention and behavior and may facilitate orientation to stimuli (Calkins, 1997; Porges, Doussard-Roosevelt, & Maiti, 1994).

Study the Complex Oscillations of HRV Data

Healthy cardiac function is characterized by irregular time intervals between consecutive heart beats. The rhythmic oscillation of the regulatory components of cardiac activity that function to orchestrate responses to challenges and to maintain cardiovascular homeostasis contributes to the complex oscillations of HRV data. An oscillatory curve can be produced when consecutive IBIs are plotted on a time scale. The "mixed oscillation" of this curve results from the rhythmic pulses of the different regulatory components, where rhythmic activities originating from the PNS exhibit higher frequency than those of the SNS. In order to analyze the complex oscillations of HRV, using data from at least 5-min of consecutive IBIs is recommended (von Borell, et al., 2007).

According to Borell and colleagues (Borell et al., 2007), recordings of IBIs should contain less than 5% of artefacts before editing and subsequent manual editing of the data should be done to a very high standard. They also identified the following areas that warrant further study in order to improve methodology and to enhance our understanding of HRV and underlying sympathovagal mechanisms in relation to stress and emotional regulation:

1) Improve ease of analysis by means of automatic elimination of artefacts.

2) Measure possible confounding effect, such as diurnal variation and effects age, sex, sleep, metabolic state and other factors on HRV, then find ways to eliminate or minimize the confounding effects (e.g., standardize the data according to age, sex and time of the day).

3) Study possible age/temperament specific ranges of variation for HRV in the populations in order to estimate subject numbers needed for studies comparing HRV in response to intrinsic and environmental/social factors.

4) A within-subject change in HRV, recorded before and after a treatment is applied, is more meaningful than between subject/group comparisons.

Conclusion

The moderating effect of individual differences must be considered in future research of environment influences on cortisol levels of young children. Moreover, considering the dynamics and individual differences in cortisol secretion, it is important to detect the interplay of environmental influences and individual characteristics.

When studying the environmental influences, consideration of the multi-faceted constructs related to early development is necessary. Possible mediating effects of environmental factors may include interaction and social-emotional regulation in the family, environmental controllability and social competence, and the childcare environment including social interaction with peers. Prospective examination is needed on the long-term outcomes of various individual characteristics and environmental influences in early experience that are related to reactivity differences and atypical patterns of cortisol, both hypercortisolism and hypocortisolism.

To avoid under-representation of the links between personal characteristics and cortisol responses, one may try a data aggregation method and determine the optimal sampling time of salivary cortisol. Considering the dynamics of cortisol levels, further research must observe and recode the activities of the individual child, peers, and caregivers in the classroom in order to investigate possible sources of variation and to explore situational effects.

Future research should provide a more complete picture of the temporal course of emotional reactivity and regulation. Measuring emotional regulation ability by using physiological indicators, one should take into account the baseline pattern, the reactive response, and the tendency of recovery to baseline.

REFERENCES

Adam, E. (2012). Emotion-cortisol transactions occur over multiple time scales in development: Implications for research on emotion and the development of emotional disorders. *Monographs of the Society for Research in Child Development, 77,* 17-27. [doi:10.1111/j.1540-5834.2012.00657.x](doi:10.1111/j.1540-5834.2012.00657.x)

Bayart, F., Hayashi, K. T., Faull, K. F., Barchas, J. D., & Levine, S. (1990). Influence of maternal proximity on behavioral and physiological responses to separation in infant rhesus monkeys (*Macaca mulatta*). *Behavioral Neuroscience, 104,* 108-115. [doi:10.1037/0735-7044.104.1.98](doi:10.1037/0735-7044.104.1.98)

Beauchaine, T. (2001). Vagal tone, development, and Gray's motivational theory: Toward an integrated model of autonomic nervous system functioning in psychopathology. *Development and Psychopathology, 13,* 183-214. [doi:10.1017/S0954579401002012](doi:10.1017/S0954579401002012)

Binder, E. B., Bradley, R. G., Liu, W., Epstein, M. P., Deveau, T. C., Mercer, K. B., Tang, Y., Gillespie, C. F., Heim, C. M., Nemeroff, C. B., Cubells, J. F., & Ressler, K. J. (2008). Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *Journal of the American Medical Association, 299,* 1-5. doi:10.1001/jama.299.11.1291

Calkins, S. D. (1997). Cardiac vagal tone indices of temperamental reactivity and behavioral regulation in young children. *Developmental Psychobiology, 31,* 125-135. doi:10.1002/(SICI)1098-2302(199709)31:2<125::AID-DEV5>3.0.CO;2-M

Calkins, S. D., & Dedmon, S. A. (2000). Physiological and behavioral regulation in two-year-old children with aggressive/destructive behavior problems. *Journal of Abnormal Child Psychology, 28,* 103-118. [doi:10.1023/A:1005112912906](doi:10.1023/A:1005112912906)

Calkins, S. D., & Fox, N. A. (2002). Self-regulatory processes in early personality development: A multilevel approach to the study of childhood social withdrawal and aggression. *Development & Psychopathology, 14,* 477-498. [doi:10.1017/S095457940200305X](doi:10.1017/S095457940200305X)

Calkins, S. D., & Swingler, M. M. (2012). Psychobiological measures of temperament in childhood. In M. Zentner, & R. L. Shiner (Eds.), *Handbook of temperament* (pp. 229-247). New York, NY: Guilford Press.

Clarke-Stewart, K. A. (1973). Interactions between mothers and their young children: Characteristics and consequences. *Monographs of the Society for Research in Child Development, 38,* 6-7. [doi:10.2307/1165928](doi:10.2307/1165928)

Coplan, R. J., Rubin, K. H., Fox, N. A., Calkins, S. D., & Stewart, S. (1994). Being alone, playing alone and acting alone: Distinguishing among reticence, and passive- and active-solitude in young children. *Child Development, 65,* 129-137. [doi:10.2307/1131370](doi:10.2307/1131370)

Crockenberg, S. (2003). Rescuing the baby from the bath water: How temperament and gender (may) moderate effects of child care on child development. *Child Development, 74,* 968-972. [doi:10.1111/1467-8624.00585](doi:10.1111/1467-8624.00585)

Crockenberg, S. C., & Leerkes, E. M. (2004). Infant and maternal behaviors regulate infant reactivity to novelty at 6 months. *Developmental Psychology, 40,* 1123-1132. [doi:10.1037/0012-1649.40.6.1123](doi:10.1037/0012-1649.40.6.1123)

Davis, E. P., Donzella, B., Krueger, W. K., & Gunnar, M. R. (1999). The start of a new school year: Individual differences in salivary cortisol response in relation to child temperament. *Developmental Psychobiology, 35,* 188-196. doi:10.1002/(SICI)1098-2302(199911)35:3<188::AID-DEV3>3.0.CO;2-K

De Kloet, E. R., Sibug, R. M., Helmerhorst, F. M., & Schmidt, M. (2005). Stress, genes and the mechanism of programming the brain for later life. *Neuroscience & Biobehavioral Reviews, 29,* 271-281. doi:10.1016/j.neubiorev.2004.10.008

Dettling, A. C., Gunnar, M. R., & Donzella, B. (1999). Cortisol levels of young children in full-day childcare centers: Relations with age and temperament. *Psychoneuroendocrinology, 24,* 505-518. [doi:10.1016/S0306-4530(99)00009-8](doi:10.1016/S0306-4530(99)00009-8)

Dettling, A. C., Parker, S. W., Lane, S., Sebanc, A., & Gunnar, M. R. (2000). Quality of care and temperament determine whether cortisol levels rise over the day for children in full-day childcare. *Psychoneuroendocrinology, 25,* 819-836. [doi:10.1016/S0306-4530(00)00028-7](doi:10.1016/S0306-4530(00)00028-7)

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin, 130,* 355-391. [doi:10.1037/0033-2909.130.3.355](doi:10.1037/0033-2909.130.3.355)

Dozier, M., Peloso, E., Lewis, E., Laurenceau, J., & Levin, S. (2008). Effects of an attachment-based intervention on the cortisol production of infants and toddlers in foster care. *Development and Psychopathology, 20,* 845-859. [doi:10.1017/S0954579408000400](doi:10.1017/S0954579408000400)

Eisenberg, N., Fabes, R. A., Murphy, B., Maszk, P., Smith, M., & Karbon, M. (1995). The role of emotionality and regulation in children's social functioning: A longitudinal study. *Child Development, 66,* 1360-1384. [doi:10.2307/1131652](doi:10.2307/1131652)

Fabes, R. A., Eisenberg, N., & Eisenbud, L. (1993). Behavioral and physiological correlates of children's reactions to others in distress. *Developmental Psychology, 29,* 655-663. [doi:10.1037/0012-1649.29.4.655](doi:10.1037/0012-1649.29.4.655)

Fabes, R. A., Eisenberg, N., Karbon, M., Troyer, D., & Switzer, G. (1994). The relations of children's emotion regulation to their vicari-

ous emotional responses and comforting behaviors. *Child Development, 65,* 1678-1693. doi:10.2307/1131287

Gillespie, C. F., Phifer, J., Bradley, B., & Ressler, K. J. (2009). Risk and resilience: Genetic and environmental influences on development of the stress response. *Depress Anxiety, 26,* 984-992. doi:10.1002/da.20605

Goeders, N. E. (2003). The impact of stress on addiction. *European Neuropsychopharmacology, 13,* 435-441. doi:10.1016/j.euroneuro.2003.08.004

Gold, P. W., & Chrousos, G. P. (2002). Organization of the stress system and its dysregulation in melancholic and atypical depression: High vs low CRH/NE states. *Molecular psychiatry, 7,* 254-275. doi:10.1038/sj.mp.4001032

Graham, Y. P., Heim, C., Goodman, S. H., Miller, A. H., & Nemeroff, C. B. (1999). The effects of neonatal stress on brain development: Implications for psychopathology. *Development and Psychopathology, 11,* 545-565. doi:10.1017/S0954579499002205

Greenspan, S. I. (2003). Child care research: A clinical perspective. *Child Development, 74,* 1064-1068. doi:10.1111/1467-8624.00591

Gunnar, M. R. (1980a). Contingent stimulation: A review of its role in early development. In S. Vevine, & H. Ursin (Eds.), *Coping and health* (pp. 101-119). New York: Plenum. doi:10.1007/978-1-4684-1042-6_6

Gunnar, M. R. (1980b). Control, warning signals, and distress in infancy. *Developmental Psychology, 16,* 281-289. doi:10.1037/0012-1649.16.4.281

Gunnar, M. R. (2001). The role of glucocorticoids in anxiety disorders: A critical analysis. In M. W. Vasey, & M. R. Dadds (Eds.), *The developmental psychopathology of anxiety* (pp. 143-159). New York: Oxford Press.

Gunnar, M. R., & Donzella, B. (2002). Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology, 27,* 199-220. doi:10.1016/S0306-4530(01)00045-2

Gunnar, M. R., Kryzer, E., Van Ryzin, M. J., & Phillips, D. A. (2010). The rise in cortisol in family day care: Associations with aspects of care quality, child behavior, and child sex. *Child Development, 81,* 851-869. doi:10.1111/j.1467-8624.2010.01438.x

Gunnar, M. R., Larson, M., Hertsgaard, L., Harris, M., & Brodersen, L. (1992). The stressfulness of separation among 9-month-old infants: Effects of social context variables and infant temperament. *Child Development, 63,* 290-303. doi:10.2307/1131479

Gunnar, M. R., Morison, S. J., Chisholm, K., & Schuder, M. (2001). Salivary cortisol in children adopted from Romanian orphanages. *Development and Psychopathology, 13,* 611-628. doi:10.1017/S095457940100311X

Gunnar, M. R., Tout, K., deHaan, M., Pierce, S., & Stansbury, K., (1997). Temperament, social competence, and adrenocortical activity in preschoolers. *Developmental Psychobiology, 31,* 65-85. doi:10.1002/(SICI)1098-2302(199707)31:1<65::AID-DEV6>3.0.CO:2-S

Gunnar, M. R., & White, B. P. (2001). Salivary cortisol measures in infant and child assessment. In L. T. Singer, & P. S. Zeskind (Eds.), *Biobehavioral assessment of the infant* (pp. 167-189). New York: Guilford Press.

Heim, C., Owen, M. J., Plotsky, P. M., & Nemeroff, C. B. (1997). The role of early adverse life events in the etiology of depression and posttraumatic stress disorder: Focus on corticotropin-releasing factor. *Annals of the New York Academy of Sciences, 821,* 194-207. doi:10.1111/j.1749-6632.1997.tb48279.x

Heinrichs, M., Baumgartner, T., Kirschbaum, C., & Ehlert, U. (2003). Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biological Psychiatry, 54,* 1389-1398. doi:10.1016/S0006-3223(03)00465-7

Hertsgaard, L., Gunnar, M. R., Larson, M., Brodersen, L., & Lehman, H. (1992). First time experiences in infancy: When they appear to be pleasant, do they activate the adrenocortical stress response? *Developmental Psychobiology, 25,* 319-334. doi:10.1002/dev.420250504

Jarvis, P. A., & Creasey, G. L. (1991). Parental stress, coping and attachment in families with an eighteen-month-old infant. *Infant Behavior and Development, 14,* 383-395. doi:10.1016/0163-6383(91)90029-R

Joffe, J., Rawson, R., & Mulick, J. (1973). Control of their environment reduces emotionality in rats. *Science, 180,* 1383-1384. doi:10.1126/science.180.4093.1383

Kirschbaum, C., & Hellhammer, D. H. (1989). Salivary cortisol in psychobiological research: An overview. *Neuropsychobiology, 22,* 150-169. doi:10.1159/000118611

Lamb, M. E. (1998). Nonparental child care: Context, quality, correlates, and consequences. In W. Damon, I. E. Sigel, & K. A. Renninger (Eds.), *Handbook of child psychology* (*Vol.* 4) *Child psychology in practice* (5th ed., pp. 73-133). New York: Wiley.

Larson, M., Gunnar, M. R., & Hertsgaard, L. (1991). The effects of morning naps, car trips, and maternal separation on adrenocortical activity in human infants. *Child Development, 62,* 362-372. doi:10.2307/1131009

Legendre, A., & Trudel, M. (1996). Cortisol and behavioural responses of young children in a group of unfamiliar peers. *Merrill-Palmer Quarterly, 42,* 554-577.

Li, I., & Shen, P. S. (2008). Internalizing disposition and preschool children's cortisol fluctuations. *Child: Care, Health, & Development, 34,* 626-630. doi:10.1111/j.1365-2214.2008.00847.x

Li, I., Chiou, H. H., & Shen, P. S. (2007). Correlations between cortisol level and internalizing disposition of young children are increased by selecting optimal sampling times and aggregating data. *Developmental Psychobiology, 49,* 633-639. doi:10.1002/dev.20239

Luby, J. L., Heffelfinger, A. K., Mrakotsky, C., Hessler, M. J., Brown, K. M., & Hildebrand, T. (2002). Preschool major depressive disorder: Preliminary validation for developmentally modified *DSM-IV* criteria. *Journal of the American Academy of Child and Adolescent Psychiatry, 41,* 928-937. doi:10.1097/00004583-200208000-00011

Mathew, S. J., Coplan, J. D., Goetz, R. R., Feder, A., Greenwald, S., Dahl, R. E., Ryan, N. D., Mann, J. J., & Weissman, M. M. (2003). Differentiating depressed adolescent twenty-four hour cortisol secretion in light of their adult clinical outcome. *Neuropsychopharmacology, 28,* 1336-1343. doi:10.1038/sj.npp.1300184

McEwen, B. S. (1998). Protective and damaging effects of stress mediators. *New England Journal of Medicine, 338,* 171-179.

Mineka, S., Gunnar, M. R., & Champoux, M. (1986). Control and early socio-emotional development: Infant rhesus monkeys reared in controllable versus uncontrollable environments. *Child Development, 57,* 1241-1256. doi:10.2307/1130447

Ouellet-Morin, I., Boivin, M., Dionne, G., Lupien, S. J., Arseneault, L., Barr, R. G., Pérusse, D., & Tremblay, R. E. (2008). Variations in heritability of cortisol reactivity to stress as a function of early familial adversity among 19-month-old twins. *Archives of General Psychiatry, 65,* 211-218. doi:10.1001/archgenpsychiatry.2007.27

Pettit, G. S., & Bates, J. E., (1984). Continuity of individual-differences in the mother-infant relationship from 6 to 13 months. *Child Development, 55,* 729-739. doi:10.2307/1130125

Porges, S. W. (1995). Orienting in a defensive world: Mammalian modifications of our evolutionary heritage. A polyvagal theory. *Psychophysiology, 32,* 301-318. doi:10.1111/j.1469-8986.1995.tb01213.x

Porges, S. W. (2001). The polyvagal theory: Phylogenetic substrates of a social nervous system. *International Journal of Psychophysiology, 42,* 123-146. doi:10.1016/S0167-8760(01)00162-3

Porges, S. W., Doussard-Roosevelt, J. A., Portales, A. L., & Suess, P. E. (1994). Cardiac vagal tone: Stability and relation to difficultness in infants and three-year-old children. *Developmental Psychobiology, 27,* 289-300. doi:10.1002/dev.420270504

Propper, C., & Moore, G. A. (2006). The influence of parenting on infant emotionality: A multi-level psychobiological perspective. *Developmental Review, 26,* 427-460. doi:10.1016/j.dr.2006.06.003

Raine, A. (2002). Biosocial studies of antisocial and violent behavior in children and adults: A review. *Journal of Abnormal Child Psychology, 30,* 311-326. doi:10.1023/A:1015754122318

Roma, P. G., Champoux, M., & Suomi, S. J. (2006). Environmental control, social context, and individual differences in behavioral and cortisol responses to novelty in infant rhesus monkeys. *Child Development, 77,* 118-131. doi:10.1111/j.1467-8624.2006.00860.x

Rosenblum, K. R., McDonough, S. C., Muzik, M., Miller, A. L., & Sameroff, A. J. (2002). Maternal representations of the infant: Ef-

fects on infant response to the still-face. *Child Development, 73,* 999-1015. doi:10.1111/1467-8624.00453

Rothbart, M. K., & Bates, J. E. (1998). Temperament. In W. Damon (Series Ed.), & N. Eisenberg (Vol. Ed.) (Eds.), *Handbook of CHILD Psychology: Vol.* 3. *Social*, *emotional and personality development* (5th ed., pp. 105-176). New York: Wiley.

Santucci, A., Silk, J. S., Shaw, D. S., Gentzler, A., Fox, N., & Cohn, J. (2008). Vagal tone and temperament as predictors of emotion regulation strategies in young children. *Developmental Psychobiology, 50,* 205-216. doi:10.1002/dev.20283

Schulkin, J. (2003). *Rethinking homeostasis.* Cambridge: MIT Press.

Segerstrom, S. C., & Miller, G. E. (2004). Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry. *Psychological Bulletin, 104,* 601-630.
doi:10.1037/0033-2909.130.4.601

Sims, M., Guilfoyle, A., & Parry, T. (2006). Children's cortisol levels and quality of child care provision. *Child: Care, Health & Development, 32,* 452-466. doi:10.1111/j.1365-2214.2006.00632.x

Sterling, P., & Eyer, J. (1988). Allostasis: A new paradigm to explain arousal pathology. In S. Fisher, & J. Reason (Eds.), *Handbook of life stress, cognition and health* (pp. 629-649). New York: John Wiley & Sons.

Stifter, C. A., & Jain, A. (1996). Psychophysiological correlates of infant temperament: Stability of behavior and autonomic patterning from 5 to 18 months. *Developmental Psychobiology, 29,* 379-391.
doi:10.1002/(SICI)1098-2302(199605)29:4<379::AID-DEV5>3.0.CO;2-N

Stifter, G. A., & Fox, N. A. (1990). Infant reactivity: Physiological correlates of newbom and 5-month temperament. *Developmental Psychology, 26,* 582-588. doi:10.1037/0012-1649.26.4.582

Suchecki, D., Rosenfeld, P., & Levine, S. (1993). Maternal regulation of the hypothalamic-pituitary-adrenal axis in the infant rat: The roles of feeding and stroking. *Developmental Brain Research, 75,* 185-192.
doi:10.1016/0165-3806(93)90022-3

Susman, E. J., & Pajer, K. (2004). Biology behavior integration and antisocial behaavior in girls. In M. Putallaz, & K. L. Bierman (Eds.), *Aggression, antisocial behaavior, and violence among girls* (pp. 23-47). New York: Guilford Press.

Susman, E. J. (2006). Psychobiology of persistent antisocial behavior: Stress, early vulnerabilities and the attenuation hypothesis. *Neuroscience and Biobehavioral Reviews, 30,* 376-389.
doi:10.1016/j.neubiorev.2005.08.002

Thayer, J. F., & Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *Journal of Affective Disorders, 61,* 201-216. doi:10.1016/S0165-0327(00)00338-4

Thayer, J. F., & Lane, R. D. (2009). Claude bernard and the heart-brain connection: Further elaboration of a model of neurovisceral integration. *Neuroscience & Biobehavioral Reviews, 33,* 81-88.
doi:10.1016/j.neubiorev.2008.08.004

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers III, J. J., & Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: Implications for heart rate variability as a marker of stress and health. *Neuroscience & Biobehavioral Reviews, 36,* 747-756.
doi:10.1016/j.neubiorev.2011.11.009

Thompson, L. A., & Trevathan, W. R. (2008). Cortisol reactivity, maternal sensitivity, and learning in three-month-old infants. *Infant Behavior and Development, 31,* 92-105.
doi:10.1016/j.infbeh.2007.07.007

Tiller, W. A., McCraty, R., & Atkinson, M. (1996). Cardiac coherence: A new, noninvasive measure of autonomic nervous system order. *Alternative Therapies in Health and Medicine, 2,* 52-65.

Tyrka, A. R., Price, L. H., Marsit, C., Walters, O. C., & Carpenter, L. L. (2012). Childhood adversity and epigenetic modulation of the leukocyte glucocorticoid receptor: Preliminary findings in healthy adults. *PLoS One, 7,* e30148. doi:10.1371/journal.pone.0030148

Tyrka, A. R., Wier, L., Price, L. H., Ross, N., Anderson, G. M., Wilkinson, C. W., & Carpenter, L. L. (2008). Childhood parental loss and adult hypothalamic-pituitary-adrenal function. *Biological Psychiatry, 63,* 1247-1254. doi:10.1016/j.biopsych.2009.08.014

Vanitallie, T. B. (2002). Stress: A risk factor for serious illness. *Metabolism, 51,* 40-45. doi:10.1053/meta.2002.33191

Von Borell, E., Langbein, J., Després, G., Hansen, S., Leterrier, C., Marchant-Forde, J., Marchant-Forde, R., Minero, M., Mohr, E., & Prunier, A. (2007). Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals—A review. *Physiology & Behavior, 92,* 293-316.
doi:10.1016/j.physbeh.2007.01.007

Watamura, S. E., Donzella, B., Alwin, J., & Gunnar, M. R. (2003). Morning to afternoon increases in cortisol concentrations for infants and toddlers at child care: Age differences and behavioral correlates. *Child Development, 74,* 1006-1020. doi:10.1111/1467-8624.00583

White, R. W. (1959). Motivation reconsidered: The concept of competence. *Psychological Review, 66,* 279-333. doi:10.1037/h0040934

Wilson, R. S., & Matheny Jr., A. P. (1986). Behavior genetics research in infant temperament: The Louisville twin study. In R. Plomin, & J. Dunn (Eds.), *The study of temperament: Changes*, *continuities and challenges* (pp. 81-97). Hillsdale, NJ: Erlbaum.