ABSTRACT: This study characterized the longitudinal evolution of HPA axis functioning from 7 to 16 months of age and identified individual and environmental factors that shape changes in HPA axis functioning over time. Participants were 167 mother–infant dyads drawn from a larger longitudinal study, recruited based on maternal history of being maltreated during childhood. Salivary cortisol levels were assessed before and after age-appropriate psychosocial stressors when infants were 7 and 16 months old. Maternal observed parenting and maternal reports of infant and environmental characteristics were obtained at 7 months and evaluated as predictors of changes in infant baseline cortisol and reactivity from 7 to 16 months. Results revealed that infants did not show a cortisol response at 7 months, but reactivity to psychosocial stress emerged by 16 months. Individual differences in cortisol baseline and reactivity levels over time were related to infant sex and maternal overcontrolling behaviors, underscoring the malleable and socially informed nature of early HPA axis functioning. Findings can inform prevention and intervention efforts to promote healthy stress regulation during infancy.

Keywords: HPA axis; infant; mother–infant relations; stress

INTRODUCTION

Hypothalamic-Pituitary-Adrenal (HPA) dysregulation has been proposed as a risk marker for lifespan psychopathology (e.g., Badanes, Watamure, & Hankin, 2011). However, attempts to identify early patterns of HPA axis functioning that convey risk have had mixed results. This may be, at least in part, due to normative changes in the functioning of this still maturing system. This paper seeks to: (1) characterize changes in cortisol baseline and reactivity levels during infancy (from 7 to 16 months) among a high-risk sample of infants whose caregivers had high rates of being maltreated during childhood; and (2) identify individual and environmental factors that prospectively predict these changes.

Infant cortisol levels have been associated with fearful, inhibited, and difficult temperament (Blair et al., 2008; Fortunato, Durbin, Granger, & Buss, 2008), prenatal stress (Leung et al., 2010), maternal psychopathology (Essex, Klein, Cho, & Kalin, 2002; Laurent, Ablow, & Measelle, 2012), caregiver sensitivity and engagement (Blair et al., 2008; Martinez-Torteya et al., 2014), neglect (Gunnar, Morison, Christholm, & Schuder, 2001), parent–child attachment (Ber-...
Highly influenced by external factors (i.e., maternal engagement), while temperamental characteristics (i.e., distress to novelty) became a significant predictor of cortisol secretion during toddlerhood, when the child’s self-regulation skills evolved.

To address these gaps, we examined changes in infant cortisol baseline and reactivity levels from 7 to 16 months of age, as well as the effects of known correlates of early HPA axis activity, including sex, temperament, maternal psychopathology, maternal parenting, and demographic factors. Due to the inconsistent findings of previous longitudinal studies and the dearth of research examining predictors of change in patterns of cortisol secretion over time, we did not specify a-priori hypotheses. We used validated, age-appropriate stress induction tasks with a sample of infants whose mothers were (74%) or were not (26%) victims of maltreatment during their own childhood. Understanding the early evolution of HPA activity and its correlates among high-risk groups is key to enhance early prevention and elucidate intervention targets to reduce the intergenerational effects of trauma.

METHOD

Participants and Procedures

Participants were 167 mother–infant dyads (56% male infants) drawn from a larger longitudinal study of stress during childbearing years (n = 269). Women were recruited in pregnancy and up to 4 months postpartum: (a) as part of a study on the effects of pregnancy PTSD on childbearing (60%; see Seng, Low, Sperlich, Ronis, & Liberzon, 2009, for details), or (b) through flyers distributed within the same Southeast Michigan community (40%). Women were classified based on a history of being maltreated during childhood and lifetime post-traumatic stress disorder (PTSD): (1) maltreatment and PTSD, (2) maltreatment without PTSD, and (3) no maltreatment, no PTSD. Participants were included in the present study if at least one infant cortisol sample (from 7 or 16 months) was available.

In the reduced sample, infants’ race/ethnicity was: 58% Caucasian, 22% African American, 12% Bi-Racial, 9% Latino, Asian/Pacific Islander, or other. Mothers were 18–45 years (M = 29.01, SD = 5.61), with median annual household incomes of $45,000–$49,999 per year (27.1% below $20,000). Sixty-three percent of mothers were Caucasian, 24% were African American, and 11% were Asian/Pacific Islander, Latina or biracial. Education was: 14% high school or less, 29% some college, 34% a Bachelor’s degree, and 23% graduate school. Seventy-seven percent were living with a partner and 71% lived with the child’s father. Seventy-four percent of the women reported being maltreated as children and about one fourth reported clinically significant levels of PTSD or depression 7 months postpartum (26% and 22%). Dyads in this reduced sample (n = 167) were not demographically different from non-participants (n = 102; all p > .05).
Data were collected during two home visits (1–2 weeks apart) when infants were 6–7 months \((M = 7)\) and at a laboratory visit when they were 15–18 months \((M = 16)\). During the 7 month assessment, mothers completed all the questionnaires and mother–infant dyads were videotaped during two 5-min free play sessions and the Still-Face Paradigm (SFP; Tronick, Als, Adamson, Wise, & Brazelton, 1978). This widely used, validated social stressor, comprises three sequential 2-min episodes – “normal” dyadic play, a maternal still-face episode (mother holds an unresponsive face and refrains from contact or communication with infant), and a re-engagement episode (dyad resumes normal play). Infants’ saliva was collected prior to, and 20-, 40-, and 60-min after the SFP, to capture baseline cortisol, stress-induced reactivity and return to baseline (Granger et al., 2007). This procedure can elicit a cortisol response even among low risk infants (Haley & Stansbury, 2003), but most studies report significant individual differences: high risk (e.g., maternal anxiety and low sensitivity), but not low risk infants, display a robust cortisol response to the SFP (Grant et al., 2009; Martinez-Torteya et al., 2014).

At the 16 month laboratory visit, dyads were videotaped in the Strange Situation Paradigm (SSP; Ainsworth, Blehar, Waters, & Wall, 1978). The SSP elicits mild, increasing stress on the dyad through introduction to an unfamiliar room, an unfamiliar “friendly stranger,” two brief separations from the mother, and two mother–infant reunions. Saliva samples were collected prior to, and 20-, 40-, and 60-min after the SSP. The procedure elicits increased cortisol for a majority of infants (Jansen, Beijers, Riksen-Walraven, & DeWeerth, 2010), including low (Goldberg et al., 2003) and high-risk samples (e.g., Laurent, Ablow, & Measelle, 2012). Significant individual differences exist, with relational (i.e., insecure/disorganized attachment) and temperamental risk heightening cortisol response to the SSP (Bernard & Dozier, 2010; Hertsgaard et al., 1995; Spangler & Grossman, 1993).

**Measures**

All individual and environmental characteristics were assessed when infants were 7 months.

**Demographics.** Following previous research (Sameroff, Seifer, Baldwin, & Baldwin, 1993), a cumulative demographic risk variable was computed based on five dichotomized self-report variables: income risk (<$20,000 yearly), minority status, age (<22 years), education (≤ high school), and partner status (not partnered). Range \(= 0–5\).

### Maternal Psychopathology

Mothers completed the Postpartum Depression Screening Scale (Beck & Gable, 2002) and the National Women’s Study PTSD Module (Resnick, Kilpatrick, Dansky, Saunders, & Best, 1993), two validated self-report measures with good reliability and links to clinical diagnoses (Beck & Gable, 2000; Resnick et al., 1993). Internal consistencies were high in our sample (Depression \(\alpha = .96\), PTSD \(\alpha = .91\)).

### Infant Temperament

Mothers completed the 25-item Negative Affectivity subscale and the 26-item Regulation subscale from the Infant Behavior Questionnaire–Revised (IBQ–R; Gartstein & Rothbart, 2003). The questionnaire has good reliability and validity (Gartstein & Rothbart, 2003; Parade & Leerkes, 2008). Subscale internal consistency was good (\(\alpha = -.88\)).

### Observed Maternal Parenting Behaviors

Trained staff blind to other variables coded the two 5-min free play sessions using 5-point Likert-style scales from the MACY Infant–Parent Coding System (MIPCS; Earls, Beeghly, & Muzik, 2009). Intra-class correlations were established through double-coding 40 random videotapes. Scores for the two sessions were significantly correlated and averaged. A conceptually-driven positive parenting composite was created by averaging the Behavioral Sensitivity, Engagement, Warmth, Affective Sensitivity, and Flexibility scales (ICC=.79–.89; \(M = .86\)). The overcontrolling behaviors scale was also used (ICC=.88).

### Infant Salivary Cortisol

Saliva was collected four times at 7 months and four times at 16 months. Samples were collected using absorbent cotton rolls, centrifuged at 7000 rpm for 10 min, stored at \(-20^\circ\text{C}\), and assayed using enzyme-linked immunosorbent assay (ELISA; 22%), radioimmunoassay (RIA; 30%), or Immulite (48%) techniques based on equipment availability. All infant samples at a specific time point were assayed in the same batch. Inter- and intra-assay coefficients of variation were all <10%. Cortisol values higher than three were deleted because they are outside of the detection range (0.03–3.0 \(\mu\text{g/dl}\)). Values higher than three \(SDs (n = 14)\) were windsorized (truncated at \(3*SD\) and \(1.0*SD\) was added sequentially to preserve rank order; windsorized range = 0.01–1.40 \(\mu\text{g/dl}\)). Values were log-transformed to normalize their distribution, following convention (e.g., Dettling, Parker, Lane, Sebanc, & Gunnar, 2000).

### Data Analysis Plan

Hierarchical Linear Model (HLM) in HLM 7.1 (Raudenbush, Bryk, Cheong, Congdon, & du Toit, 2011)
was used. It can accommodate an uneven number of data points per individual and test complex interactions with a limited sample size (Littell, Henry, & Ammerman 1996). Following Raudenbush & Bryk (2002); models with increasing complexity were estimated in a step-wise fashion, beginning with the unconditional model, adding covariates (i.e., assay type, time of day, time from eating/sleeping), then level-1 (i.e., time varying) fixed effects one by one (Sample number, Age, and Age-by-Sample interactions), and level-1 random effects one at the time. Finally, 7-month level-2 predictors (i.e., person-level; Demographic Risk, Infant Negativity, Infant Regulation, Maternal Depressive Symptoms, PTSD Symptoms, Positive Parenting, and Overcontrolling Behavior) were added. To avoid multicollinearity and enhance power, independent models were estimated for each level-2 predictor. Model fit was assessed using the Deviance $\chi^2$ difference test. Full Maximum Likelihood (FIML) estimation was used to compare the fit of models with additional fixed effects; Restricted Maximum Likelihood (REML) was used to compare models with additional random effects (Verbeke & Molenberghs, 2000).

Sample number was recoded into three dummy-coded variables using baseline as the reference group: (a) “Sample2” captured change from pre- to 20-min post-task; (b) “Sample3” captured change from pre- to 40-min post-task; and (c) “Sample4” captured change from pre- to 60-min post-task. Age was dichotomized (0 = 7 months; 1 = 16 months). Three Age-by-Sample interactions were included to test for changes in reactivity and regulation over time. For example, a significant positive effect for the Age-by-Sample2 interaction suggests that cortisol reactivity 20-min post-stress increased from 7 to 16 months. Random effects evaluate significant person-level variation in the estimated parameters. The random effects for the Age-by-Sample interactions were not estimated to avoid extreme model complexity, based on sample size. The random effects for Lab2 and Lab3 were not included in the models because there was no expectation of person-level differences in the effect if assay type.

RESULTS

Missing Data and Descriptive Statistics

Each of the 167 infants provided at least one saliva sample, but sample numbers from each varied due to attrition, naps, inadequate volume, or technical problems. Seventeen percent of dyads ($n = 29$) dropped out between assessments due to moving away, time conflicts, or not being reached despite several attempts. A total of 545 saliva samples were available. Out of eight possible samples, most infants provided 3–4 (53%), 16% provided 5–8, and 31% provided 1–2. The number of samples available was not significantly associated with any predictor variable. HLM 7.1 can accommodate datasets with uneven data points per individual, but necessitates complete person-level data. Less than 9% of person-level data points were missing at random (MAR; based on nonresponse analyses). Data were imputed using the Expectation Maximization algorithm (Dempster, Laird, & Rubin, 1977) in SPSS 21. Means, standard deviations, and correlations are shown in Table 1.

Model Testing

Assay type was associated with cortisol levels, but other potentially relevant variables (i.e., collection time and recent feeding or sleeping) were not. Thus, HLMs were estimated controlling only for assay type, using two dummy coded variables (Lab 2 and Lab 3).

Baseline Cortisol and Reactivity From 7 to 16 Months. Based on fit statistics, the best fitting model included the fixed effects of Age, Sample2, Sample3, Sample4, and the Age-by-Sample interactions, as well as random components for the intercept, for the main effect of Age on the intercept, and for the main effects of Sample2 and Sample3. The final model had a significantly better fit than the unconditional model (Deviance = 84.38, $df = 9$, $p < .01$), the model with only age and sample number predictors (Deviance = 7.60, $df = 3$, $p < .05$) and the model without random effects (Deviance = 49.28, $df = 12$, $p < .01$). Sample2, $\beta = -0.03$, $p < .05$; Sample3, $\beta = -0.03$, $p < .05$; and the Age-by-Sample2 interaction, $\beta = 0.07$, $p < .01$ had a significant effect on cortisol levels. Results were plotted following Cohen, Cohen, West, and Aiken (2013); (Fig. 1). Seven-month-old infants’ cortisol levels decreased from baseline to the 20- and 40-min post-SFP samples. An opposite pattern was observed at the 16-month laboratory visit, as cortisol levels increased from baseline to 20-min post-SSP.

Predictors of Cortisol Baseline and Reactivity Levels. Estimated HLMs included all components of the level-1 best fitting model, and added the main effect of the level-2 predictor on the intercept and the slopes of all level-1 predictors. There was a significant effect of Sex on the Age Slope, $\beta = .01$, $p < .01$; boys’ baseline cortisol levels had a smaller decrease from 7 to 16 months than girls’ (Girl $M = 23.23$–.04 μg/dl; Boy $M = 19.19$–.10 μg/dl). There was an effect of maternal Overcontrolling Behavior on the Age-by-Sample2 inter-
action slope, $\beta = -0.08$, $p < .05$. More maternal overcontrolling behavior was associated with less change in infant reactivity from 7 to 16 months. In contrast, less overcontrolling behavior was associated with increasing infant reactivity from 7 to 16 months (Fig. 2). All other predictors were not significantly associated with infant cortisol.

**DISCUSSION**

This study examined infant patterns of baseline and reactivity cortisol from 7 to 16 months in a high-risk sample of infants. We also examined individual and environmental factors that predict these changes over time. Overall, infants did not show a cortisol response to psychosocial stress at 7 months, but displayed reactivity at 16 months. Infant sex and maternal overcontrolling behavior during free-play at 7 months significantly moderated the changes in cortisol secretion patterns. Girls’ baseline cortisol levels declined more than boys’ from 7 to 16 months. Also, low levels of maternal overcontrolling behavior were associated with emergence of reactivity at 16 months, whereas infants of mothers with more overcontrolling behaviors failed to show a stress response at 7 and 16 months.

Girls’ dampening of baseline cortisol over time is consistent with findings that girls have lower baseline levels at 6 and 15 months, as compared to boys (Gunnar et al., 1996). However, past research has not examined sex differences in the evolution of HPA axis functioning in early life. This finding may reflect differential maturation of the HPA axis for boys and girls, and is consistent with previous findings of

![FIGURE 1](image)
enhanced behavioral regulation in toddler girls', relative to boys (Calkins, Dedmon, Gill, Lomax, & Johnson, 2002; Else-Quest, Hyde, Goldsmith, & Van Hulle, 2006). This finding is also consistent with adult literature that documents higher stress-induced HPA activity among males (for a review, see Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004). Although all infants provided baseline cortisol first in the lab (7 months) and then at home (16 months), this change in setting could have influenced results: the circumstances leading to the lab visit (i.e., mother’s anxiety about the visit, car ride) may have affected girls’ and boys’ differently, as girls may be better able to regulate daily hassles and novelty at this age (Kochanska & Murray, 2001). Thus, sex differences in baseline cortisol changes between the first and second year of life need to be replicated.

Overall, there was no cortisol reactivity at 7 months, but there was at 16 months. Lack of reactivity at 7 months was also reported by two longitudinal studies with low risk samples (Davis & Granger, 2009; Gunnar et al., 1996), but contrasts with the findings of Blair et al. (2008) who assessed a high-risk, low SES sample. Specific to the SFP, this finding is somewhat inconsistent with previous reports that healthy 5–6 month-olds show cortisol reactivity (Haley & Stansbury, 2003). However, in that study the method was twice as long and potentially more stressful. The reactivity observed post-SSP in our sample is consistent with Blair et al.’s (2008) results with 16-month-olds from their low SES sample. Specific to the SSP, findings are similar to those obtained with other high-risk samples (i.e., low SES; Laurent et al., 2012) and among infants with temperamental or relational risk (Hertsgaard et al., 1995; Nachmias et al., 1996). Findings challenge previous suggestions that reactivity to psychosocial stress gradually dampens from high in early infancy to low by 7 months, and very low in the second year of life (Gunnar et al., 2009).

Importantly, we found significant individual differences in the pattern of reactivity over time. More maternal overcontrolling behavior predicted lack of reactivity and little change over time, but infants not exposed to overcontrolling maternal behaviors showed increased cortisol reactivity from 7 to 16 months. A “healthier” parenting style may enhance infant emotional and physiological stress, as proposed by the “maternal buffering” hypothesis (Gunnar & Donzella, 2002). Thus, mobilization of the cortisol response during the SSP may reflect adaptive recruitment of physiological resources when the caregiver is unavailable. On the other hand, overcontrolling parenting is associated with chronic stress and contextual risk (Grolnick, Weiss, McKenzie, & Wrightman, 1996) and predicts infant negativity (Ispa et al., 2004), which may all contribute to difficulties mobilizing the physiological stress response in the face of threat.

In our sample, most mothers were victims of childhood maltreatment, and thus at risk for parenting problems, harsh parenting, and more infant temper-
mental difficulty (DiLillo & Damashek, 2003; Lang, Gartstein, Rodgers, & Lebeck, 2010). Findings can inform prevention and intervention efforts with this population, targeting overcontrolling maternal parenting to promote healthy infant cortisol regulation. Notably, parenting was the only significant predictor of infants’ reactivity, highlighting the importance of caregiving and its potential to normalize HPA axis functioning when other adversity is present.

The current study is not without limitations. The use of different settings for the 7 and 16 month assessments and different stress-induction tasks at each time point (SFP and SSP) complicate interpretation of age-related changes in HPA functioning. Due to novelty, the laboratory setting may have influenced baseline cortisol more than the home setting. Moreover, there is no single task that is equivalent to the SFP for 16-month-olds or to the SSP for 7-month-olds. However, both are developmentally appropriate and widely used to elicit stress, and both include short maternal unavailability. Second, findings obtained with this high risk sample may not be generalizable to the general population. Third, due to reasons outside of the investigators’ control three different methods for cortisol assaying were used (20% RIA, 30% Immulite, and 50% ELISA). To ensure that results were unaffected by assay methodology used, lab number was controlled for in all statistical modeling. Nevertheless, the fact that no infant samples were tested by all three methods is a limitation. Finally, the limited sample size prevented examination of the additive or interactive effects of individual and environmental risk over time. Larger samples are needed to replicate our findings and evaluate complex models.

In sum, the current longitudinal study evaluated changes in baseline and reactivity cortisol from ages 7 to 16 months among a high-risk sample of infants. Infants did not show a cortisol response to the SFP at 7 months but showed reactivity to the SSP at 16 months. Individual differences in cortisol secretion patterns were related to infant sex and maternal overcontrolling behaviors, underscoring the malleable and socially informed nature of early HPA axis functioning trajectories. Findings can inform prevention and intervention efforts to promote healthy stress regulation during infancy.

REFERENCES


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