EXAMINING INFANTS' CORTISOL RESPONSES TO LABORATORY TASKS AMONG CHILDREN VARYING IN ATTACHMENT DISORGANIZATION: STRESS REACTIVITY OR RETURN TO BASELINE?

by

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This manuscript is dedicated to:

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ABSTRACT

Cortisol is a hormone involved in mounting a stress response in humans. During infancy, however, cortisol appears to be relatively unresponsive to a number of stressors. This developmental period of cortisol hypo-reactivity in human infants may be similar to the stress hyporesponsive period (SHRP) in rodent pups. In both rodent and human models, maternal care has been implicated as an important factor in the regulation of infants' physiological responses to stressors. However, findings regarding this effect in humans have been relatively ambiguous due to lack of adequate baseline measures of cortisol activity. In the present study, the order of two laboratory tasks (i.e., Strange Situation and play) was counterbalanced and home saliva samples were obtained in order to control for basal activity of cortisol more systematically. Saliva samples were also collected upon arrival at lab, and at 40, 65, and 80 minutes after arrival. Changes in cortisol were examined using piecewise hierarchical linear modeling, testing whether observed increases reflected a return to baseline or stress reactivity. An interaction between attachment disorganization and task emerged, such that disorganized infants showed increases in cortisol in response to the stressor compared to play, whereas organized infants did not show cortisol reactivity to either task. Implications for the buffering effects of maternal care on the maintenance of hyporesponsivity in infants are discussed.

Chapter 1

INTRODUCTION

Infancy marks a unique period of development during which important changes take place in the neurobiology of the human stress response (Gunnar & Donzella, 2002). Across the first year of life, children appear to dampen their cortisol reactivity to acute stressors. Rodents experience a similar period of relative hypo-reactivity, termed the stress hyporesponsive period (SHRP) (Sapolsky & Meaney, 1986). Research focused on this developmental period in human infants contributes to our broader understanding of the neurobiology of the stress response. Given associations between dysregulated cortisol production and psychopathology (DeBellis et al., 1999; Goodyer, Park, & Herbert, 2001; Yehuda, 2001), it is important to have a clear understanding of how the stress response changes throughout development and what factors contribute to these changes.

Glucocorticoids (cortisol in humans, corticosterone in rodents) are steroid hormones produced as an end product of the hypothalamic-pituitary-adrenocorical (HPA) system. In addition to mounting a stress response, glucocorticoids serve a major role in maintaining circadian patterns of daily activity, such as waking and sleeping (Gunnar & Cheatham, 2003). Basal, or diurnal, levels of cortisol vary across the day.

In humans, diurnal cortisol levels peak about 30 minutes after wake-up, decrease sharply by mid-morning, and continue to decrease gradually until bedtime (Gunnar & Donzella, 2002). This diurnal pattern remains relatively consistent from around 3 months of age through adulthood (Larson, White, Cochran, Donzella, & Gunnar, 1998; Price, Close, & Fielding, 1983), although the gradual decline from mid-morning to afternoon is less reliably observed in children under 4-years-old (Bruce, Davis, & Gunnar, 2002; Watamura, Donzella, Alwin, & Gunnar, 2003). Atypical diurnal patterns are associated with a number of adverse early experiences, such as foster care and institutional care (Carlson & Earls, 1997; Cicchetti & Rogosch, 2001; Dozier et al., 2006), as well as psychopathology later on (Charney, 2004; Young, Carlson, & Brown, 2001).

Responses of cortisol to stressors are superimposed upon this diurnal pattern. The norepinephrine-sympathetic adrenomedullary (NE-SAM) system and the hypothalamic-pituitary-adrenocortical (HPA) system both function in the mounting of a stress response (Gunnar & Quevedo, 2007). Following a stressor, corticotrophin-releasing hormone (CRH) is released by the paraventricular nuclei of the hypothalamus, which travels through the bloodstream to the anterior pituitary. Adrenocorticotropic hormone (ACTH) is then released, which signals production and release of glucocorticoids (i.e., cortisol in humans) by the adrenal gland (Gunnar & Quevedo, 2007). This cascade of biochemical reactions promotes immediate survival by preparing the organism for a fight or flight response. Thus, energy is directed away

from processes less critical to immediate survival, such as immune functioning, growth, digestion, and reproduction (Gunnar & Cheatham, 2003).

Developmental Changes in Response to Stress

The past two decades of research regarding HPA function have helped shape our current understanding of important developmental changes in the stress response. Elevations in cortisol among adults are elicited fairly consistently when situations include elements of unpredictability, uncontrollability, and social-evaluative threat (for a review, see Dickerson & Kemeny, 2004). Studies of cortisol reactivity in children, however, suggest a far more complex story. A variety of paradigms have been used to examine children's cortisol responses to stress, including mild pain tasks (e.g., inoculations, in Lewis & Ramsay, 1995), fear and anger tasks (e.g., still face paradigm, in Lewis & Ramsay, 2005), and maternal separation tasks (e.g., Strange Situation, in Spangler, 1993). Given the marked changes in cognitive, social, and emotional abilities during childhood, the ineffectiveness of particular tasks in eliciting cortisol reactivity may in part reflect choices of inappropriate stressors (see Gunnar, Talge, & Hererra, 2009). However, important patterns emerge across tasks, offering converging support of the development of a period of cortisol hypo-reactivity during infancy. From birth until about 3 or 4 months of age, infants reliably demonstrate a rise in cortisol following various stressor tasks, including mild pain tasks (Gunnar, Porter, Wolf, Rigatuso, & Larson, 1995; Lewis & Ramsay, 1995; Mantagos, Koulouris, &

Vagenakis, 1991; Ramsay & Lewis, 1994) and physical examinations (Gunnar, Brodersen, Krueger, & Rigatuso, 1996; Gunnar, Connors, & Isensee, 1989; Keenan, Gunthorpe, & Young, 2002). By 6 months of age, however, stressor tasks elicit increases in cortisol less reliably, and by 12 months it is even more difficult to elicit an increase in cortisol. This apparent dampening of cortisol reactivity over the first year has been reported for mild pain tasks (Gunnar, Brodersen, Krueger et al., 1996; Gunnar & Nelson, 1994; Jacobson, Bihun, & Chiodo, 1999; Lewis & Ramsay, 1995), fear and anger tasks (Buss et al., 2003; Goldberg et al., 2003; Lewis & Ramsay, 2005; Ouellet-Morin et al., 2008) and maternal separation tasks (Dozier, Peloso, Lewis, & Laurenceau, 2008; Gunnar, Mangelsdorf, Larson, & Hertsgaard, 1989; Gunnar & Nelson, 1994).

Relative Stress Hyporesponsive Period

The human developmental period marked by an inhibited cortisol response may be analogous to a period in rodents called the relative stress hyporesponsive period (SHRP). Rodents experience a period from about 4 to 14 days postnatally during which cortisol increases are not observed following a variety of stressors (Rosenfeld, Suchecki, & Levine, 1992; Sapolsky & Meaney, 1986). This dampening of the cortisol response may serve to protect the developing brain from a number of deleterious effects of elevated glucocorticoids (Gunnar, 1998; Gunnar, Fisher, & The Early Experience, Stress, and Prevention Network, 2006). In rodents, high levels of

glucocorticoids during this developmental period produce changes in brain regions associated with memory and learning (McEwen et al., 1992; Sapolsky & Meaney, 1986; Sapolsky, Romero, & Munck, 2000) in addition to apoptosis, or cell death, and dendritic atrophy (Gunnar & Donzella, 2002; Sapolski, 2002a, 2002b). Thus, the relative stress hyporesponsive period is likely an evolutionary adaptation that serves to protect the infant during particularly sensitive periods of development.

Human development may be characterized by a similar period of hypo-reactivity, emerging across the first year of development. As with the rodent, the maintenance of a stress hyporesponsive period may be important to the healthy development of specific brain regions, including the hippocampus and prefrontal cortex (Gunnar et al., 2006). It is unclear how long this period lasts in human children, although Gunnar et al. (2006) have suggested that it extends throughout childhood.

Effects of Quality of Care on Cortisol Response

In rodents, maternal care plays a critical role in the maintenance of the stress hyporesponsive period (Francis & Meaney, 1999; Gunnar et al., 2006). Maternal deprivation studies with rats offer a compelling model for the buffering effects of maternal care for rodent pups. During the stress hyporesponsive period, corticosterone increases are not elicited by novelty stressors following short separations (e.g., 1 hour); however, pups exhibit elevations in corticosterone to novelty stressors following longer separations (e.g., 24 hours) (Graham, Heim, Goodman, Miller, & Nemeroff,

1999). The magnitude of the corticosterone response to the stressor is positively correlated with the duration of the preceding maternal deprivation (Stanton, Gutierrez, & Levine, 1988). Furthermore, maternal behaviors, such as licking and grooming, appear to be important in these buffering effects on infant stress reactivity (Caldji et al., 1998, Rosenfeld et al., 1992; Suchecki, Rosenfeld, & Levine, 1993). Thus, in rodents, the dam (i.e., mother) serves as an important regulator of infant physiology during the stress hyporesponsive period.

The quality of maternal care in humans has also been suggested to serve an important role in cortisol reactivity in infants. Although this claim fits with the rodent literature, there is limited empirical evidence. Attachment quality, assessed using the Strange Situation procedure, reflects the quality of maternal care received by infants (Ainsworth, Blehar, Waters, & Walls, 1978). Whereas most infants develop organized strategies for managing stressful circumstances in the presence of a parent, some infants lack a coherent strategy. Disorganized attachment reflects a "breakdown" of a strategy, and is associated with specific parenting behaviors, such as maltreatment and frightening behavior (Main & Solomon, 1990). A few studies have reported associations between attachment disorganization and cortisol reactivity. Spangler and Grossman (1993) examined cortisol reactivity to the Strange Situation in a low-risk sample of 41 twelve-month-olds. Relative to infants with secure attachments, infants with insecure attachments, and especially those with disorganized attachments, showed higher cortisol levels following the Strange Situation. In a similar study of 38

nineteen-month-old infants, Hertsgaard, Gunnar, Farrell, Erickson, & Nachmias (1995) found that infants with disorganized attachments had significantly higher levels of cortisol after the Strange Situation compared to infants with organized attachments. Both of these studies offer exciting preliminary evidence of the effects of quality of maternal care on the stress response. However, their results are difficult to interpret in and of themselves, primarily due to the lack of baseline or control measures of cortisol activity, a critical methodological issue described in more detail below. Additional studies have also found associations between insecurity and cortisol reactivity, but only when insecure children were also fearful (Gunnar, Brodersen, Nachmias, Buss, & Rigatuso, 1996) or temperamentally inhibited (Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996).

Appropriate Baseline Samples

In order to ensure that rises in cortisol represent reactivity to a stressor, it is important to include appropriate measures of baseline levels of cortisol during non-stress conditions. Of the four studies cited above that reported associations between attachment classification and cortisol reactivity, three relied on a pretest measure of cortisol obtained immediately before the stressor (i.e., Gunnar et al., 1995; Nachmias et al., 1996; Spangler & Grossman, 1993), and one did not include a baseline measure of cortisol (i.e., Hertsgaard et al., 1995). Although a pretest measure may seem sufficient, Gunnar et al. (1989) reported an unanticipated finding regarding differences

in basal levels of cortisol that is important to take into account. Specifically, comparison of cortisol samples collected upon arrival at the laboratory and home samples collected at the same time on a different day revealed that cortisol levels of laboratory baseline samples were significantly lower than those of home baseline samples. Larson, Gunnar, & Hertsgaard (1991) further examined this finding by having mothers collect cortisol samples before and after a 40-minute car ride to the laboratory. They found a significant decrease in cortisol levels following the car ride, resulting in "baseline" levels at laboratory arrival that were significantly lower than "baseline" levels at home. These results were not associated with napping or drowsiness during the car ride. It is not clear what aspect of riding in the car produced decreases in cortisol below baseline, but some have suggested that it may be associated with visual and auditory stimulation, physical restraint in a car seat, or behavioral calming (Gunnar & Donzella, 2002; Larson et al., 1991). Other studies have reported lowering of cortisol below baseline in response to novel situations, such as mother-infant swim classes (Hertsgaard, Gunnar, Larson, Brodersen, & Lehman, 1992). Although the purpose for suppressions in cortisol is unclear, these studies highlight the need for appropriate baseline measures of cortisol. Increases in cortisol in the laboratory may be imposed on already lowered baselines, making findings ambiguous and difficult to interpret. Without adequate comparison samples, it is unclear whether increases in cortisol following a laboratory task represent cortisol reactivity or simply return to baseline (i.e., home) levels.

The Present Study

Despite accumulating support for a hyporesponsive period, researchers continue to use cortisol as a measure of response to stress during infancy. The present study returned to basic questions regarding the stress response in young infants. Specifically, the present study was designed to systematically examine whether changes in cortisol levels during a laboratory visit reflect reactivity to a stressor or return to baseline levels (following a decrease during the car ride). Infants were brought into the laboratory for two tasks, including a period of free play and a stressor (i.e., Strange Situation). The order of tasks was counterbalanced such that half of the participants experienced the stressor first followed by free play, and half experienced free play followed by the stressor. Time of day was controlled, with laboratory visits scheduled as close to 9 a.m. as possible. Saliva samples were obtained at home prior to the car ride to the laboratory, upon arrival at the laboratory, and at 40, 65, and 80 minutes following arrival at the lab.

The design of the study allowed for examination of competing hypotheses. If increases in cortisol reflect a return to baseline levels, we would expect that infants' cortisol levels would increase following the first task, regardless of whether it was a stressor or period of play. This return to baseline would presumably follow a drop in cortisol experienced prior to arrival at the laboratory. On the other hand, increases in cortisol may reflect reactivity to a stressor. If this were the case, we would expect levels of cortisol to increase following the stressor, but not following the play,

regardless of the episode order. Thus, the main hypotheses tested in the present study examined the main effect of type of task (i.e., stressor or play) versus the main effect of episode order (i.e., first or second).

Additionally, the present study examined the association between attachment disorganization and cortisol reactivity. Given findings of Spangler & Grossman (1993) and Hertsgaard et al. (1995), if some children do show stress reactivity as opposed to return to baseline, we might expect to see this especially for infants with disorganized attachments. Specifically, attachment disorganization may moderate the association between type of task and increases in cortisol, with children higher on attachment disorganization showing cortisol reactivity to the stressor compared with play, and children lower on attachment disorganization showing lack of cortisol reactivity in both contexts.

Chapter 2

METHOD

Participants

Participants included 32 infants who ranged in age from 11.3 to 20 months (*M* = 15.2, *SD* = 2.3). Participants were recruited from community daycare centers, local moms' groups, and through announcements posted on a University website. Nineteen (59%) of the children were female. Twenty-two of the children were White/non-Hispanic (69%), 5 were African American (16%), 2 were Biracial (6%), 2 were Hispanic (6%), and 1 was Asian American (3%). Parents ranged in age from 21 to 42.9 years (*M* = 32.6, *SD* = 5.3). All parents were mothers, except for one father. Most parents were married (88%), had completed college or earned an advanced degree (84%), and were employed outside of the home (69%). Family income ranged from the lowest category (< $10,000) to the highest (> $100,000) with most (53%) identifying themselves as earning more than $100,000.

Procedure

The purpose of the study and description of research activities were described by phone or email. During an initial visit, research assistants reviewed the consent

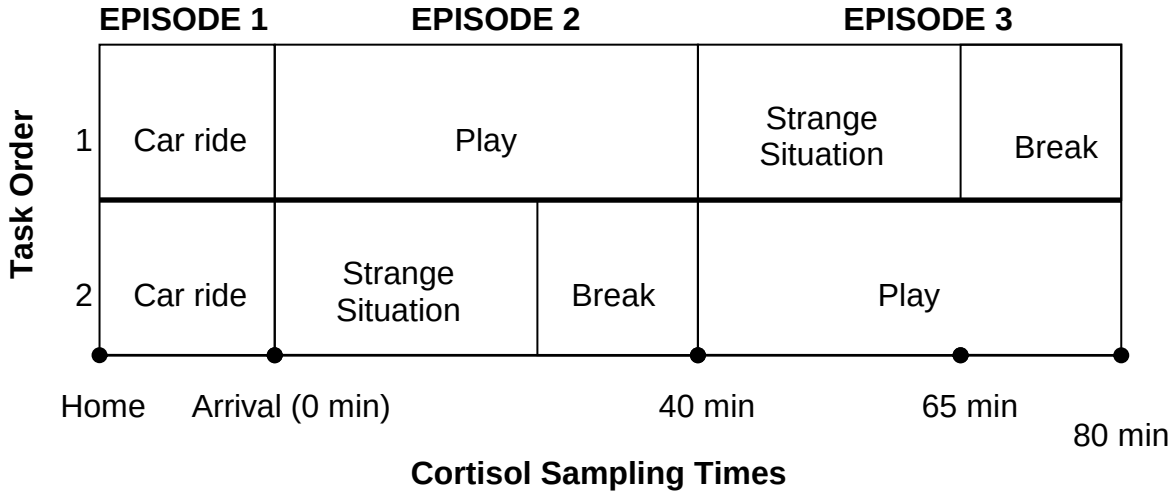
form, which described participant activities, confidentiality, and risks and benefits. This visit took place at participants' homes or another convenient location, such as the child's daycare center or parent's workplace. In addition to a review of the consent form, parents were given instructions and materials for taking a home saliva sample and directions to the laboratory. The laboratory visit was scheduled following this visit.

Laboratory visits were scheduled for the morning hours, in an attempt to control for diurnal fluctuations in cortisol production. Most visits were scheduled to start between 9:00 a.m. and 9:30 a.m. Arrival times ranged from 7:58 a.m. to 10:00 a.m. (*M* = 9:13 a.m.). The laboratory visits lasted a total of 80 minutes, and were divided into two 40-minute episodes. One of the episodes involved approximately 40 minutes of free play. Participants were brought into a room that resembled a daycare or childcare playroom, with a number of very attractive toys such as an inflatable ball pit, Little Tikes slide, and farm set. A camera in the corner of the room was set up to film the play space. There was an adult-size chair in the play room, but parents were not instructed where to sit, only to play with their child as they normally would. The experimenter waited in an adjacent room and dyads were left alone to play. The other episode involved the Strange Situation procedure, described more fully below. For this procedure, participants were brought to a different room that resembled a waiting room at a doctor's office, with chairs along the wall and several age-appropriate toys on the floor. There was a one-way mirror between this room and the observation room

used for filming. Participants were randomly assigned to the order of the tasks. Half of the participants participated in the Strange Situation followed by free play, whereas half of participants participated in free play followed by the Strange Situation. Participants changed rooms between activities, such that each episode took place in its respective room. Saliva samples were obtained 5 times, including at home before leaving, upon arrival at the lab, and at 40, 65, and 80 minutes post-arrival (See Figure 1). These intervals were chosen because peak levels of cortisol are detectable in peripheral measurements about 20 minutes after a stressor (Pollard & Ice, 2007). For infants who experienced the Strange Situation first, the 40-minute sample was expected to capture cortisol reactivity. Given that the Strange Situation is expected to be an increasingly stressful experience, the timing of this post sample (i.e., about 20 minutes after the final episode) was expected to capture effects of the mounting stress, rather than stress experienced during a particular episode (e.g., reunion versus separation). Sampling approximately 20 minutes after the Strange Situation is consistent with procedures used in previous studies (e.g., Spangler & Grossman, 2003). For those who experienced the Strange Situation second, the 80-minute sample was expected to capture cortisol reactivity. During the time between the end of the Strange Situation and the cortisol sample (approximately 20 minutes), the parent was asked to complete demographic and infant health status questionnaires while remaining in the room with the child. The inclusion of multiple samples (e.g., 65

minutes) further increased the reliability of estimating of changes in cortisol during the visit. Saliva sampling procedures are described more fully below.

Figure 1. Timeline for lab tasks and saliva samples.



Strange Situation

The Strange Situation (Ainsworth et al., 1978) is divided into eight episodes, designed to be increasingly stressful for infants. During the first episode, the dyad is brought into the room and procedures are reviewed with the parent. After the experimenter leaves, the parent and child are left alone for 3 minutes, the parent remaining relatively uninvolved as the child plays on the floor (episode 2). In episode

3, the “stranger” (i.e., unfamiliar female) enters the room. The stranger sits quietly for the first minute, interacts with the parent for the second minute, and then interacts with the child for the third minute. The parent is then signaled to leave the room for the first separation (episode 4). The child is left alone with the stranger for 30 seconds to 3 minutes, depending on the child’s level of behavioral distress. In episode 5, the parent returns to the room and the stranger leaves the room. After three minutes, the parent is signaled to leave the room for the second separation (episode 6). The stranger returns in the seventh episode after 30 seconds to 3 minutes, depending on the child’s level of distress. Finally the parent returns after 30 seconds to 3 minutes in episode 8. The Strange Situation typically lasts between 15 minutes and 25 minutes, depending on the child’s level of distress.

Attachment quality was coded from digital recordings of the Strange Situations. Infants were classified as secure (B), avoidant (A), or resistant (C) according to procedural guidelines of Ainsworth et al. (1978). Infants also received a continuous score for disorganization, ranging from 1 to 9. Infants could receive a primary classification of disorganized (D) as per procedures described by Main and Solomon (1990). All videotapes were coded by a primary coder, blind to other participant information. The primary coder (MD) had attended a training course and passed reliability tests for classifying organized and disorganized attachment strategies. A second blind coder, who also attended the Strange Situation coding training course, coded 20% of tapes for reliability. Reliability for the major

classifications (including D) for this subset of tapes was 100%. The Spearman correlation for inter-rater agreement on the continuous scale of disorganization was .82. For cases that were double-coded, continuous scores for disorganization were averaged. Additional cases were conferenced when the primary coder was unsure of classification.

Saliva sampling

Procedures for saliva sample collection were first reviewed with parents at the initial consent visit. Parents were instructed to hold one end of a dental cotton roll and moisten the other end in the child's mouth. Flavored drink crystals (Pathmark cherry-flavored drink mix) were provided to facilitate sampling. After moistening the cotton briefly in the child's mouth, parents dipped the cotton into a cup with 0.03 g of the flavored drink crystals. The cotton was then placed back in the child's mouth until it was sufficiently wet with the child's saliva. Some parents chose not to use the flavored drink crystals due to ease of sampling or due to food allergies. Recent controlled studies have reported minimal effects of sweeteners on values on the radioimmunoassay (Gordon, Peloso, Auker, & Dozier, 2004; Talge, Donzella, Kryzer, Gierens, & Gunnar, 2005).

Five saliva samples were obtained for each participant. One sample was collected at home before the drive to the lab. Parents were instructed not to give the child anything to eat or drink 30 minutes prior to sampling. The second sample was

collected immediately upon arrival at the laboratory. The remaining samples were collected at 40-, 65-, and 80-minutes post arrival. The timing of samples was chosen to capture peak levels of cortisol response, typically occurring 20 minutes after the onset of a stressor. Multiple samples allowed for analyses that model the shape of change over time. Parents completed questionnaires about infant health status variables, such as whether children were teething, sick, or had eaten prior to sampling.

Cortisol assay

All saliva samples were stored in a freezer at -20 C prior to assay procedures. Samples were assayed using Salimetrics, Inc. High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit. To minimize variability of results, all samples for one child were assayed in duplicate on the same plate. Pairs of samples with coefficients of variation greater than 10% were rerun in a later assay. For this study, inter- and intraassay coefficients of variation fell below 7% and 14%, respectively.

Chapter 3

RESULTS

Data Preparation

Before running analyses, cortisol data were examined for outliers. Although instructed otherwise, several parents reported that their children had food or drink within the 30 minutes prior to taking the home sample, or during the car ride within 30 minutes of taking the arrival sample. Values were excluded in these cases, resulting in five excluded home values and two excluded arrival at lab values. No parent identified significant illness of their child on the day of the visit, although 61% reported that the child had a runny nose or cold. Approximately 39% of children were reported to be teething. Cortisol values were not associated with either cold or teething; therefore cortisol values were not excluded for these conditions. For each sample time (home, arrival, 40-, 65-, 80-minutes), cortisol values of 3 standard deviations above the mean were considered outliers and excluded prior to further analyses, consistent with procedures used in similar studies (e.g., Dettling, Gunnar, & Donzella, 1999). This resulted in excluding one home sample, one arrival sample, and one 65-minute sample. Finally, because cortisol values were positively skewed, log 10 transformation was used to normalize the distributions, consistent with procedures used in similar

studies (Dettling, Parker, Lane, Sebanc, & Gunnar, 2000). Descriptive statistics for salivary cortisol values are presented in Table 1. Of the 32 children, all had at least three samples, with 29 having four samples, and 25 having all five samples.

Table 1. Descriptive Statistics for Raw Cortisol Levels (in µg/dl)

Sample	*n*	*M*	*SD*	*Min.*	*Max*
Home	26	.20	.19	.03	.93
Arrival at lab	28	.28	.38	.02	1.37
40 minutes post arrival	31	.33	.43	.02	1.57
65 minutes post arrival	31	.24	.37	.03	1.57
80 minutes post arrival	30	.22	.23	.04	1.35

Preliminary Analyses

Demographic variables were examined to determine whether caregiver or child characteristics were associated with cortisol values. Caregiver age, ethnicity, marital status, income, and education level, and child age and ethnicity were not associated with cortisol values at any of the time points. Cortisol was not associated with time of arrival at the laboratory, likely because the range in arrival time was already restricted

to control expected time of day effects. As a result, time of day was not included in further analyses.

Associations between attachment disorganization and demographic variables were also examined. Approximately half of the children (44%) were classified as secure in the Strange Situation, with most other children classified as disorganized (25%) or resistant (25%). Two children (6%) were classified as avoidant. Of those classified as disorganized, most children (50%) were assigned a secondary classification of resistant, with 25 percent receiving secondary classifications of each secure and avoidant. Given that hypotheses related to attachment disorganization, primary analyses included the continuous measure of attachment disorganization, rather than categorical classifications of security. The continuous scale of disorganization may better represent the dimensional nature of attachment behaviors (Fraley & Speiker, 2003), and has been used previously when examining correlates of this construct (e.g., Carlson, 1998). Attachment disorganization ranged from 1 to 6 (M = 3.6, SD = 1.77). Disorganization was not associated with child or caregiver demographic variables.

Primary Analyses

Piecewise linear growth modeling was used to examine changes in cortisol levels across three time periods: car ride to the lab, first episode in lab, second episode in lab. Hierarchical linear modeling (HLM; Raudenbush & Bryk, 2002) accounts for

the non-independence of repeated measures within individuals. HLM treats multiple observations over time as nested within persons, allowing for variability in the number and spacing of time points. Thus, the approach allows for inclusion of participants who are missing one or more points of data. Furthermore, piecewise linear growth modeling allows for the division of growth trajectories (i.e., patterns of cortisol production) into separate linear components. Rather than estimating the rate of change in cortisol (i.e., slope) across the entire visit, this approach estimated slopes for each distinct period. Therefore, it allowed for the examination of between-individual correlates of change during each period, such as type of task. A piecewise approach is a common strategy for simultaneously modeling separate components of a process, such as reactivity and recovery (e.g., Llabre, Spitzer, Saab, & Schneiderman, 2001).

The dependent variable was the log-10 transformed cortisol value measured at each time point. Time was recoded into three separate level-1 predictors to form a three-piece linear model, as depicted in Table 2. The first linear component (EPISODE 1) captured change between the home sample and arrival at lab sample. The second linear component (EPISODE 2) captured change between the arrival at lab sample and the 40-minute sample. The third linear component (EPISODE 3) captured change across the 40-, 65-, and 80-minute post arrival samples. Thus, the level-1 model was of the form

$$\text{Log cort}_{ti} = \pi_{0i} + \pi_{1i}(\text{EPISODE 1}) + \pi_{2i}(\text{EPISODE 2}) + \pi_{3i}(\text{EPISODE 3}) + e_{ti}$$

where π_{0i} represents child *i*'s log cort upon arrival at lab (coded as zero), π_{1i} represents the rate of linear change in log cort over EPISODE 1 (car ride) for child *i*, π_{2i} represents the rate of linear change in log cort over EPISODE 2 (first episode in lab) for child *i*, π_{3i} represents the rate of linear change in log cort over EPISODE 3 (second episode in lab) for child *i*, and e_{ti} represents the within-individual error in child *i*'s log cort that cannot be accounted for by initial cort (π_{0i}) or by linear change in log cort over time.

Table 2. Coding Scheme for Three-Piece Linear Model

	Sample					
Variable	Home	Arrival	40	65	80	Interpretation of πs:
EPISODE 1	-.47 [a]	0	0	0	0	Rate of change during car ride
EPISODE 2	0	0	1	1	1	Rate of change during first task
EPISODE 3	0	0	0	.625	1	Rate of change during second task

Note. One unit of time is equal to 40 minutes.

[a] The length of the car ride varied between participants, such that the recoded time of the home sample ranged from -1.25 to -.125 (50 to 5 minutes before arrival). The average amount of time spent in the car (*M* = 18.6 minutes, recoded as -.47 units) is presented above, however HLM analyses allowed for variability in TIME 1duration for each individual.

Return to baseline hypothesis

First, we examined an unconditional level 2 model with π_{0i}, π_{1i}, π_{2i}, and π_{3i} random. This allowed for examination of mean linear rates of change in cortisol during each episode using the whole sample. If infants' cortisol levels increased following the first episode, regardless of the type of task, this would support the *return to baseline* hypothesis. This return to baseline would presumably follow a drop in cortisol experienced prior to arrival at the laboratory. Results for the unconditional model are presented in Table 3. Mean rates of change were not significantly different than zero at any of the time periods (p >.05). These results do not provide evidence of a decrease in baseline levels following a car ride or an associated return to baseline levels, at least when averaging across individuals. However, variance components were significant for the slope of each TIME variable, indicating significant variability between individuals.

Table 3. Three Piece Unconditional Linear Model for Changes in Salivary Cortisol

	Salivary Cortisol				
Effect	Coefficient	*SE*	*t*	*df*	*p*
Intercept, β_{00}	-.79	.11	-7.48	31	.00
Slope, β_{10}	-.11	.17	-.63	31	.53
Slope, β_{20}	-.03	.05	-.51	31	.61
Slope, β_{30}	-.01	.08	-.11	31	.92

Note. β_{00} represents the baseline measure of cortisol at arrival to lab. β_{10}, β_{20}, and β_{30} represent the changes in salivary cortisol across EPISODE 1 (car ride), EPISODE 2 (first lab task), and EPISODE 3 (second lab task), respectively.

Stress reactivity hypothesis

Next, we examined between-individual predictors of rates of change at level 2, to explore individual differences in the slopes for each time period. This model allowed us to examine whether the type of task (i.e., Strange Situation versus play), attachment disorganization, or an interaction of the two were associated with changes in cortisol levels. The level 1 model remained the same as that described above. At

level 2, the model was expanded to include several between-subject predictors, including task (TASK; a dummy variable indicating lab task: 1 = SS, 0 = Play), attachment disorganization (DISORG; a continuous measure of disorganization score), and the interaction of task and attachment disorganization (TASXxDISORG). As the TIME 1 period represented the rate of change during the car ride, minutes in the car (CARTIME) was included as a predictor in this equation, and predictors associated with lab tasks (i.e., TASK and TASKxDISORG) were excluded. Due to concern that changes in cortisol levels may depend on baseline cortisol levels (Laws of Initial Values or LIV; Wilder, 1958), baseline cortisol values were included to control for possible LIV effects on slopes. The cortisol sample collected at home was included as the baseline measure in the slope equation for the car ride episode (i.e., EPISODE 1, π_{1i}) and the cortisol sample collected at arrival to lab was included as the baseline measure in the slope equations for the laboratory episodes (i.e., EPISODE 2, π_{2i} and EPISODE 3, π_{3i}). The resulting level 2 model can be represented as

$$\pi_{0i} = \beta_{00} + \beta_{01}(\text{TASK}) + \beta_{02}(\text{DISORG}) + \beta_{03}(\text{TASKxDISORG}) + r_{0i}$$

$$\pi_{1i} = \beta_{10} + \beta_{11}(\text{BASELINE}) + \beta_{12}(\text{CARTIME}) + \beta_{13}(\text{DISORG}) + r_{1i}$$

$$\pi_{2i} = \beta_{20} + \beta_{21}(\text{BASELINE}) + \beta_{22}(\text{TASK}) + \beta_{23}(\text{DISORG}) + \beta_{24}(\text{TASKxDISORG}) + r_{2i}$$

$$\pi_{3i} = \beta_{30} + \beta_{31}(\text{BASELINE}) + \beta_{32}(\text{TASK}) + \beta_{33}(\text{DISORG}) + \beta_{34}(\text{TASKxDISORG}) + r_{3i}$$

where π_{0i} represents the initial value of log cort at arrival to the lab for an individual, and π_{1i}, π_{2i}, and π_{3i} represent individual rates of linear change in log cort over time

during each separate episode. The term β_{00} estimates the mean lab arrival log cort value for participants when other predictors equal zero. The term β_{01} represents the difference in initial log cort value between infants who had the play episode versus those that had the SS first (i.e., main effect of task on intercept). The term β_{02} represents the change in initial log cort value as scores of attachment disorganization increase (i.e., main effect of disorganization on intercept). The term β_{03} represents the interaction of task and attachment disorganization in predicting the initial cortisol value. The equations for linear change (i.e., π_{1i}, π_{2i}, π_{3i}) can be similarly broken down in order to understand the relative contributions of each predictor on each episode's slope.

Initial cortisol values, defined as arrival at lab, differed significantly with respect to attachment disorganization. Specifically, as attachment disorganization increased, the initial cortisol value decreased significantly ($p < .05$). As this finding was unanticipated, post-hoc analyses were conducted to test whether attachment disorganization was also negatively correlated with the home sample. Indeed, although the findings were not significant, attachment disorganization tended to be inversely correlated with the home sample of cortisol ($r = -.34$, $p < .08$). Type of task (i.e., play or SS) was not associated with the initial cortisol value upon arrival at lab, suggesting that there were no pre-task differences between groups assigned to each order. Regarding the rate of change across the car ride, individual differences in slope were

not associated with time spent in the car, attachment disorganization, or baseline levels obtained at home.

The primary effects of interest were those associated with the slopes of the laboratory episodes (i.e., EPISODE 2 and EPISODE 3). The interaction of task and attachment disorganization emerged as a predictor of the rate of cortisol change for episode 3 ($p < .01$), and approached significance for episode 2 ($p = .06$) (see Table 4). As seen in Figure 2, attachment disorganization moderated the association between task and cortisol reactivity. To further examine this effect, models were analyzed separately for children with a primary classification of disorganized and children with an organized primary classification (i.e., secure, avoidant, resistant). Results for disorganized children and organized children are presented together in Table 5. For infants with a disorganized classification, there were differences in change in cortisol (slope) depending on the task, which reached statistical significance for EPISODE 3 ($\beta_{21} = .91$, $p < .01$) and approached significance for EPISODE 2 ($\beta_{11} = .37$, $p = .08$). Specifically, the Strange Situation elicited an increase in cortisol for these infants, compared with the period of play. For infants with an organized classification, differences in cortisol response between the tasks did not approach significance.

Table 4. Linear Piecewise Modeling Coefficients of Between-individual Effects on Salivary Cortisol

	Salivary Cortisol				
Effect	Coefficient	*SE*	*t*	*df*	*p*
Intercept, β_{00}	-.51	.18	-2.91	28	.01
TASK, β_{01}	-.21	.14	-1.45	28	.16
DISORG, β_{02}	-.14	.06	-2.44	28	.02
TASKxDISORG, β_{03}	.07	.05	1.43	28	.16
Slope, β_{10}	-1.32	.37	-3.62	28	.00
BASELINE, β_{11}	-1.19	.16	-7.28	28	.00
CARTIME, β_{12}	.01	.01	2.78	28	.36
DISORG, β_{13}	-.12	.09	-1.43	28	.16
Slope, β_{20}	.56	.28	1.99	27	.06
BASELINE, β_{21}	1.15	.08	14.74	27	.00
TASK, β_{22}	-.08	.25	-.32	27	.75
DISORG, β_{23}	.12	.09	1.46	27	.16
TASKxDISORG, β_{24}	.16	.08	1.89	27	.06
Slope, β_{30}	-.12	.16	-.77	27	.45
BASELINE, β_{31}	-.40	.07	3.33	27	.00
TASK, β_{32}	-.14	.21	-.67	27	.51
DISORG, β_{33}	-.18	.05	-3.52	27	.00
TASKxDISORG, β_{34}	.22	.07	-3.55	27	.00

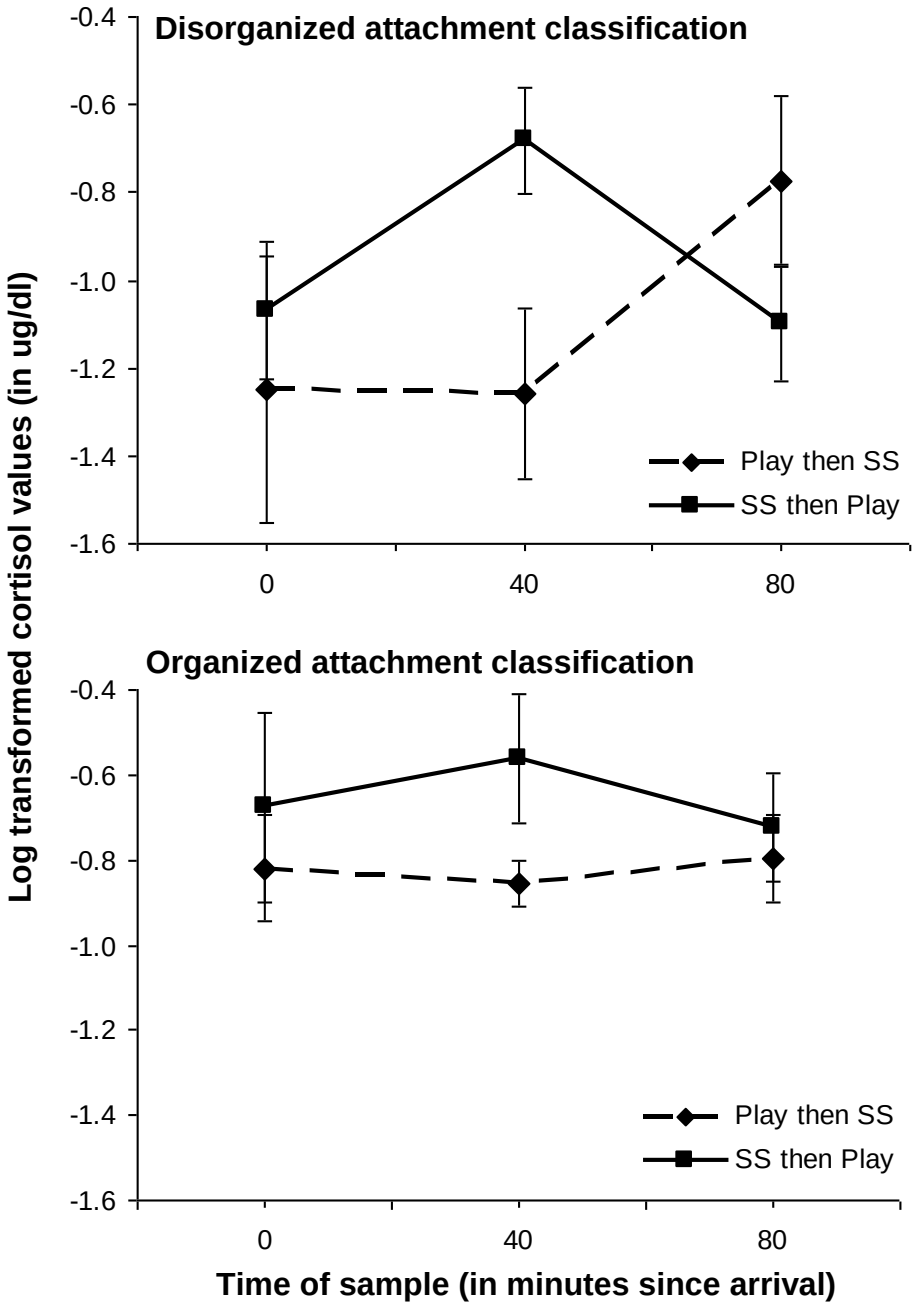
Note. β_{00} represents the baseline measure of cortisol at arrival to lab. β_{10}, β_{20}, and β_{30} represent the changes in salivary cortisol across EPISODE 1 (car ride), EPISODE 2 (first lab task), and EPISODE 3 (second lab task), respectively.

Table 5. Linear Piecewise Modeling Coefficients of Salivary Cortisol during Laboratory Tasks for Children with Disorganized and Organized Attachment Classifications

	Salivary Cortisol				
Effect	Coefficient	*SE*	*t*	*df*	*p*
Disorganized Attachment Classification					
Intercept, β_{00}	-1.25	.25	-4.95	5	.00
TASK, β_{01}	.19	.39	.49	5	.64
Slope, β_{10}	-.01	.11	-.04	5	.97
TASK, β_{11}	.37	.17	2.09	5	.08
Slope, β_{20}	-.42	.12	-3.70	5	.02
TASK, β_{21}	.91	.15	5.99	5	.00
Organized Attachment Classification					
Intercept, β_{00}	-.85	.16	-5.38	22	.00
TASK, β_{01}	.25	.23	1.12	22	.27
Slope, β_{10}	-.18	.09	-2.10	22	.05
TASK, β_{11}	.21	.13	1.60	22	.12
Slope, β_{20}	-.14	.14	-.97	22	.34
TASK, β_{21}	.31	.20	1.60	22	.12

Note. β_{00} represents the baseline measure of cortisol at arrival to lab. β_{10} and β_{20} represent the changes in salivary cortisol across EPISODE 2 (first lab task) and EPISODE 3 (second lab task), respectively.

Figure 2. **Changes in cortisol levels as a function of type of task and attachment disorganization.**



Chapter 4

DISCUSSION

In the present study, children's levels of cortisol were examined before and after two tasks in the laboratory (i.e., Strange Situation and free play). An interaction of attachment disorganization and task emerged as a significant predictor of the slope (or change) of cortisol during one of the two episodes and approached significance for the other episode. Specifically, attachment disorganization moderated the association between task and cortisol response. For children with disorganized attachment classifications, the Strange Situation elicited increases in cortisol that were significantly different than changes in cortisol elicited during the play episode. For children with organized attachment classifications, there were no significant differences in cortisol changes associated with the type of task. For these children, neither the Strange Situation nor the play elicited an increase in cortisol. These findings fit within the growing body of research supporting the development of a stress hyporesponsive period in human infants, and offer further indication of the role of attachment relationships in the maintenance of this period of hypo-responsivity. Previous studies have reported similar findings regarding the association between disorganized attachment and stress reactivity (i.e., Hertsgaard et al., 1995; Spangler &

Grossman, 1993). The present study is exciting because, to our knowledge, it is the first study of cortisol reactivity to counterbalance the order of the Strange Situation and a comparison laboratory task (i.e., play), which allowed for more systematic investigation of observed changes in cortisol.

Children with disorganized attachments showed cortisol reactivity to the Strange Situation in the present study. Compared to children with organized attachments, disorganized children appear to lack a coherent strategy to manage stress on a behavioral level. Taken together with findings from previous studies (Gunnar et al., 1995; Hertsgaard et al., 1995; Nachmias et al., 1996; Spangler & Grossman, 1993), the present study suggests that disorganized children may also have difficulty coping with stress on a biological level. For children with organized attachments, however, their relationship with that parent may serve as a buffer of the glucocorticoid response, such that children remain relatively hypo-responsive to stressors. Disorganized attachment is associated with the paradoxical experience of a parent who is both a source of security and a source of fright (van Ijzendoorn, Schuengel, & Bakermans-Kranenburg, 1999). In a meta-analysis, van Ijzendoorn et al. (1999) described several precursors of disorganized attachment, including maltreatment, parental unresolved loss or trauma, and unpredictably frightening behavior. It is unclear from the present study whether disorganized infants have failed to develop effective ways of coping with stressful situations in general, or whether something about the Strange Situation (e.g., presence of frightening parent) exacerbates this inability to cope effectively.

Similarly, it is not clear what aspect of having an organized attachment (e.g., presence of parent, expectation of parent's response, behavioral strategy) may inhibit cortisol reactivity at this age.

An alternative explanation of the findings is that disorganized infants experienced the Strange Situation as stressful, whereas organized infants did not. Although other indices of stress reactivity were not measured in the present study, previous studies have reported that even securely attached infants show elevated heart rate during the Strange Situation (Spangler & Grossman, 1993). Nevertheless, it is possible that the Strange Situation was more stressful for children with disorganized attachment classifications than other children.

Previous studies of infants' cortisol reactivity to the Strange Situation have relied on baseline measures of cortisol collected upon arrival at the lab, immediately before the stressor (e.g., Spangler & Grossman, 1994). However, as demonstrated by Larson et al. (1991), these "baseline" measurements may not be the most appropriate measure of typical cortisol levels. The present study did not find evidence of a drop in cortisol following a car ride to the laboratory. Cortisol levels at home and at arrival to lab were comparable, and the change in cortisol during the car ride was not significantly different from zero. Given that Larson et al. (1991) found the "car ride effect" following a timed 40-minute car ride, it is possible that participants in the present study were not in the car long enough to experience a similar decline in cortisol. Although a substantial range in travel times was reported (i.e., 5-50 minutes),

only 3 children (9%) experienced a car ride lasting 40 minutes or longer, with the majority of car rides (72%) lasting for only 5 to 20 minutes. Future studies should further test this effect using procedures more similar to Larson et al. (1991), specifically with regard to the amount of time spent in the car. Nevertheless, these results allowed us to systematically assess the possibility that differential rises in cortisol reflected return to baseline.

A significant main effect of attachment disorganization on the baseline level of cortisol (intercept) also emerged. There was a significant negative association, such that as attachment disorganization increased, arrival at lab cortisol levels decreased. The negative correlation between home levels of cortisol and attachment disorganization approached significance. Although this finding was not hypothesized, previous studies have reported similar effects. Specifically, pre-stressor measures of cortisol were found to be higher for infants with secure attachments compared with those with insecure attachments (Nachmias et al., 1996; Spangler & Grossman, 1993). Though researchers have been reluctant to interpret this unexpected finding previously (Gunnar, Brodersen, Nachmias, et al., 1996), replication in the present study provides converging evidence for the effect. Lowered basal levels of cortisol may be the result of down-regulation of the HPA system, serving a protective adaptation to elevated levels (Gunnar & Vasquez, 2001). A growing body of literature suggests that atypically low levels of basal cortisol and flattened daytime rhythms may be an indicator of risk for later health problems (Heim, Ehlert, & Hellhammer, 2000),

antisocial behavior (Vanyukov et al., 1993), aggression (McBurnett, Lahey, Rathouz, & Loeber, 2000), and anxiety disorders (Yehuda et al., 2000). Thus, future research should examine mechanisms underlying the development of lowered basal cortisol levels.

Given that higher attachment disorganization was associated with lower baseline levels of cortisol, it was important to consider possible Law of Initial Values (LIV) effects. When comparing relative effects of a stimulus on physiological responses, it is critical to control or adjust for baseline differences between groups (Oken & Heath, 1963; Wilder, 1958). Several methods for addressing LIV effects have been recommended in the literature, such as subtracting pre-test from post-test measures (i.e., difference scores) or covarying pre-test measures (Wainer, 1991). In the present study, baseline measures of salivary cortisol were included as predictors of the slope of cortisol change, in order examine main effects for level 2 predictors (e.g., task) while controlling for possible LIV effects. It was not the case that increases in cortisol were observed for disorganized children immediately upon entering the laboratory. Rather, an interaction between task and disorganization emerged (controlling for baseline cortisol levels), such that increases in cortisol levels appeared to result specifically from experiencing a stressor.

Limitations and Conclusions

It should be noted that the present study had a relatively small sample size. Given that our sample represented a middle class, nonclinical group, there were a higher percentage of children classified as disorganized (25%) than expected given rates in comparable samples (15%). The rates of disorganized attachment were significantly higher in the present study compared with distributions for comparable samples reported in a recent meta-analysis (van Ijzendoorn et al., 1999), $\chi^2 = 7.84$, $p < .01$. The atypically high proportion of disorganized attachments may have increased our ability to detect the reported effect within a relatively small sample size. Nevertheless, given the small sample size, replication of the findings will be important.

It is also important to consider the findings in the context of typical diurnal fluctuations in cortisol. In the present study, children's cortisol levels were measured in the morning, a time when decreases in cortisol are typically observed (Larson et al., 1998). Thus, a slight increase or flat pattern of cortisol production might reflect cortisol reactivity at this time of day. Although counterbalancing the presentation of tasks allowed us to control for order effects, future studies could further address this issue by collecting time-matched cortisol samples at home. Additionally, obtaining parents' reports of infants' wake-up times would be helpful in considering how findings fit within the expected diurnal rhythms of cortisol production.

Taken together, results of this study add to our understanding of the neurobiology of the human stress response during infancy. Several methodological

strengths facilitated interpretability of findings, including restricting the time of laboratory visits, using multiple baseline samples, including a comparison (non-stress) laboratory task, and counterbalancing the order of laboratory tasks. Many questions remain regarding HPA functioning in the human infant, which should be addressed in future studies. More longitudinal research should address the duration and timing of the period of stress hyporesponsivity in typically developing populations. Given support for the moderating effects of attachment disorganization, future studies should also examine what aspects of maternal care (e.g., sensitivity, frightening behavior, intrusiveness) are associated with the hypo- versus hyper-reactivity to stressors. Future studies should also investigate associations between cortisol reactivity and diurnal regulation of cortisol within individuals. Although these functions are considered to be relatively orthogonal, developmental changes may reflect more interdependent processes. Finally, the present study highlights the need for longitudinal studies that examine the effects of early cortisol regulation on later outcomes.

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