ABSTRACT

Title of Thesis: STABILITY AND CHANGE OF CORTISOL REACTIVITY TO A LABORATORY STRESSOR FROM EARLY TO MIDDLE CHILDHOOD

Katherine A. Leppert, Master of Science, 2015

Thesis Directed By: Dr. Lea R. Dougherty, Associate Professor
Department of Psychology

This study examined the stability and change of children's cortisol reactivity to a laboratory stressor from early to middle childhood and moderators of change. Ninety-six children completed stress-inducing laboratory tasks and provided five salivary cortisol samples at preschool age (T1; *M* = 49.88 months, *SD* = 9.51 months) and three years later (T2; *M* = 87.44 months, *SD* = 11.42 months). At T1, parents completed clinical interviews assessing child and parent psychopathology.

Cortisol reactivity patterns significantly changed from decreasing to increasing reactivity from early to middle childhood. Moreover, preschool psychopathology moderated this change. Children with fewer preschool psychiatric symptoms demonstrated more stable reactivity patterns, whereas children with preschool psychiatric comorbidity demonstrated more unstable reactivity patterns across assessments. Findings suggest a developmental shift from decreasing to increasing cortisol reactivity from early to middle childhood, and highlight early preschool psychopathology as a moderator of change in cortisol reactivity.

STABILITY AND CHANGE OF CORTISOL REACTIVITY TO A LABORATORY STRESSOR FROM EARLY TO MIDDLE CHILDHOOD

by

Katherine A. Leppert

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Advisory Committee:
Professor Lea R. Dougherty, Chair
Professor Laura MacPerson
Dr. Julia M. Shadur

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Chapter 1: Introduction

Developmental psychoneuroendocrine research has supported the widespread significance of children's biological responses to stress across the lifespan (Gunnar & Herrera, 2013). In response to a stressor, the hypothalamic-pituitary-adrenal (HPA) axis, one of the body's major stress response systems, initiates a cascade of hormonal pathways resulting in the release of the primary stress hormone cortisol, which then typically attenuates following the stressor. Individual differences in children's cortisol reactivity to a laboratory stressor have been linked to numerous factors relevant to developmental psychopathology (for a review see Gunnar & Vazquez, 2006), including child temperament (e.g., Donzella, Gunnar, Krueger, & Alwin, 2000), parenting (e.g., Dougherty, Klein, Rose, & Laptook, 2011), life stress and maltreatment (e.g., Elzinga et al., 2008) and children's concurrent emotional and behavioral problems (e.g., Kryski, Smith, Sheikh, Singh, & Hayden, 2013). Moreover, children's cortisol reactivity has been shown to prospectively predict later emotional and behavioral problems (e.g., Hastings et al., 2011). While this measure holds great significance in developmental research, we know little about its stability and change across development, particularly through middle childhood.

Research provides evidence that the HPA-axis undergoes considerable change across development. Both longitudinal and cross-sectional studies demonstrate that basal cortisol levels decrease across the first few years of life and then exhibit a significant increase between middle childhood and adolescence (Gunnar, Brodersen, Krueger, & Rigatuso, 1996; Lupien, King, Meaney, & McEwen, 2001). However, much less is known about the developmental trajectory of cortisol reactivity. Cross-

sectional studies suggest that cortisol reactivity varies as a function of age, but the data is rather limited. For instance, young infants are able to mount stress-related cortisol increases, though the magnitude of the cortisol response to a stressor appears to decrease across infancy (Davis & Granger, 2009; Gunnar & Vazquez, 2006) and into the second year of life (Urasche, Blair, Granger, Stifter, & Voegtline, 2014). During early childhood (ages 3-5 years), results are mixed. Several studies have found that preschoolers' cortisol levels decrease following a laboratory stressor (Dougherty et al., 2011; for a review see Gunnar, Talge, & Herrera, 2009a; Hankin, Badanes, Abela, & Watamura, 2010; Luby et al., 2003), while one study found that preschoolers' cortisol levels increase following a stressor presented in the home environment (Kryski, Smith, Sheikh, Singh, & Hayden, 2011). By middle childhood, findings suggest that children demonstrate increases in cortisol levels in response to a laboratory stressor (de Veld, Riksen-Walraven, & de Weerth, 2012; Hankin et al., 2010; Lopez-Duran, Hajal, Olson, Felt, & Vazquez, 2009), and cortisol reactivity appears to increase further through adolescence, possibly coinciding with pubertal onset and hormonal fluctuations (Gunnar, Wewerka, Frenn, Long, & Griggs, 2009b; Stroud et al., 2009). Taken together, these cross-sectional studies suggest cortisol reactivity increases across development.

Despite the cross-sectional research, surprisingly few longitudinal studies have examined the stability and change in cortisol reactivity across development. In infants, cortisol reactivity to a psychosocial stressor (e.g., doctor's visit, parental separation) demonstrated low to moderate stability across one week (Spearman's rho = 0.42; Goldberg et al., 2003) and two-months (Pearson's r = 0.26; Lewis & Ramsay,

1995). In older children (9-15 years-old), Hankin and colleagues (2015) reported moderate test-retest stability (partial $r = 0.41$) of cortisol reactivity over 18 months. Moreover, adolescent girls evidenced greater decreases in cortisol reactivity over one year than adolescent boys, suggesting that girls and boys may differ in their cortisol response to stress over time (Susman, Dorn, Inoff-Germain, Nottelmann, & Chrousos, 1997). Only a few longitudinal studies have been conducted in adults, with some evidence demonstrating short-term stability of cortisol reactivity over periods of up to two weeks (Pearson's *r*s = 0.17-0.60; Cohen et al., 2000; Kirschbaum et al., 1995), though stability estimates decrease over a one-year follow-up (Burleson et al., 2003). While these longitudinal studies provide initial data on the stability and change of cortisol reactivity across development, major questions remain.

To date, no previous study has examined the stability and change of the cortisol response to a laboratory stressor from early to middle childhood. Investigating neuroendocrine development across childhood is critical for several reasons. First, we may gain a crucial understanding of children's physiological adaptation to the increasing social demands and academic stressors from early to middle childhood. Second, increased cortisol reactivity has been proposed to be an early emerging biomarker, or possibly an endophenotype, for the development of psychopathology (Gottesman & Gould, 2003; Hasler, Drevets, Manji, & Charney, 2004). If cortisol reactivity demonstrates stability over time, this may lend support for stress reactivity as an intermediate phenotype for risk. Third, given that previous longitudinal studies include follow-up periods limited to eighteen months or less, longer follow-up periods are necessary to characterize the developmental course of

the body's stress system over time, which may shed light on the biological processes underlying the emergence of psychopathology. Lastly, no study has examined factors associated with change in cortisol reactivity, which may highlight developmental risk factors for physiological dysregulation.

To address the gaps, the current study employed a longitudinal design to investigate the continuity and change in cortisol reactivity from early (3-5 years, T1) to middle childhood (6-10 years, T2). We recruited an ethnically diverse sample of 96 children from the community, a subsample of which was targeted based on a maternal lifetime history of depression. Cortisol responses to a laboratory stressor were measured during the preschool period and again approximately three years later using age-appropriate standardized laboratory stressor paradigms. We used different laboratory stressor paradigms across assessment waves to maintain the potency of each task's stress-inducing properties across the different age groups. Nevertheless, both tasks included similar performance and social evaluation components known to induce cortisol responses in older children and adults (Dickerson & Kemeny, 2004; Gunnar et al., 2009a).

At both assessment waves, baseline salivary cortisol samples were collected before the stressor, and four salivary cortisol samples were collected following the tasks to assess the cortisol response and recovery from the stressors Previous studies examining the stability of cortisol reactivity have reported correlations between average cortisol levels across all cortisol reactivity samples or a summary statistic of cortisol reactivity across assessments. In contrast to these studies, which only estimated population-level change and were limited to subjects without missing data,

we employed mixed linear modeling (MLM) to examine both individual- and population-level patterns of cortisol reactivity over time. MLM offers many advantages, including the ability to examine two or more levels of data, model alternate covariance structures, and handle missing values (Singer & Willett, 2003).

We hypothesized that children's cortisol reactivity to a laboratory stressor would evidence low stability and a developmental shift from decreasing cortisol levels in response to the stressor in early childhood (i.e., negative linear slope and positive curvature) to increasing cortisol levels in response to the stressor in middle childhood (i.e., positive linear slope and negative curvature) These hypotheses were based on cross-sectional data showing that while preschoolers' cortisol levels tend to decrease following a laboratory stressor (e.g., Donzella et al., 2000; Tolep & Dougherty, 2014), older children evidence the typical rise in cortisol following a laboratory stressor (e.g., Hankin et al., 2010).

We further investigated whether the stability or change of cortisol reactivity was related to several early individual- and environmental-level factors previously linked to children's cortisol response to stress. First, we explored whether early child mental health problems predicted change in cortisol reactivity from early to middle childhood. In children as young as preschool-age, alterations in the cortisol response to stress have been linked to their concurrent emotional and behavioral problems (Hastings et al., 2011; Kryski et al., 2013; Luby et al., 2003). Thus, it is possible that children's early mental health problems may moderate changes in the cortisol response to stress over time, which could help explain developmental pathways of risk and adaptation involving the HPA-axis. Second, despite findings that males

demonstrate higher cortisol reactivity than females in both adolescence and adulthood (e.g., Klimes-Dougan, Hastings, Granger, Usher, & Zahn-Waxler, 2001; Lopez-Duran, Mayer, & Abelson, 2014), no clear evidence exists for gender differences during early childhood (Dougherty et al., 2011; Kryski et al., 2011; Kudielka & Kirschbaum, 2005). However, given that increased cortisol reactivity has been observed for males in infancy (Davis & Emory, 1995) and middle childhood (Dahl et al., 1992), we explored the role of child gender as a moderator of change in cortisol reactivity from early to middle childhood.

Lastly, we examined whether the stability and change of cortisol reactivity varied as a function of maternal depression history. Maternal depression is associated with numerous disruptions in the early rearing environment, including parenting problems, marital discord, and socioeconomic disadvantages, and reflects a contextual risk factor for later maladjustment (Goodman & Gotlib, 1999; Lovejoy, Graczyk, O'Hare, & Neuman, 2000; Weissman et al., 2006). Moreover, disruptions in the early rearing environment, including maternal depression, have been linked to abnormalities in offspring's cortisol reactivity (Brennan et al., 2008; Dougherty, Tolep, Smith, & Rose, 2013; Feldman et al., 2009). Thus, we explored whether maternal depression history moderated the change in cortisol reactivity over time.

Given the paucity of research, we tentatively hypothesized that greater increases in children's cortisol reactivity from early to middle childhood would be observed for children with high levels of preschool psychiatric symptoms and comorbid diagnoses, for boys, and for the offspring of depressed mothersGiven the paucity of research, we tentatively hypothesized that greater increases in children's

cortisol reactivity from early to middle childhood would be observed for children with high levels of preschool psychiatric symptoms and comorbid diagnoses, for boys, and for the offspring of depressed mothers.

Chapter 2: Methods

Participants

The sample was drawn from an initial sample of 156 preschool-aged children (*M* = 49.88 months, *SD* = 9.51 months, range = 36-71 months, 49.4% male) who completed a laboratory-based cortisol reactivity assessment at T1 (Dougherty et al., 2013). Of the 156 children, 99 (*M* = 87.44 months, *SD* = 11.42 months, range = 66-120 months, 52.5% male) returned for the T2 visit and completed a second laboratory-based cortisol reactivity assessment. At T1, participants were recruited from the Washington D.C. metropolitan area through flyers (73.1%) and a commercial mailing list (26.9%). Eligible children were between three to five years of age, had an English-speaking biological parent with at least 50% legal custody, and had no significant physical or developmental conditions. The initial sample targeted a subset of children whose mothers had a lifetime history of depression (major depressive disorder and/or dysthymic disorder) based on the Structured Clinical Interview for DSM-IV, Non-Patient version (SCID-NP; First, Spitzer, Gibbon, & Williams, 1996) (Kappa = 1.00, *n* = 15). Of the 96 participants included in the current study (see below), 48 (50.5%) biological mothers met criteria for a lifetime history of maternal depression.

At T1, one participant was excluded due to extreme cortisol values (>3 SD above the mean; Gunnar & White, 2001). At T2, two participants were excluded due to extreme cortisol values or use of antibiotics. Thus, 96 children were included in the final sample for this study. We compared participants who completed both assessments to those who only completed T1 on child gender, race, ethnicity, age,

parent marital status, parental education, maternal depression history, and child psychiatric symptoms, and only one significant difference emerged: families who completed both waves were more likely to have a parent with at least a 4-year college degree (77.8%) than families who only completed the first wave (54.4%) (χ^2 (1, N = 154) = 9.292, p = 0.002). This study was approved by the Institutional Review Board. Informed consent was obtained from parents at both assessments, and child assent was obtained for children seven years and older at T2. Table 1 presents the sample's demographic characteristics.

Procedure

The first wave of data collection (T1) occurred between February 2010 and July 2011. The second wave of data collection (T2) occurred approximately three years later, between March 2013 and October 2014. At both data collection points, children participated in stress-inducing laboratory procedures. At T1, parents completed clinical interviews assessing both parent and child psychopathology.

Early Childhood Assessment (T1)

Children's Psychiatric Symptoms and Disorders. Children's current psychiatric symptoms over the previous three months were assessed with the Preschool Age Psychiatric Assessment (PAPA; Version 1.4; Egger, Ascher, & Angold, 1999), a semi-structured diagnostic interview for children ages 2-6. All parents (94.8% mothers) completed the PAPA at T1. A total preschool symptoms scale score (M = 22.45, SD = 11.59, range = 0 - 60) was created by summing items in each of the following diagnostic categories: depression (major depressive disorder, dysthymia, or depression-not otherwise specified [NOS]), anxiety (specific phobia,

separation anxiety, social phobia, generalized anxiety disorder, agoraphobia, panic disorder, selective mutism), attention-deficit/hyperactivity disorder (ADHD), and oppositional defiant disorder (ODD). The total symptoms scale score had excellent internal consistency and inter-rater reliability ($\alpha = .97$; *ICC* = .94, $n = 15$). In addition, given the significant comorbidity between psychiatric disorders in preschoolers (Bufferd, Dougherty, Carlson, & Klein, 2011), we assessed children's total number of psychiatric diagnoses at T1: 55 (57.3%) children did not meet diagnostic criteria for any T1 psychiatric disorder; 28 (29.2%) children met diagnostic criteria for one disorder; and 13 (13.5%) children met criteria for two or more comorbid disorders. PAPA diagnoses evidenced good inter-rater reliability for any diagnosis (Kappa = 0.64, $n = 15$).

Stressor Paradigm and Cortisol Reactivity Assessment. At T1, children's cortisol reactivity was assessed using a developmentally appropriate acute stressor paradigm (Kryski et al., 2011). See Dougherty et al. (2013) for a complete description of the task. The preschool stressor paradigm consisted of a timed matching task in the presence of the experimenter ($M = 8.11$ minutes, $SD = 1.96$). The child was told that the matching task was easy and that most children experienced no difficulty completing it in the allotted time. The experimenter manipulated the timer such that the child failed three trials, while also pretending to take notes on the child's performance to elicit feelings of social evaluation. After the third failed trial, the experimenter informed the child that the timer was broken and presented the child with a prize. The task incorporates several elements shown to evoke a cortisol response in older children and adults, including uncontrollability and social

evaluation (for reviews see Dickerson & Kemeny, 2004; Gunnar et al., 2009a). Salivary cortisol samples were collected from children at multiple time points: prior to the stressor paradigm (baseline; S1), and 20 (S2), 30 (S3), 40 (S4), and 50 (S5) minutes after the stressor task was completed.

Middle Childhood Assessment (T2)

Stressor Paradigm and Cortisol Reactivity Assessment. At T2, children were exposed to novel, developmentally appropriate laboratory stressor tasks. After choosing a favorite and least favorite prize, children were told that an unfamiliar "judge" would determine their prize based on their performance on a series of games. Participants completed a modified version of the Trier Social Stress Task for Children (TSST-C; Buske-Kirschbaum et al., 1997), a task shown to effectively evoke a cortisol response in children (Gunnar et al., 2009a), and a puzzle task (M = 10.21 minutes, SD = 0.52).[1] Children were presented with a picture book and instructed to tell a 4.5 minute story after a 30 second preparation period. In instances where children paused for over 15 seconds or completed their story before the 4.5 minutes elapsed, they were instructed to continue telling their story. At the end of the story-telling task, children were presented with an unsolvable puzzle to assemble within three minutes and were told that the puzzle was easy for young children. The puzzle contained pieces from two highly similar but different puzzles, rendering the task impossible to complete. For both tasks, the judge pretended to take notes on the child's performance to elicit feelings of social evaluation. Following the puzzle task, the judge left the room to deliberate on which prize the participant had earned while

[1] The T2 stressor task was significantly longer in duration than the T1 stressor task, $t(95) = -10.747$, $p < 0.001$. However, results were similar when controlling for the duration of the stressor tasks in analyses.

the child was allowed to color freely. Four and a half minutes into the coloring period, the experimenter also left the room for 30 seconds to confer with the judge on which prize she had decided to give the child. Upon re-entry, the experimenter told the participants they had done well and presented them with their favorite prize. The experimenter also informed the children about the mixed-up puzzle pieces and apologized for the mistake. Similar to procedures used at T1, participants provided five salivary cortisol samples: prior to the stressor paradigm (baseline; S1), and 20 (S2), 30 (S3), 40 (S4), and 50 (S5) minutes after the stressor tasks were completed.

Salivary Cortisol Data Collection

At T1 and T2, parents were asked to refrain from feeding their child for one hour prior to coming to the laboratory, and refrain from giving their child caffeine two hours prior to their laboratory visit to minimize influences of factors known to affect cortisol levels (Gunnar & Talge, 2007). In addition, given that cortisol levels vary as a function of time of day, the majority of laboratory visits were scheduled for the afternoon (78.1% at T1; 93.8% at T2). Saliva samples were obtained by having participants dip a cotton dental roll into a few grains (0.025 mg) of Kool-Aid® mix, and then chew the cotton roll until saturated (~1 minute). Kool-Aid® was chosen to facilitate the production of saliva, and the saturated cotton was expressed into a vial by the experimenter. These sampling procedures have been shown to yield little-to-no effect on salivary cortisol concentrations (Talge, Donzella, Kryzer, Gierens, & Gunnar, 2005). Vials were frozen at -20º Celsius until assayed in duplicate using a time-resolved fluorescence immunoassay with fluorometric end-point detection (DELFIA). Salivary cortisol samples were assayed at the Biochemical Laboratory at

the University of Trier, Germany. Inter- and intra-assay coefficients were 7.1%-9.0% and 4.0-6.7%, respectively.

Data Analysis Plan

Due to the nested nature of cortisol samplings, we modeled cortisol reactivity at each wave and across waves using MLM with the MIXED procedure in SPSS v. 21 (West, Welch, & Galecki, 2014). We applied a growth-modeling framework to examine the linear and quadratic effects of time, which was indexed by the five cortisol samplings at each wave. All five cortisol samples were nested within wave and person. Intercepts and slopes were modeled as random to capture individual differences in baseline cortisol (intercept) and cortisol reactivity (slope and curvature). Cortisol values were positively skewed and thus log10-transformed cortisol levels served as the dependent variable in all analyses, though untransformed cortisol values are presented in figures and tables for ease of interpretation.

First, we examined cortisol reactivity at each individual wave by modeling the five individual cortisol assessments as outcomes that were predicted by the linear and quadratic effects of time relative to the laboratory stressor (within each wave). This was accomplished by including both time (linear slope) and time^2 (quadratic curvature) as predictors of the individual cortisol assessments. Time was indexed by the five cortisol samplings at each wave (baseline, 20 minutes, 30 minutes, 40 minutes, and 50 minutes), and the intercept represented the baseline sample (S1) at each wave. Next, we examined whether children's cortisol reactivity curves varied as a function of assessment wave. All ten cortisol samples across both waves served as the dependent variable, and time was indexed by the five cortisol samplings nested

within each wave (coded T1 = 0 and T2 = 1). We examined the wave X time and wave X time^2 interactions to determine how the slope and curvature of children's cortisol reactivity profiles differed by assessment wave.

Finally, we examined whether children's T1 psychiatric symptoms and comorbidity, child gender, or maternal lifetime depression history moderated the association between T1 and T2 cortisol reactivity in separate models. Children's total T1 PAPA symptoms was a continuous variable. Preschool psychiatric comorbidity (no disorder vs. one disorder vs. two or more disorders) was dummy coded into two variables for one psychiatric disorder only (0 = absent, 1 = present) and for two or more psychiatric disorders (0 = absent, 1 = present), and both dummy-coded variables were included as independent variables in the psychiatric comorbidity models. Child gender (0 = male, 1= female) and maternal lifetime depression (0 = absent, 1 = present) were coded dichotomously. To test these predictors of change, the individual cortisol samples at T2 were modeled as a function of the cortisol samples at T1. T1 cortisol was partitioned into between-subjects and within-subjects components. To assess between-subjects variation in cortisol, the T1 cortisol assessments were averaged ("average T1 cortisol") for each person, to obtain a measure of individual differences in cortisol at T1. A positive effect of average T1 cortisol indicates that between-person differences in average cortisol were stable over time. To assess within-subjects variation in cortisol, we used the individual T1 cortisol samples after centering them on each participant's average T1 cortisol ("person-centered T1 cortisol"). Given that we used this centering strategy, variation in this variable only reflects within-person temporal change or reactivity in cortisol and is purged of all

between-person variance (Enders & Tofighi, 2007). A positive effect of person-centered T1 cortisol on T2 cortisol indicates that participants had a similar pattern of cortisol reactivity to the stressor at T1 and T2, regardless of whether their average cortisol levels changed. Both average and person-centered cortisol values at T1 were included in all moderation models and examined for their interactions with each predictor variable. Significant interaction terms were probed using simple slopes analyses (Aiken & West, 1991; Curran, Bauer, & Willoughby, 2006).

Chapter 3: Results

Pearson product-moment correlations between study variables are presented in Table 2. All correlations between cortisol samples within each wave were significant (ps < 0.001), while no correlations between T1 cortisol values and T2 cortisol values were significant (ps > 0.164).

We examined associations between cortisol values and potential covariates, including age at each assessment, race/ethnicity, parental education, and parental marital status. We also assessed multiple factors previously shown to affect children's cortisol levels, including time of sampling, medication use, and food and caffeine intake (Gunnar & Talge, 2007). Of these potential confounds, time of sample collection was negatively associated with the cortisol reactivity growth curve models using MLM and thus was included as a covariate in all analyses.

Children's cortisol reactivity from early to middle childhood

Figure 1 illustrates the growth curves for cortisol reactivity at each wave. At T1, we observed a significant negative linear effect (b = -0.023, SE = 0.006, t = -3.643, p < 0.001) and a significant positive quadratic effect of cortisol reactivity (b = 0.003, SE = 0.001, t = 2.858, p = 0.004), indicating that cortisol levels at T1 were highest at baseline and decreased after the stressor until leveling off around 40 minutes post-stressor. In contrast, at T2 the linear effect of cortisol reactivity over time was not significant (b = 0.014, SE = 0.008, t = 1.764, p = 0.079); however, there was a significant negative quadratic effect of time (b = -0.003, SE = 0.001, t = 2.151, p = 0.032), indicating that cortisol increased from baseline to 30 minutes post-stressor before declining.

We then investigated whether cortisol reactivity significantly changed from T1 to T2.[2] As seen in Table 3, assessment wave was a significant moderator of the slope and curvature of cortisol reactivity over time. Compared to children's cortisol reactivity at T1, children's cortisol reactivity at T2 demonstrated an increased slope and more negative quadratic curvature. Moreover, we observed a low intraclass correlation coefficient (ICC) ($\rho = -0.10$) between the two waves, indicating that cortisol samples at T1 demonstrated low negative correlations with their respective T2 cortisol samples.

Early predictors of change in cortisol reactivity from early to middle childhood

Early childhood psychopathology. Table 4 presents the main and interactive effects of average and person-centered T1 cortisol and T1 child psychiatric symptoms on children's T2 cortisol reactivity. There were no significant main effects for average T1 cortisol or person-centered T1 cortisol, indicating that cortisol reactivity at T1 did not significantly predict cortisol reactivity at T2. However, we did observe a significant interaction between average T1 cortisol and T1 total PAPA symptoms in predicting T2 cortisol reactivity. When probed, the association between average T1 cortisol and T2 cortisol reactivity was not significant for either children high in T1 total PAPA symptoms ($b = -0.084$, $SE = 0.045$, $t = -1.873$, $p = 0.064$) or for children low in T1 total PAPA symptoms ($b = 0.074$, $SE = 0.044$, $t = 1.691$, $p = 0.094$) at T1. As seen in Table 4, there was also a significant interaction between children's person-

[2] Given that children ranged in age at each assessment wave, we also conducted analyses examining whether child age, rather than assessment wave, moderated the change in cortisol reactivity over time. Similar to results when examining assessment wave as the moderator, child age significantly moderated the linear ($b = 0.002$, $SE = 0.001$, $t = 3.099$, $p = 0.002$) and quadratic ($b < 0.001$, $SE < 0.001$, $t = -2.631$, $p = 0.009$) effects of time on cortisol reactivity: as child age increased, children's cortisol reactivity demonstrated a significant increase in slope and a more negative curvature.

centered T1 cortisol and T1 total PAPA symptoms in predicting cortisol reactivity at T2. For children low in T1 total PAPA symptoms, person-centered T1 cortisol showed a positive association with T2 cortisol reactivity (b = 0.017, SE = 0.008, t = 2.038, p = 0.042), indicating a stable pattern of cortisol reactivity from T1 to T2. In contrast, for children high in T1 total PAPA symptoms, person-centered T1 cortisol did not significantly predict T2 cortisol reactivity (b = -0.013, SE = 0.008, t = -1.639, p = 0.102).

We further examined whether children's early psychiatric comorbidity at T1 moderated the change in cortisol reactivity from T1 to T2. Two dummy-coded variables were entered as independent variables (dummy coded absent vs. present for one T1 PAPA diagnosis and dummy coded absent vs. present for two or more T1 PAPA diagnoses), along with average T1 cortisol and person-centered T1 cortisol, and their respective interaction terms. There were no significant interactions between one T1 PAPA diagnosis and average T1 cortisol (b = 0.010, SE = 0.080, t = 0.128, p = 0.898) or person-centered T1 cortisol (b = -0.019, SE = 0.013, t = -1.476, p = 0.141). The interaction between two or more comorbid T1 PAPA diagnoses and average T1 cortisol was also not significant (b = 0.029, SE = 0.101, t = 0.273, p = 0.785), whereas the interaction between two or more comorbid T1 PAPA diagnoses and person-centered T1 cortisol was significantly associated with T2 cortisol reactivity (b = -0.045, SE = 0.017, t = -2.649, p = 0.008). For children with two or more T1 PAPA diagnoses, person-centered T1 cortisol was negatively associated with T2 cortisol reactivity (b = -0.033, SE = 0.016, t = -2.120, p = 0.035), which demonstrates an unstable pattern of cortisol reactivity for children with T1 preschool

psychiatric comorbidity. In contrast, there were no significant associations between person-centered T1 cortisol and T2 cortisol reactivity for children with no T1 PAPA diagnoses (b = 0.012, SE = 0.007, t = 1.662, p = 0.097) or for children with one T1 PAPA diagnosis (b = -0.007, SE = 0.011, t = -0.650, p = 0.516).

Gender. We examined whether gender (dummy coded 0 = boys and 1 = girls) moderated the change in cortisol reactivity from early to middle childhood. Gender did not significantly moderate the relation between T2 cortisol reactivity and average T1 cortisol (b = -0.032, SE = 0.064, t = -0.506, p = 0.614) or person-centered T1 cortisol (b = -0.022, SE = 0.011, t = -1.952, p = 0.052).

We further examined whether boys and girls demonstrated different patterns of cortisol reactivity at each wave. At T1, child gender was not significantly associated with the linear (b = -0.001, SE = 0.013, t = -0.111, p = 0.912) or quadratic (b = -0.001, SE = 0.018, t = -0.676, p = 0.500) effects of time on cortisol reactivity. However, at T2 gender significantly interacted with the linear effect (b = -0.033, SE = 0.016, t = -2.039, p = 0.042) but not the quadratic effect (b = 0.004, SE = 0.002, t = 1.615, p = 0.107) of time on cortisol reactivity. Boys demonstrated a significant linear increase in cortisol from baseline (b = 0.030, SE = 0.011, t = 2.696, p = 0.007), whereas girls did not demonstrate a significant linear effect of cortisol reactivity at T2 (b = -0.003, SE = 0.012, t = -0.248, p = 0.804).

Maternal Depression. We did not observe a significant interactive effect between maternal lifetime depression and person-centered T1 cortisol (b = 0.007, SE = 0.011, t = 0.651, p = 0.515) or average T1 cortisol (b = -0.045, SE = 0.064, t = -0.709, p = 0.480) in predicting cortisol reactivity at T2.

Chapter 4: Discussion

This study examined the stability and change of cortisol reactivity to standardized laboratory stressor paradigms from early to middle childhood and moderators of change in children's cortisol reactivity. We did not observe a significant relation between cortisol reactivity across the childhood assessments, suggesting little to no stability in cortisol reactivity from early to middle childhood. Rather, we observed distinct cortisol trajectories at each wave: cortisol levels decreased after the stressor in early childhood, but increased after the stressor by middle childhood. Moreover, children's cortisol reactivity demonstrated a significant increase from early to middle childhood. In addition, early childhood psychiatric symptoms moderated the change in cortisol reactivity. Specifically, for children low in preschool psychiatric symptoms, cortisol reactivity from early to middle childhood demonstrated greater intra-individual stability, and for children who had comorbid psychiatric diagnoses in early childhood, cortisol reactivity from early to middle childhood demonstrated greater intra-individual instability. Our data suggest a possible developmental shift in cortisol reactivity profiles from the preschool to elementary school period.

Consistent with previous cross-sectional studies, we observed decreasing cortisol reactivity to a laboratory stressor in early childhood (Dougherty et al., 2011, 2013; Hankin et al., 2010; Luby et al., 2003) and increasing cortisol reactivity to a laboratory stressor in middle childhood (Lopez-Duran et al., 2009). Although we found little evidence for stability across the three-year follow-up, we observed significant change in children's cortisol reactivity over time, specifically an increase

in slope and a more negative curvature from early to middle childhood. In contrast to our findings, previous longitudinal studies of infants and adults have reported low to moderate stability of cortisol reactivity over short follow-up periods of two months or less (Cohen et al., 2000; Goldberg et al., 2003; Lewis & Ramsay, 1995). In addition, Hankin et al. (2015) observed moderate stability in older children ages 9-15 years-old across an 18-month follow-up. Nevertheless, the three-year follow-up period for this study was longer than any other longitudinal investigation of cortisol reactivity, and to our knowledge, the current study is the only study to examine stability and change in cortisol reactivity from early to middle childhood. Thus, it is possible that cortisol reactivity has little stability over longer follow-up periods, or perhaps that the low stability in cortisol reactivity may be related to developmental changes in children's neuroendocrine functioning during this developmental period.

First, the change in reactivity may be evidence of maturational processes occurring from early to middle childhood, including increases in self-regulation and social, emotional, and cognitive skills (Best, Miller, & Jones, 2009; Del Giudice, Ellis, & Shirtcliff, 2011; Sameroff & Haith 1996). Second, it is possible that the early to middle childhood period may reflect a developmental shift to increasing cortisol reactivity. The large increases in cortisol reactivity may be similar to those observed during the pubertal transition (Gunnar et al., 2009b; Stroud et al., 2009). Lastly, the change in reactivity might also reflect the plasticity of the stress system. Cortisol reactivity, although moderately genetically determined (Steptoe, van Jaarsveld, Semmler, Plomin, & Wardle, 2009), is also shaped by multiple environmental and biological (e.g., hormonal, neurobiological) processes. Thus, the change in children's

cortisol reactivity may indicate the use of adaptive physiological mechanisms in order to adjust to environmental and biological demands (McEwen, 1998; Del Giudice et al., 2011). Future research should identify the multiple mechanisms involved in children's change in cortisol reactivity from early to middle childhood and whether specific trajectories predict children's developmental outcomes.

We also examined whether preschool psychiatric symptoms moderated the change in cortisol reactivity from early to middle childhood. We found that for children low in preschool psychiatric symptoms, patterns of cortisol reactivity remained stable from early to middle childhood, whereas for children high in preschool psychiatric symptoms, there was no association between their cortisol reactivity profiles from early to middle childhood. We further examined whether psychiatric comorbidity moderated the change in cortisol reactivity, given that comorbidity is associated with poorer psychosocial functioning and worse treatment outcomes in preschoolers (Bufferd et al., 2011; Ezpeleta, de la Osa, & Doménech, 2014; Ghuman et al., 2007). We found that for children with two or more preschool psychiatric disorders, their patterns of cortisol reactivity evidenced instability from the early childhood assessment to middle childhood assessment. Taken together, these findings suggest that preschool psychopathology may be related to how the HPA-axis changes over time. Children with few psychiatric symptoms in early childhood may have more effective behavioral and physiological regulation strategies that are maintained through middle childhood and which contribute to the stability of their cortisol reactivity patterns over time. Conversely, it is possible that children with early comorbid psychiatric conditions experience significant impairment and distress

that interferes with the development of effective regulation strategies through middle childhood, possibly leading to unstable and variable physiological trajectories. The associations between early psychiatric symptoms and comorbidity and the development of cortisol reactivity are likely bidirectional. It is important for future research to determine the directionality of these effects and to explore whether the stability or instability of stress reactivity over time predicts psychiatric outcomes later in development.

Next, we investigated the moderating role of maternal depression and child gender on the stability of cortisol reactivity from early to middle childhood. Although neither maternal depression nor child gender moderated the change in children's cortisol reactivity, child gender was associated with cortisol reactivity in middle childhood only. We observed no gender differences in cortisol reactivity in early childhood, which is consistent with previous findings in preschool-aged children (Dougherty et al., 2011; Kryski et al., 2011; Lewis & Ramsay, 2002; Talge, Donzella, & Gunnar, 2008). However, by middle childhood, boys demonstrated a greater linear increase in cortisol responses to the stressor than girls. Consistent with our findings, adolescent and adult males also demonstrate higher rates of cortisol reactivity to psychosocial stressors than adolescent and adult females (for a review, see Kudielka & Kirschbaum, 2005). Thus, our results suggest that gender differences in the stress response emerge in middle childhood and may reflect gender differences in children's self-regulation abilities. Evidence supports that girls tend to demonstrate more inhibitory control and better attention shifting in middle childhood compared to boys (Murphy, Eisenberg, Fabes, Shephard, & Guthrie, 1999; Raffaelli, Crockett, & Shen,

2005), which may result in more rapid adaptation to the stressor and thus smaller stress-induced cortisol increases for girls. Boys may also be more reactive to performance-based laboratory assessments, such as those used in this study, resulting in greater increases in cortisol levels for boys than for girls (Lopez-Duran et al., 2014; Stroud, Salovey, & Epel, 2002). However, given that gender did not influence the stability of cortisol reactivity over time, it is possible that the differential maturation of the stress response for boys and girls may be emerging in middle childhood, and thus further investigation across middle childhood and adolescence is warranted.

This study had a number of methodological and statistical strengths. This is the first study, to our knowledge, to examine the stability of cortisol reactivity over three years, which is the longest follow-up period to date, and to examine moderators of developmental change in cortisol reactivity. Second, we employed an MLM framework to examine both population- and individual-level differences in children's patterns of cortisol reactivity over time. Third, our standardized laboratory stressor paradigms at each assessment wave were both developmentally appropriate and included similar performance and social evaluation components. Finally, we collected multiple post-stressor cortisol samples in order to capture individual differences in children's cortisol responses to the laboratory stressor.

This study also had limitations. First, although we used a three year follow-up design, we only examined cortisol reactivity at two assessment points. Conducting more assessments over the follow-up period could provide greater insight into when the shift in cortisol reactivity occurs. Second, it is possible that the stressors were not equally potent to the children at each assessment wave. Nevertheless, we incorporated

developmentally appropriate stressor tasks which resulted in significant variability in children's cortisol reactivity curves at each wave. Third, our cortisol assessments were conducted in the laboratory, but findings may have differed in other settings, such as home or school settings (Gunnar, Tout, de Haan, Pierce, & Stansbury, 1997; Kryski et al., 2011). Fourth, we examined only one aspect of stress reactivity; it will be important for future research to investigate the stability and change of other physiological responses to acute stress from early to middle childhood, such as respiratory sinus arrhythmia, epinephrine, and dehydroepiandrosterone (DHEA). Lastly, although we did not find that maternal depression predicted changes in children's cortisol reactivity, it is possible that a combination of early rearing factors (e.g., parenting, socio-economic stressors, parent psychopathology) impacted the change in children's cortisol reactivity over time. Future research exploring how more comprehensive measures of the early rearing environment might affect the stability or change in children's cortisol reactivity is warranted.

In sum, our findings highlight the change in children's cortisol responses to a laboratory stressor from early childhood to middle childhood and underscore the importance of early mental health problems as modulators of this change. Understanding how early childhood factors, including preschool psychopathology, impact the stability or change of cortisol reactivity holds great promise in elucidating the mechanisms underlying physiological dysregulation and developmental pathways to risk and resiliency. Given the plasticity of the HPA-axis early in life (Boyce & Ellis, 2005), interventions targeting preschool problems may alter children's

trajectories of cortisol reactivity and perhaps mediate changes in later psychopathology and psychosocial functioning.

Tables

Table 1.

Demographic characteristics of study sample

Demographic variable	
Child age at T1, mean (SD), months	50.27 (9.86)
Child age at T2, mean (SD), months	87.21 (11.55)
Mother age T1, mean (SD), years	36.23 (5.97)
Father age at T1, mean (SD), years	38.36 (6.56)
Child gender, male [n (%)]	51 (53.1%)
Child race [n (%)]	
White, European-American	46 (47.9%)
African-American	28 (29.2%)
Asian	1 (1.0%)
Other	21 (21.8%)
Child ethnicity [n (%)]	
Hispanic/Latino descent	15 (15.8%)
Biological parents' marital status at T1 [n (%)]	
Married	66 (68.8%)
Living together	4 (4.2%)
Divorced or separated	8 (8.4%)
Never married	18 (18.8%)
Family income [n (%)]	

< $20,000	5 (5.4%)
$20,001 to $40,000	7 (7.5%)
$40,001 to $70,000	18 (19.4%)
$70,001 to $100,000	30 (32.3%)
> $100,000	33 (35.5%)
Parental education: graduated 4-year college [n (%)]	
Mothers	65 (68.5%)
Fathers	61 (67.1%)
PAPA interview respondent [n (%)]	
Mother	86 (89.6%)
Father	5 (5.2%)
Both parents	5 (5.2%)

Note. $N = 96$. Of the sample, 1 family (1.0%) did not report the child's ethnicity. 6 (6.2%) families did not report parental education. 3 (3.1%) families did not report their yearly income.

Table 2.

Correlations among all major study variables

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1. Wave 1 Baseline sample	-														
2. Wave 1 20 min. sample	.77***	-													
3. Wave 1 30 min. sample	.69***	.90***	-												
4. Wave 1 40 min. sample	.62***	.83***	.90***	-											
5. Wave 1 50 min. sample	.58***	.78***	.85***	.88***	-										
6. Wave 2 Baseline sample	-.03	-.08	-.01	.06	.03	-									
7. Wave 2 20 min. sample	-.04	-.05	.02	.06	.04	.78***	-								
8. Wave 2 30 min. sample	-.08	-.10	-.04	.02	<.001	.76***	.87***	-							
9. Wave 2 40 min. sample	-.11	-.14	-.07	-.03	-.04	.75***	.86***	.93***	-						
10. Wave 2 50 min. sample	-.12	-.12	-.05	-.01	-.04	.70***	.82***	.86***	.93***	--					
11. Maternal lifetime depression	.04	.03	.02	-.02	-.13	.02	-.03	-.09	-.09	-.09	-				
12. Total T1 psychiatric symptoms	.04	-.002	.02	.05	.04	-.05	-.01	-.06	-.09	-.09	.25*	--			
13. Child gender	.11	.06	.03	.003	-.03	-.13	-.22*	-.20	-.22*	-.17	-.05	.06	--		
14. Child age at T1 (months)	-.16	-.10	-.13	-.18	-.24*	-.02	.01	.04	.07	.10	-.14	-.03	-.04	--	
15. Child age at T2 (months)	-.20*	-.12	-.15	-.19	-.23*	-.03	-.09	-.03	.03	.08	-.23*	-.10	-.07	.83***	
Mean	2.96	2.45	2.32	2.22	2.31	2.28	2.54	2.38	2.37	2.39	--	22.45	--	50.27	87
SD	4.49	3.78	3.19	2.78	2.68	3.19	4.09	2.93	3.46	3.68	--	11.64	--	9.86	11
N	96	96	96	96	96	96	96	96	96	96	95	96	96	96	9

Note: N =96. Correlation analyses used log10 transformed cortisol values; however, means and standard deviations
(SD) for cortisol levels are reported as raw cortisol levels in nmol/L; Maternal lifetime depression disorder: 0 = no
maternal lifetime depressive disorder (n = 47), 1 = maternal lifetime major depressive disorder or dysthymic
disorder (n = 48); PAPA = Preschool Age Psychiatric Assessment; Child gender: 0 = male, 1 = female;
$^{*}p < .05$, $^{**}p < .01$.

Table 3.

Assessment wave moderates the change in children's cortisol reactivity from early to middle childhood.

Variable	*B*	*SE*	*t*
Time	-0.064	0.015	-4.295***
Time x Time	0.011	0.003	3.323**
Wave	-0.061	0.043	-1.418
Baseline sample collection time	<0.001	<0.001	-2.108*
Time x Wave	0.076	0.021	3.574***
Time x Time x Wave	-0.015	0.005	-3.101**

Note. Child cortisol values were $\log_{10}$ transformed. Wave = 0 at age 3-5 assessment (T1), wave = 1 at age 6-10 assessment (T2); *$p < .05$; **$p < .01$; ***$p < 0.001$.

Table 4.

Preschool psychopathology predicts the change in children's cortisol reactivity from early to middle childhood.

Variable	*b*	*SE*	*t*
Time	0.010	0.014	0.682
Time x Time	-0.004	0.003	-1.103
Baseline sample collection time	<0.001	<0.001	-2.020*
Average T1 cortisol	-0.005	0.028	-0.182
Person-centered T1 cortisol	0.002	0.006	0.337
Total T1 PAPA symptoms	-0.007	0.029	-0.248
Average T1 cortisol x Total T1 PAPA symptoms	-0.079	0.035	-2.291*
Person-centered T1 cortisol x Total T1 PAPA symptoms	-0.015	0.006	-2.561*

Note. Child cortisol values were $\log_{10}$ transformed. PAPA = Preschool Age Psychiatric Assessment; *$p < 0.05$.

Figures

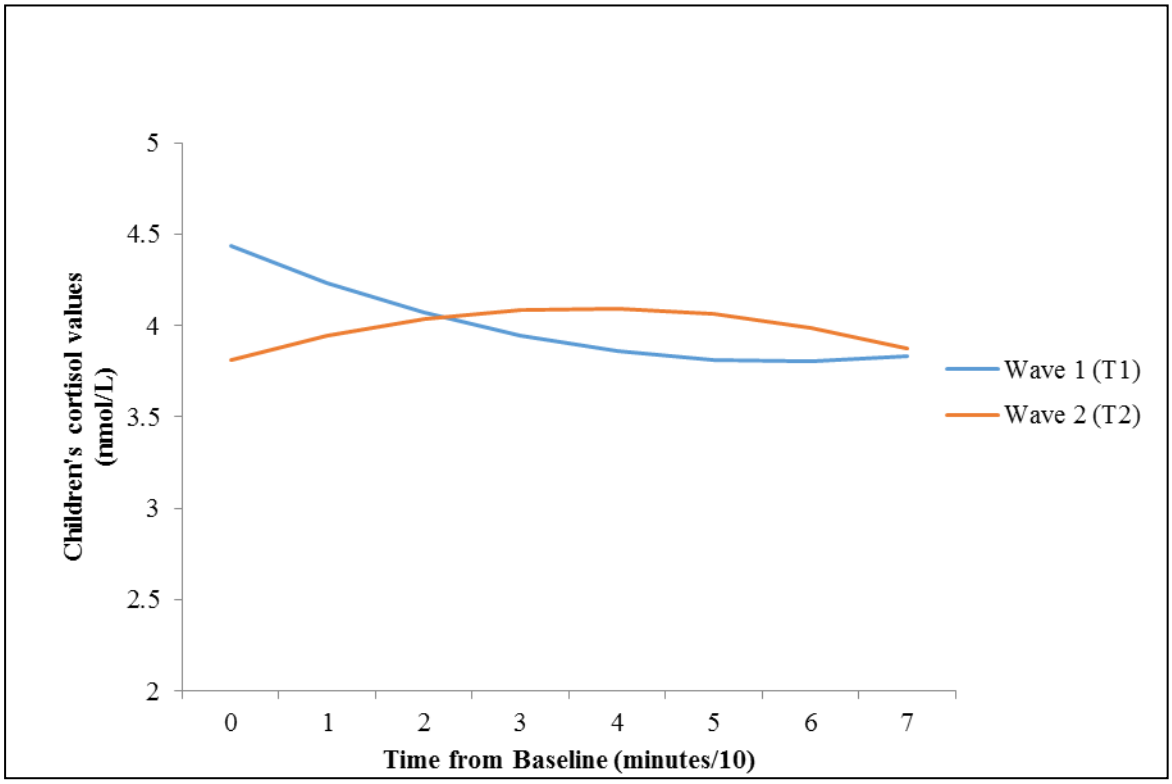

Figure 1. Mean child cortisol level (nmol/L) as a function of sampling time. The graph shows mean cortisol values at each wave for each of the five reactivity samples: baseline, 20 minutes post-stressor, 30 minutes post-stressor, 40 minutes post-stressor, and 50 minutes post-stressor.

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