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Maternal-Child Adrenocortical Attunement in Early Childhood: Continuity and Change

ABSTRACT: This study evaluated continuity and change in maternal-child hypothalamic-pituitary-adrenal axis attunement in early childhood. Participants were drawn from a prospective study of 1,292 mother-child dyads, which were racially diverse, predominantly low-income, and non-urban. Child focused stress tasks designed to elicit anger, fear, and frustration were administered during early infancy, later infancy, and toddlerhood. Mothers' and children's saliva samples (later assayed for cortisol) were collected before and after the tasks. The strength of mother-child adrenocortical attunement was conserved across infancy and toddlerhood. The magnitude of maternal-child adrenocortical attunement decreased in response to the child-focused stress tasks. Maternal sensitivity and the child's task-related emotional reactivity moderated adrenocortical attunement across the task, with greater maternal sensitivity during a free-play, and lower levels of child emotional reactivity during the stress tasks, stabilizing attunement from pre- to post-task levels. The findings advance our understanding of individual differences in the social regulation of adrenocortical activity in early childhood. © 2014 Wiley Periodicals, Inc. *Dev Psychobiol.* 57: 83–95, 2015.

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INTRODUCTION

Evolutionary theory places primacy on the pressures associated with successfully mating and producing viable offspring. Primates maximize success in these two areas through the formation of uniquely large groups characterized by intense social bonds that allow individuals to carefully choose reproductive mates who will share in the burden of postnatal parental investment (e.g., Dunbar & Shultz, 2007). Social groups pose

distinct strains compared to independent living because group living requires complex cognitive functions to perceive social information, decipher social cues, and respond in socially appropriate ways to maintain cohesion and defuse conflict (e.g., Byrne & Whiten, 1988). The social skills required for successful group living are not innate, and regulation of behavioral, emotional, and physiological responses to social stresses are primarily learned through mother-child interactions (e.g., Peterson & Rollins, 1987; Bretherton & Munholland, 2008). So important are these skills that the life history stage of childhood in humans is thought to have evolved for the purpose of developing and acquiring these regulatory abilities (e.g., Flinn, 2011; Geary & Bjorklund, 2000).

Human infants are born with a stress response system that relies to a large degree on caregivers to soothe and calm heightened emotional and physiological arousal (Gunnar & Donzella, 2002; Hofer, 1987). Mother-child interactions during infancy up and down regulate children's behavioral and biological stress

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reactivity. Over time these exchanges are thought to calibrate and re-calibrate the threshold and set-point of the child's stress response system to "match" the demands of the child's social environments (e.g., Blair & Raver, 2012; Champagne & Meaney, 2001; Del Giudice, Ellis, & Shiryckoff, 2011; Lupien, McEwen, Gunnar, & Heim, 2009). Not surprisingly, children's behavior is also thought to have specifically evolved to induce changes in maternal behavior (Lorenz, 1935; Out, Pieper, Bakermans-Kranenburg, Zeskind, & van IJzendoorn, 2010) and physiology (Joosen et al., 2013; Out, Bakermans-Kranenburg, van Pelt, & van IJzendoorn, 2012; Swain, Lorberbaum, Kose, & Strathearn, 2007). Contemporary theorists assume that mother-child interactions reflect an intricately matched exchange in which both members of the dyad's behavior and physiology are transactionally influenced (e.g., Bornstein, 2009; Hofer, 1987).

In 1998, Granger and colleagues presented evidence suggesting that a major component of the psychobiology of the stress response, the hypothalamic-pituitary-adrenal (HPA) axis, played a role in this dynamic mother-child biobehavioral exchange. Specifically, maternal behavior and cortisol levels (the end product of HPA axis activation) were associated with child adrenocortical activity (Granger et al., 1998). Recently there has been increased interest in this phenomenon. Studies reveal "attunement" or "coregulation" in mother-child cortisol at multiple time points across the day (Hibel, Trumbell, & Mercado, 2014; Middlemiss, Granger, Goldberg, & Nathans, 2012; Papp, Pendry, & Adam, 2009) and across childhood (Atkinson et al., 2013; Hibel, Granger, Blair, & Cox 2009; Laurent, Ablow, & Measelle, 2012; Ruttle, Serbin, Stack, Schwartzman, & Shirtcliff, 2011; Sethre-Hofstad, Stansbury, & Rice, 2002). A core component of human and nonhuman primate sociality, which is particularly characteristic of mother-child relationships, rests on the deeply emotional connection experienced by bonded individuals. Thus, the attunement of adrenocortical activity between mothers and children is thought to be the physiological manifestation of a dyad's shared emotional and behavioral experiences (e.g., Feldman, 2007; Hibel et al., 2014).

We currently face limited understanding of the fine details of how attunement is established, interrupted, and then re-established as mother-child dyads adjust and adapt to the demands of their everyday social worlds. Originally, studies examined individual differences in the overall level of attunement across an event (Atkinson et al., 2012; Hibel et al., 2009; Sethre-Hofstad et al., 2002) focusing on between-dyad differences in attunement strength. Subsequent investigations revealed within-dyad fluctuations in attunement, finding stronger

attunement on weekends compared to workdays (Hibel et al., 2014), during attachment-related stressors compared to temperament-related tasks (Laurent et al., 2012), during a child directed cognitive test compared to a free-play interaction (Ruttle et al., 2011), and when mothers were aware, compared to not aware, of their child's distress (Middlemiss et al., 2012). These studies make clear that attunement is not a continuous steady state. The strength of the association is dependent on behaviors specific to the situation and features of the social environment. However, no studies have examined how the association between mother and child cortisol levels strengthens (or weakens) in response to stress, and strengthens (or weakens) during the stress recovery period. Ruttle and colleagues (2012) suggest that the process of adapting to demanding environmental challenge may enhance adrenocortical attunement. Yet the timing of saliva sampling (immediately post-task, as opposed to accounting for the delay in cortisol reactivity) and the nature of the stressor employed in that study (a cognitive test not explicitly designed to elicit cortisol reactivity) make this assertion difficult to decipher. Generally speaking, if physiological attunement is a manifestation of behavioral cooperation between individuals, we expect that intense stress experienced by either member of a mother-child dyad will disrupt adrenocortical attunement between them.

Previous studies have examined maternal receptivity to child cues as a moderator of adrenocortical attunement (Atkinson et al., 2013; Sethre-Hofstad et al., 2002; van Bakel & Riksen-Walraven, 2008). It was assumed that mothers who are able to accurately read their child's cues may also be able to behaviorally, emotionally, and physiologically match their child's state (Feldman, 2007; Sethre-Hofstad et al., 2002). Studies with community samples reveal that maternal sensitivity strengthens adrenocortical attunement (Atkinson et al., 2013; Sethre-Hofstad et al., 2002; van Bakel & Riksen-Walraven, 2008). However, certain pathologies (e.g., maternal depression; Laurent, Ablow, & Measelle, 2011) and risky contexts (e.g., partner violence; Hibel et al., 2009) also appear to foster attuned mother-child cortisol reactivity during times of child stress. Together, it appears that mothers regulate child physiology for better and for worse, with both positive and negative maternal behaviors impacting child physiology (Blair & Raver, 2012), as well as adrenocortical attunement. To date, the available empirical information regarding how attunement fluctuates in response to environmental demands and the impact of the dyad's behavior on the intra-dyadic fluctuations in adrenocortical attunement is scant. Building off previous studies, the present study examines whether maternal sensitivity buffers stress induced interruptions in cortisol attunement.

For mothers (sensitive or not) to be physiologically attuned to their child's internal state, a child's behavioral profile must be a fairly accurate representation of their emotional and physiological experience. In a test of this idea, Middlemiss and colleagues (2011) examined mother-child cortisol attunement before and after infants were separated from their mothers during a sleep training intervention. On the first night of intervention, infants were separated from mothers and taken by a nurse to a different room. The infants expressed long and intense bouts of negative affect until they fell asleep. Mothers were alone in an adjacent room, could hear their infants distress, but as part of the intervention did not respond to these cues. Mother and child cortisol was attuned before and after the child fell asleep. By the third night of the intervention, infants were still being separated from their mothers for the sleep routine but no longer vocalized distress. Prior to the sleep routine mother-child cortisol was attuned, but after the sleep routine on this night the dyad's cortisol levels were no longer attuned (though the authors do not report if this is a significant change in attunement from before to after sleep). In the present study, we will examine the degree to which higher levels of child behavioral distress to an emotion eliciting stress task serve to attune mother-child cortisol levels.

Development over the first 2 years of life presents a major shift in the child's regulatory abilities. While infants are primarily dependent on caregivers for external regulation, toddlers' increasing capacity to self-regulate reduces dependence on caregiver's support (Calkins, Smith, Gill, & Johnson, 1998; Feldman, Greenbaum, & Yirmiya, 1999). Likewise, the degree to which mothers and their children are adrenocortically attuned may vary by the child's age, particularly during the transition from infancy to toddlerhood. Cross-sectional studies show the existence of adrenocortical attunement during infancy (Hibel et al., 2009; Middlemiss et al., 2012) and toddlerhood (Atkinson et al., 2013; Laurent et al., 2012; Sethre-Hofstad et al., 2002); however, no study has longitudinally followed dyads across this developmental period examining the stability of cortisol attunement across early childhood. Mothers may perceive their infant as less capable of self-regulation, and may be more inclined to calm and soothe their infant, as opposed to their toddler (Landry, Smith, Swank, Assel, & Vellet, 2001). Toddler distress may not fully engage maternal caregiving, and thus stress may induce larger disruptions in mother-child cortisol attunement. To fill this knowledge gap, we will longitudinally follow mother-child dyads from early infancy into toddlerhood, examining the impact of the child's age on mother-child adrenocortical attunement during a stressor.

The Current Study

The main study goals are to determine 1) if mother-child cortisol attunement strengthens or dampens when the child is challenged with an emotion eliciting stress task, 2) whether maternal or child behaviors moderate changes in adrenocortical attunement in response to the stress task, and 3) if mother-child task-related adrenocortical attunement changes as the child ages. The stress task (Lab-TAB; Goldsmith & Rothbart, 1988), was designed to induce fear, frustration, and anger and was administered during a home interview when the children were approximately 7 months (early infancy), 15 months (later infancy), and 24 months (toddlerhood; see Blair et al., 2011; Towe, Goodman, Stifter, Mills-Koonce, Granger, & The Family Life Project Key Investigators, 2012; Ursache, Blair, Granger, Stifter, & Voegtline, 2013). We expect attunement to be a product of shared behaviors and emotions, and thus a child-only oriented stressor will disrupt attunement. Second, building off of the idea that shared emotions facilitate attunement, we hypothesize that more sensitive mothers will be better able to match their child's emotional state. Similarly, we expect that children with more clear and intense distress signals (i.e., greater emotional distress) will facilitate mother's attunement to child physiology. Lastly, we will compare changes in attunement across the stress task at early infancy, later infancy, and toddlerhood. Based on behavioral studies of mother-child coregulation, we expect stress induced divergence in attunement to expand as the child enters toddlerhood and mothers perceive their children as more capable of self-regulation.

METHOD

Overview

The Family Life Project (NICHD, P01HD39667) was designed to study families in two areas of high child poverty: the Rural South (North Carolina) and Northern Appalachia (Pennsylvania). Complex sampling designs were employed to recruit a representative sample from the target counties, oversampling for low-income families in both states, and African American families in North Carolina. Families were recruited from six hospitals (three from each state) at the time of the child's birth. Mothers were screened for income and education, intentions to move out of the state, her child's race, and English as a primary language. Recruitment occurred 7 days per week for 1 year beginning September 15, 2003. Further details on the FLP sampling plan and recruitment procedures are available in Vernon-Feagans, Cox, and the FLP Investigators (2013).

Participants

Data for this analysis came from the larger project's home interview and assessments at three time points across early childhood. Recruitment efforts resulted in 1,292 families consenting to take part in the study, of these, 1,204 dyads participated in the first visit (early infancy), 1,169 participated in the second visit (later infancy), and 1,144 participated in the third visit (toddlerhood). For the current analyses, pregnant or non-biological mothers of the target child were excluded, resulting in 1,141, 1,058, and 1,036 dyads at the early infancy, later infancy, and toddler visits, respectively. At the early infancy assessment, child age ranged from 5.0 to 15.4 months ($M = 7.7$ months, $SD = 1.4$), 41.5% were African American, and 48.8% were male. Mothers were approximately 26.4 years old ($SD = 5.8$), 47.4% were single and had an average income-to-needs ratio of 1.96 (range: 0–16.49, $SD = 1.73$). At the later infancy assessment children were approximately 15.7 months old ($SD = 1.3$; range 14.0–22.3 months), 43.8% were African American and 49.4% were male. Mothers were approximately 27.1 years old ($SD = 5.8$), 47.6% were single and had an average income-to-needs ratio of 1.89 (range: 0–16.76, $SD = 1.66$). At the toddlerhood assessment children were approximately 24.8 months old (range 22.2–34.9 months, $SD = 1.8$), 41.4% were African American and 49.8% were male. Mothers were approximately 28 years old ($SD = 5.8$), 45.5% were single and had an average income-to-needs ratio of 1.80 (range: 0–16.2, $SD = 1.58$).

Procedures and Measures

Home visits lasted approximately 2–3 hr and included self-report measures, semi-structured interviews, mother–child interaction tasks, and a stress task directed at the child. The mother–child interactions occurred toward the beginning of the visit, while the child stress task, taken from the Laboratory Temperament Assessment Battery (Lab-TAB, Goldsmith & Rothbart, 1988), was presented toward the end of the visit. Saliva from the mother and child was collected surrounding the Lab-TAB (for more details on the structure of these visits, please see Vernon-Feagans, Cox, & the FLP Investigators, 2013).

Laboratory Temperament Assessment Battery (Lab-TAB). Stress tasks designed to elicit emotional reactivity were administered to the child (see Blair et al., 2011). The tasks are from the Laboratory Temperament Assessment Battery (Lab-TAB; Goldsmith & Rothbart, 1988), and procedures have been previously validated (e.g., Buss & Goldsmith, 1998; Kochanska, Tjebkes, & Fortnan, 1998; Stifter & Braungart, 1995). At early

infancy, three tasks were presented to the infant: the barrier task, the mask task, and the arm restraint task. First, for the barrier task (conducted three consecutive times) infants were presented with an attractive toy and encouraged to play with it, after which the experimenter then placed the toy behind a clear plastic barrier. For the mask presentation the experimenter wore four unusual masks while calling the child's name and moving from side to side. For the arm restraint task the experimenter crouched behind the child and gently restrained his or her arms for 2 min or until 20 s of hard crying ensued. Mothers watched the infant during the task from a vantage point out of the infant's line of sight, but were asked to not intervene. At later infancy and toddlerhood, the toy removal and mask tasks were conducted. At later infancy when the toy was taken away it was held by the experimenter, while at toddlerhood the toy was placed in a clear container with a tightened lid and the child was allowed to hold the container. The mask task was carried out using the same protocol as at the early infancy visit.

Child Emotional Reactivity. Assessments of peak emotional arousal in response to the stress tasks were completed via independent coders' ratings of low, moderate, and high negative reactivity using Better Coding Approach software (Danville, PA). A composite score for negative reactivity (i.e., crying) for each task was created by summing the seconds of low, moderate, and high negative reactivity and then calculating the proportion by dividing the sum of all negative reactivity scores by the total time of the task (e.g., Voegtline, Stifter, & the FLP Investigator, 2012). Coders were trained to achieve a Cohen's k (reliability) of at least 0.75 on the reactivity coding. Subsequent inter-rater reliability was calculated on 15% of cases using kappa coefficients, resulting in a kappa of 0.94 for the masks task, 0.89 for the barrier task, and 0.86 for the arm restraint task.

Maternal Behaviors. Mothers and children engaged in a 10 min semi-structured free-play. Mothers were instructed to play with their child as they would if they had a small amount of free time. All mothers were given three standardized toys ranging in intensity. Interactions were video recorded and later coded to assess levels of mother's sensitivity, detachment, intrusiveness, positive regard, negative regard, and animation in interacting with the child (see NICHD ECCRN, 1999). Reliability was determined by calculating the intra-class correlation coefficients for ratings made by pairs of trained coders. A minimum of 30% of all observations were double coded; discrepant codes were resolved by conferencing. Ratings for each code were

made on a scale ranging from 1 (not at all characteristic) to 5 (highly characteristic) at the infancy assessments and ranging from 1 to 7 at the toddler assessment (scores from the toddler observations were rescaled to a 1 to 5 range for the current analyses; see Hibel, Granger, Blair, Cox, & The Family Life Project Investigator, 2011). On the basis of the oblique rotation (i.e., Promax) factor analysis, two broad-based parenting factors emerged: positive maternal behaviors and negative maternal behaviors. Positive behaviors included five parental ratings: sensitivity, reverse scored detachment, positive regard, animation, and stimulation of development. Negative behaviors included two parental characteristics: intrusiveness and negative regard. For the current analyses only the positive parenting factor was utilized. Intra-class correlations were 0.85–0.91 for positive parenting across the three child ages.

Salivary Cortisol. The collection of the mother and child saliva samples was coordinated to occur simultaneously. The first cortisol sample (pre-task) was collected immediately prior to the stress task. The second sample (post-task1) was collected 20 min after the final stress task was completed (or prior, if the child reached peak emotional arousal), and the last (post-task2) was collected 20 min after post-task1. The sample collection paradigm was designed to capture the mother and child's reactivity and regulation to the stressor. On average, mothers collected their samples 13 s (SD = 0:00:08) before their child's sample was collected. Due to the diversity of the participants' work schedules, visits were scheduled at the family's convenience. Saliva samples were collected at approximately 1:30pm (early infancy, range: 8am–8pm), 2pm (later infancy, range: 8:45am–9pm), and 1:45pm (toddlerhood, range: 8:20am–8:45pm).

After collection, samples were immediately placed on ice, then stored frozen (-20°C) until shipped on dry-ice. Samples were then stored frozen at -80°C until assayed. All samples were assayed for salivary cortisol using a highly-sensitive enzyme immunoassay (Salimetrics, State College, PA). The test used 25 μl of saliva, had a range of sensitivity from .007 to 3.0 $\mu\text{g}/\text{dl}$, and average intra- and inter-assay coefficients of variation less than 10 and 15%. All samples were assayed in duplicate and the average of the duplicates was used in all analyses. Cortisol levels were natural log transformed to correct for skewed distributions (skew range after transformation: -0.127 – 0.348).

Analytical Strategy

The three main objectives of these analyses were to 1) determine the change in mother–child cortisol attune-

ment during a stress task, 2) determine the behavioral moderators of the change in adrenocortical attunement during a stress task, and 3) determine if task-related attunement changes from early infancy to late infancy to toddlerhood. Previous analyses of these data (e.g., Blair et al., 2008; Hibel et al., 2011) have highlighted the importance of controlling for saliva collection time of day, race (African American vs. not), and income-to-needs ratio when examining child cortisol. All models with cortisol as the outcome will control for these variables.

Our primary analyses employed one main model with child cortisol during the stress task as the primary outcome. For this model, the outcome was the child's three cortisol samples around the stress task (pre-task, post-task1, and post-task2), across the three child ages (early infancy, later infancy, and toddlerhood). The maximum number of samples for each child was nine. The Level 1 submodel described the within child cortisol change across the stress task; the Level 2 submodel described how these within child changes differ across the three ages; the Level 3 submodel described the between child variations (Singer & Willet, 2003). We used a piecewise approach in which we included dummy codes of the pre- and post-task2 collections, to estimate change in cortisol over the task. The first dummy code (i.e., pre-task) estimated change from post-task1 to pre-task and the second (i.e., post-task2) estimated change from post-task1 to post-task2. (The pre-task coefficient estimates the change in attunement from post-task1 to pre-task, thus a negative pre-task coefficient indicates lower levels of attunement at the pre-task collection compared to the post-task1 collection. In other words, a negative pre-task coefficient indicates lower attunement at pre-task than post-task1). With these designations, the intercept (or reference point) is cortisol at the post-task1 collection. Maternal cortisol around the stress task (pre-task, post-task1, and post-task2), across the three child ages (early infancy, later infancy, toddlerhood) was also a Level 1 variable, allowing for the model to determine the relation between intra-individual change in maternal and child cortisol (i.e., attunement). The Level 1 interaction between maternal cortisol and the pre-task dummy variable, predicting child cortisol, will reveal the degree to which the stress task potentiates or attenuates mother–child cortisol attunement (objective 1). Similarly, the interaction between maternal cortisol and the post-task2 dummy variable will assess the degree to which attunement “recovers” after the stress task.

Measurements that vary across the three child ages were modeled at Level 2. Cortisol trajectories have been shown to differ across the three ages, therefore

dummy codes representing the early infancy and toddlerhood assessments were included, with the later infancy assessment designated as the reference group (intercepts, therefore are cortisol levels at the post-task1 collection for the later infancy assessment). The maximum number of entries for Level 2 variables was three, and included dummy codes for the ages (early infant or toddler), family's income-to-needs ratio (income ratio), pre-task saliva collection time of day (time of day), as well as maternal positive behavior (positivity) and child negative emotional reactivity (emotional reactivity). The pre-task sample collection time (time of day) was group mean standardized within assessment to control for the diurnal rhythm of cortisol while all other variables were grand mean standardized across assessments. To determine if certain maternal or child behaviors potentiated the mother-child cortisol attunement during the task (objective 2) as well as if attunement across the task waned as the child aged (objective 3), interaction terms were created. These interaction terms consisted of maternal cortisol, the saliva collection (either pre-task or post-task2), and either the behavior or age of interest (e.g., pre-task X positivity X maternal cortisol).

Finally, at Level 3, nontime varying, between-subject variables, such as demographic controls (e.g., race), were entered. Variables that were not significantly associated with cortisol activity were not retained in the model in order to conserve degrees of freedom. Best fitting models (lowest AIC and BIC) included random intercepts.

To provide initial descriptive statistics on maternal cortisol across the stress task, and across the three child ages, a model with maternal cortisol as the outcome was created. This model was analogous to the child cortisol model just described. Nine maternal cortisol samples (three cortisol samples across the stress task, collected at all three child ages) were modeled at Level 1, with dummy codes indicating the saliva collections (pre-task and post-task2). At Level 2 the child ages (early infancy and toddlerhood) were entered along with child behavior (as a control). Finally, maternal demographics were entered at Level 3, also as control variables.

Missing Data

The exclusion of pregnant or non-biological mothers resulted in the elimination of 63 mothers at the early infancy assessment (52 pregnancies, 11 non-biological mothers), 111 mothers at the later infancy assessment (92 pregnancies, 19 non-biological mothers), and 108 mothers at the toddler assessment (87 pregnancies, 21 non-biological mothers). To retain as much data as

possible, data were excluded within assessment waves, meaning if a biological mother participated in the early infancy assessment, but not in the later infancy assessment, her data were included for the early infancy assessment. Likewise, data were only excluded for the visits the mother reported being pregnant. Cortisol levels three standard deviations above the mean were excluded (total $n=36$ maternal saliva samples, 169 child saliva samples out of approximately 8,000 samples collected over the three time points). In addition, at each assessment roughly 10% of the mothers, and 20% of the child's cortisol was missing due participant refusal or insufficient sample quantity for testing. To statistically account for the random and non-random missingness of these data, full information maximum likelihood estimation (MLE) was carried out in SAS 9.2. MLE provides the correct likelihood for the missing parameters based on the distribution of the observed data and provides valid standard errors when calculated based on the observed information matrix (Schafer & Graham, 2002).

RESULTS

Descriptive Statistics for Cortisol Levels During the Stress Task, at the Early Infancy, Later Infancy, and Toddlerhood Assessments

Descriptive statistics for the main variables of interest are displayed in Table 1. To determine cortisol change during the stress task, cortisol differences were examined at the pre-task (i.e., cortisol reactivity) and post-task2 (i.e., cortisol regulation) collections, relative to the post-task1 collection. Maternal and child behavioral variables, in addition to demographic variables, were first entered to control for their effect on child cortisol reactivity (e.g., behavior X pre-task; see Tab. 2, Model A). Across the three ages, greater child emotional reactivity ($b = -.09$, $p = .007$) and higher levels of positive maternal behavior ($b = -.05$, $p < .001$) were related to higher post-task1 cortisol (i.e., reactivity). Controlling for these effects, the change in cortisol from pre- to post-task1 differed significantly across the three ages (see Tab. 2, Model A). Specifically, while cortisol did significantly increase from pre- to post-task1 at early infancy, maternal sensitivity and child emotional reactivity completely accounted for this reactivity ($b = .01$, ns; $b_{\text{pre} \times \text{early infant}} + b_{\text{pre}}$), but at later infancy significant cortisol reactivity was evident above and beyond these controls ($b = -.13$, $p = .007$; b_{pre}). At toddlerhood children did not exhibit significant cortisol reactivity ($b = .02$, ns; $b_{\text{pre} \times \text{toddler}} + b_{\text{pre}}$), even without controlling for the behaviors. Cortisol levels did not

Table 1. Descriptive Information of the Main Variables of Interest. Cortisol Values (pre-task, post-task1, and post-task2) Are in $\mu\text{g/dl}$

	Early Infant			Later Infant			Toddler		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Child									
Pre-task	1044	0.20	0.18	887	0.18	0.19	832	0.16	0.14
Post-task1	948	0.22	0.19	833	0.21	0.20	828	0.16	0.15
Post-task2	887	0.20	0.16	769	0.21	0.19	811	0.15	0.12
Age (months)	1119	7.76	1.43	1039	15.74	1.34	976	24.81	1.84
Emo React ^a	1065	2.17	3.01	980	2.28	3.21	904	2.22	3.35
Mother									
Pre-task	1069	0.21	0.16	821	0.13	0.10	760	0.16	0.12
Post-task1	1001	0.16	0.13	818	0.10	0.09	748	0.11	0.08
Post-task2	1061	0.14	0.10	794	0.10	0.07	913	0.10	0.08
Positivity ^b	1073	2.90	0.79	989	2.78	0.80	921	2.90	0.81

^aEmo React: Child's Negative Emotional Reactivity to the stress task.

^bPositivity: Positive maternal behaviors during the free-play.

change from post-task1 to post-task2 at later infancy ($b = .07$, ns), a pattern that was consistent at early infancy ($b = -.10$, ns) and toddlerhood ($b = -.09$, ns).

The maternal cortisol outcome model was employed to describe the change in maternal cortisol across the task, controlling for maternal race, income level, time of day, and child emotional reactivity. Maternal cortisol consistently declined across the task at the early infancy and later infancy visits, with pre-task cortisol being significantly higher than post-task1 levels ($b = .25$, $p < .0001$). The pre- to post-task1 cortisol decline was even steeper at the toddlerhood visit ($b = .42$, $p < .001$). Maternal cortisol levels continued to decrease from post-task1 to post-task2 at the later infancy visit ($b = -.11$, $p < .001$), a pattern that was consistent at the early infancy ($b = -.08$, ns) and toddlerhood visits ($b = .02$, ns; see Fig. 1). Thus, maternal cortisol exhibited similar trajectories across the three ages, with decreases from pre- to post-task1, and from post-task1 to post-task2.

Change in Attunement Across the Stress Task

To determine the change in mother-child cortisol attunement across the stress task, differences in cortisol attunement were examined at the pre- and post-task2 collections, relative to the post-task1 collection (Tab. 2; Model B). Controlling for age and the maternal and child behavioral effects on child cortisol reactivity, maternal cortisol was related to child cortisol. At pre-task, maternal cortisol was strongly attuned to child cortisol ($b = .47$, $p < .0001$; $b_{\text{pre} \times \text{MCortisol}}$), but attunement significantly decreased ($b = .23$, $p = .02$; $b_{\text{pre} \times \text{MCortisol} + \text{MCortisol}}$) by the post-task1. Despite the

decrease, maternal cortisol was still significantly attuned to child cortisol at the post-task1 collection ($b = .23$, $p = .02$; $b_{\text{MCortisol}}$). Cortisol attunement remained stable from the post-task1 to the post-task2 ($b = .05$, ns; $b_{\text{post2} \times \text{MCortisol}}$). Thus, across the three ages, adrenocortical attunement decreased from pre to post-task1, and remained stable from post-task1 to post-task2.

Behavioral Moderators of Task-related Adrenocortical Attunement

Inter-individual differences in maternal and child behaviors were examined as possible moderators of intra-dyadic changes in mother-child cortisol attunement. Models examining the interactive power of positive maternal behavior, and child negative emotional reactivity to the stress task on mother-child cortisol attunement across the task were employed (see Table 2, Model C). Both positive maternal behavior and child emotional reactivity moderated mother-child attunement. Specifically, the change in attunement from pre- to post-task1 was moderated by child emotional reactivity ($b = .28$, $p = .006$) as well as maternal positive behavior ($b = -.23$, $p = .01$). Further, the child's emotional reactivity to the task moderated the change in attunement from post-task1 to post-task2 ($b = .28$, $p = .008$). Attunement coefficients at each of the saliva collections split by high (above the mean) and low (below the mean) groupings on maternal positive behavior and child emotional reactivity were graphed (see Fig. 2). Examination of the graph revealed cortisol attunement was stable across the task for dyads with high positive maternal behavior, but decreased from pre- to post-task1 in dyads with low positive

Table 2. Multi-Level Models With Child Cortisol as the Outcome. Models Are Examining: A) Change in Child Cortisol During the Stress Task; B) Change in Attunement During the Task; C) Mother and Child Behaviors as Moderators of Change in Attunement During the Stress Task; and D) Child Age as a Moderator of Change in Attunement During the Task

Effect	Model A		Model B		Model C		Model D	
	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Intercept	-0.018	0.03	0.010	0.04	0.030	0.05	0.038	0.04
Pre-task ^a	-0.132*	0.05	-0.105*	0.05	-0.120*	0.06	-0.118	0.06
Post-task2 ^b	0.066	0.05	0.069	0.05	0.055	0.06	0.052	0.06
Time of Day	-0.209***	0.02	-0.207***	0.02	-0.199***	0.02	-0.200***	0.02
Early Infant ^c	0.045	0.05	0.053	0.05	0.035	0.05	0.028	0.05
Toddlerhood ^d	-0.296***	0.05	-0.296***	0.05	-0.278***	0.06	-0.307***	0.06
Race	0.193***	0.04	0.193***	0.04	0.193***	0.04	0.182***	0.04
Income	-0.001	0.02	-0.001	0.02	0.001	0.02	0.002	0.02
Emotional Reactivity ^e	0.070**	0.02	0.070	0.02	0.069**	0.02	0.065**	0.02
Positive Parenting ^f	-0.017	0.02	-0.020	0.02	-0.015	0.02	-0.004	0.02
Maternal Cortisol ^g	0.329***	0.05	0.234**	0.08	0.431**	0.09	0.409**	0.09
Pre-task × Emo Reactivity	-0.090**	0.03	-0.046	0.03	-0.083**	0.03	-0.081**	0.03
Post-task2 × Emo Reactivity	-0.033	0.03	0.034	0.03	-0.027	0.03	-0.024	0.03
Pre-task × Positivity	-0.051*	0.03	-0.050*	0.03	-0.055*	0.03	-0.064	0.03
Post-task2 × Positivity	0.029	0.03	-0.030	0.03	0.021	0.03	0.014	0.03
Pre-task × Infant	0.145*	0.06	0.112	0.07	0.127*	0.07	0.127	0.07
Pre-task × Toddler	0.156*	0.07	-0.104	0.07	0.137*	0.08	0.155*	0.08
Post-task2 × Infant	-0.105	0.07	0.149*	0.07	-0.090	0.07	-0.084	0.07
Post-task2 × Toddler	-0.091	0.07	-0.091	0.07	-0.099	0.07	-0.017	0.08
Pre-task × Mcortisol			0.231*	0.10	0.059	0.13	0.096	0.21
Post-task2 × Mcortisol			0.048	0.10	-0.114	0.13	-0.102	0.22
Infant × Mcortisol					-0.054	0.14	0.175	0.17
Toddler × Mcortisol					0.195	0.17	0.494	0.31
Positivity × MCortisol					0.054	0.09	0.037	0.08
Emo React × MCortisol					-0.084	0.10	-0.100	0.09
Pre-task × Positivity × Mcortisol					-0.220*	0.10	-0.162	0.10
Post-task2 × Positivity × Mcortisol					-0.114	0.10	-0.116	0.10
Pre-task × Emo React × Mcortisol					0.278**	0.10	0.301**	0.11
Post-task2 × Emo React × Mcortisol					0.281**	0.11	0.312**	0.11
Pre × Infant × Mcortisol							-0.115	0.20
Pre × Toddler × Mcortisol							0.118	0.23
Post2 × Infant × Mcortisol							-0.070	0.21
Post2 × Toddler × Mcortisol							0.227	0.24

^aPre-task (abbreviated as Pre).

^bPost-task2 (abbreviated as Post2).

^cEarly Infant (abbreviated as Infant).

^dToddlerhood (abbreviated as Toddler).

^eEmotional Reactivity (abbreviated as Emo React): Child's Negative Emotional Reactivity to the stress task.

^fPositive Parenting (abbreviated as Positivity): Positive maternal behaviors during the free-play.

^gMaternal Cortisol (abbreviated as MCortisol).

* $p < .05$.

** $p < .01$.

*** $p < .001$.

maternal behavior. In dyads with a highly emotionally reactive child, cortisol attunement decreased from pre- to post-task1 and remained low and stable to the post-task2 collection. On the other hand, in dyads with children with low levels of emotional reactivity, attunement was stable from pre- to post-task1, but decreased from post-task1 to post-task2.

Age as a Moderator of Task-Related Adrenocortical Attunement

To examine whether mother-child attunement changed differently across the task, dependent on the child's age, we included the dummy code of child assessment as a moderator of mother-child attunement during

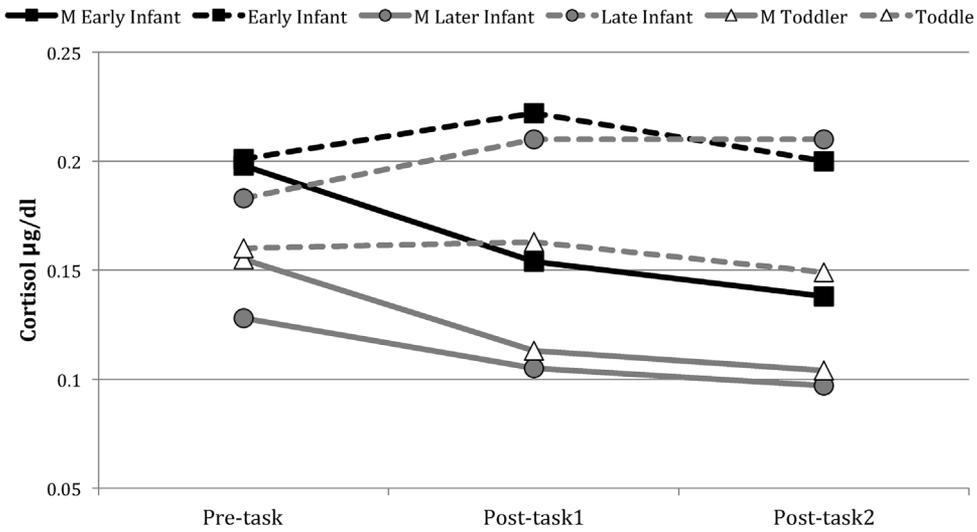


FIGURE 1 Mean cortisol levels in µg/dl of mothers’ (M) and children’s three cortisol samples around the stress task (pre-task, post-task1, and post-task2), across the three child ages (early infancy, later infancy, toddlerhood). Children exhibited cortisol reactivity to the stressor, mothers, however, declined across the three sampling occasions.

the task. Thus, this analysis used mother–child cortisol attunement at the later infancy assessment as a reference to examine differences at the early infancy and toddlerhood visits. There was no difference in the

change in attunement from pre- to post-task1 from early infancy to later infancy ($b = -.12$, ns) or from later infancy to toddlerhood ($b = .12$, ns). Similarly, there was no difference in the change in attunement

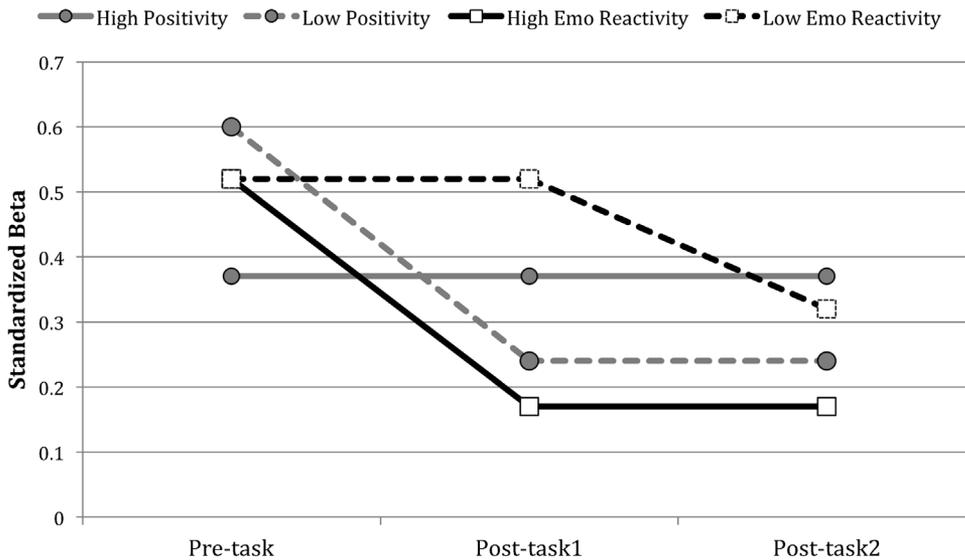


FIGURE 2 The standardized beta between mother and child cortisol levels are shown at each time point across the task (pre-task, post-task1, and post-task2). Lines represent dyads above the mean (High) and below the mean (Low) in positive maternal caregiving behavior and child emotional reactivity. In dyads with high child emotional reactivity or low maternal positive behavior, cortisol attunement decreases across the task.

from post-task1- to post-task2 from early infancy to later infancy ($b = -.07$, ns) or from later infancy to toddlerhood ($b = -.23$, ns; see Tab. 2, Model D).

DISCUSSION

The strength of mother–child adrenocortical attunement was conserved across infancy and toddlerhood, suggesting that attunement in cortisol levels between mothers and their offspring, in general, reflects a developmentally stable phenomenon across early childhood. Consistent with expectations, the magnitude of maternal–child adrenocortical attunement decreased substantially in response to situationally induced change in child negative emotion. This observation highlights the possibility that adrenocortical attunement is sensitive to situational variation in social experience. The degree of “interruption” in attunement in response to this child-focused situation was large when children expressed higher amounts of distress during the stress task, and when mothers expressed low levels of sensitive and responsive maternal behaviors during the free-play. Taken together, the findings underscore that mother–child adrenocortical attunement reflects an underlying steady state which can be temporarily interrupted to varying degrees by characteristics of the dyadic relationship, immediate social experiences, and situational demands. The findings have several noteworthy implications and advance our understanding of individual differences in the social regulation of adrenocortical activity in early childhood.

Highly social animals require the close coordination of behavior across individuals, in part, to maintain group cohesion. Family members synchronize eating, sleeping, waking, and other routines as they go about their daily lives together (Fiese et al., 2002). Within the context of this group or family level behavioral synchrony, mothers and children also engage in moment-by-moment synchronous exchanges. It is perhaps the combination of diurnal and momentary synchronicity that fosters adrenocortical attunement revealed in previous studies (e.g., Hibel et al., 2009) as well as in the current analyses. Specifically, mother and child cortisol was strongly attuned before the child engaged in the emotion eliciting stress task, and the strength of attunement was consistent from early infancy to toddlerhood.

However, group living is not always harmonious (Sussman & Garber, 2010), family life is not always stable and routine-oriented (Damaske, Smyth, & Zawadzki, 2014; Fiese et al., 2002), and mother–child interactions are not always synchronous (Feldman, 2007). Across a typical day, mothers dynamically shift

their attention across multiple competing demands, adjusting the degree to which they are behaviorally and emotionally engaged with their children (Smith & Pederson, 1988). Likewise, the developmental abilities of young children compared to their mothers ensure differential experiences, such that typical routines for the mother (e.g., talking to a neighbor) may be a fear inducing event for the young child (e.g., stranger anxiety). In light of these constant fluctuations in mothers’ and children’s shared and differential experiences throughout the day, it is understandable that attunement was not stable in the face of child distress. In the current study, children were subjected to an emotion eliciting stressor meant to mimic everyday challenges a child may face (e.g., arm restraint: being immobilized in a car seat or high chair; masks: meeting new people). For children who found these events particularly distressing, producing moderate to high levels of negative affect, mother–child adrenocortical attunement was completely eliminated. Further, for these dyads, attunement still had not re-established 40 min after the tasks were completed, and by this time, even children exhibiting lower levels of emotional reactivity had reductions in attunement. These findings suggest intense stressors may cause immediate and prolonged interruptions in shared mother and child physiological rhythms, while divergences in attunement from mild to moderate stressors may develop more slowly.

The current analyses do not elucidate the length of time or conditions necessary to re-couple mother and child cortisol, yet there are two potential explanations for why changes in child affective states un-couple mother–child cortisol attunement. Parenting a young child during times of child distress can be particularly challenging (Brame, Nagin, & Temblay, 2001) and even sensitive parents exhibit a reduction in sensitive and responsive caregiving when children are distressed (Mills-Koonce et al., 2007). Thus, it could be that heightened child emotional reactivity taxes mother’s behavioral and physiological abilities to accurately respond to her child’s state. Conversely, given that higher emotional reactivity was associated with greater cortisol reactivity, it could be that for these children, their heightened cortisol reactivity exceeds the mother’s ability to match their child’s state. In other words, the children’s emotional and physiological state is too profoundly different than the mother’s state, precluding mother’s ability to physiologically match her child.

Our analyses of attunement across the three developmental time points may shed light on this latter interpretation. Overall, children do not exhibit significant cortisol reactivity at toddlerhood, however, children do exhibit a significant increase in cortisol at both early and later infancy. Despite these different patterns

of child adrenocortical activity, there is no difference in how attunement unfolds across the task at these different ages. In other words, even though children have a significant increase in cortisol in response to the stressor at infancy, attunement does not show a greater decline from before to after the task at this age. Likewise, despite no significant cortisol reactivity in the toddlers, mother-child attunement does not remain stable across the stressor. This suggests that the decrease in attunement across the stressor is not solely due to the increase in child cortisol across the stressor, and supports the idea that children's heightened emotional reactivity, independent of their physiological reactivity, disrupts attunement. Thus, it is not merely the child's change in physiology that is responsible for the attunement disruption, but perhaps may be a product of intra-individual changes in physiology in the child and the mother as they both physiologically respond to the child's distress.

Positive maternal behaviors appear to be critical in fostering and maintaining mother-child cortisol attunement, by buffering reductions in attunement in response to the child focused stressor. Specifically, when mothers are more sensitive and positively engaged with their child during the free-play interaction, adrenocortical attunement persisted across the stress task. Thus, it seems that despite mothers' and children's overall divergent cortisol trajectories across the stressor, highly sensitive mothers are able to physiologically match their child's state. This finding corroborates multiple past examinations of mother-child adrenocortical attunement that have found more sensitive mothers to exhibit higher levels of attunement (e.g., Atkinson et al., 2013; Sethre-Hofstad et al., 2002; van Bakel & Riksen-Walraven, 2008). These past studies have suggested that mothers who are better able to behaviorally match might also be more adept at physiologically matching to their child's state. In other words, highly sensitive mothers are able to accurately recognize their child's biobehavioral state, and behaviorally and physiologically act on those perceptions. Most importantly, our data are the first to show that these sensitive mothers are dynamically adjusting their behavioral and physiological responses, even in the context of stress induced emotional changes in the child. This perhaps means that while all mother-child dyads have bouts of physiological attunement, dyads with sensitive mothers may spend a greater portion of their time physiologically attuned with their child.

Limitations and Future Directions

The sample in the current analyses is an epidemiologically valid representation of children and families

living in rural Pennsylvania and North Carolina. While the sample is representative of a substantial segment of the non-urban, low-income population in the United States; generalizability to urban populations is limited. Furthermore, the age of the sample and the continuing development of the HPA axis (Gunnar & Vazquez, 2006), may make the findings specific to children in the first two years of life.

Experimental studies specifically designed to disentangle the attunement process are needed. For example, systematic manipulation of each member of the dyad with stressors of varying degrees could begin to reveal who couples and un-couples physiological processes and the intensity of the stressor necessary to eliminate attunement. With saliva collection designs specifically targeting the attunement process, the timing of when a dyad becomes asynchronous, and how long it takes to resynchronize, could be uncovered. Similarly, comparing adrenocortical attunement across groups with qualitatively different interaction patterns (e.g., attachment classifications, night-shift working parents, and maternal depression) could elucidate systematic differences in the ability to establish or re-establish attunement. Lastly, studies should attempt to examine moment-to-moment changes in behaviors surrounding physiological assessments. In the current study, maternal behaviors were assessed during a mother-child free-play interaction that occurred during the home visit, but prior to the child-focused stressor. Future studies should assess maternal behavior immediately following the stressor to better understand the maternal behaviors responsible for task-related adrenocortical attunement, potentially examining moment-to-moment mother-child behavioral synchrony.

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