Prefrontal cortical thickness mediates the association between cortisol reactivity and executive function in childhood

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Highlights

- At school-age, greater cortisol release was related to lower executive function (EF).
- School-age cortisol change negatively related to inferior frontal cortical thickness (CT).
- Cortisol release in preschool positively related to middle frontal CT at school-age.
- Greater middle frontal CT mediated negative association of cortisol release and EF.

Abstract

The impact of stress hormones, such as cortisol, on the brain is proposed to contribute to differences in executive function of children from impoverished backgrounds. However, the association between cortisol reactivity, prefrontal cortex, and executive function is relatively unexplored in young children. The current longitudinal study examined whether 63 children's early preschool-age (3-5 years, Time 1) and concurrent school-age (5-9 years, Time 2) salivary cortisol reactivity were associated with executive function and prefrontal cortical thickness at school-age. Two measures of cortisol reactivity were calculated: area under the curve with respect to ground (AUCg; total cortisol release) and with respect to increase (AUCi; total change in cortisol). Results demonstrated that Time 2 total cortisol release was negatively associated with executive function, Time 1 total cortisol release positively related to right middle frontal cortical thickness, and Time 2 total cortisol change was negatively associated with right inferior frontal cortical thickness. Moreover, greater right middle frontal cortical thickness mediated the association between greater Time 1 total cortisol release and lower executive function. This study provides support for cortisol-related variation in the prefrontal cortex thickness underlying individual differences in executive function.

Keywords: Cognition; Stress; Development; Frontal Cortex; Salivary Cortisol

1. Introduction

Theories argue that the experience of toxic stress throughout childhood leads to gaps in cognitive abilities and achievement for those growing up in impoverished settings (Blair and Raver, 2012; Danese and McEwen, 2012; Farah, 2017; Hackman et al., 2010; Pakulak et al., 2018; Shonkoff, 2010). In combination with genetic risk (Derijk, 2009), the biological embedding of the stress response can lead to alterations in stress response systems, variations in brain development, and ultimately impact child cognitive development (Blair and Raver, 2012; Danese and McEwen, 2012; Johnson et al., 2016; Pakulak et al., 2018; Shonkoff, 2010). Prolonged or cumulative exposure to stress can lead to alterations in brain regions with a high density of glucocorticoid receptors and reduced dendritic arborization in the prefrontal cortex (PFC), the major region supporting executive function (McEwen, 2007; McEwen and Gianaros, 2011, 2010; McEwen and Morrison, 2013; Teicher et al., 2003). Despite a wealth of studies depicting correlations between measures of stress, brain development, and executive function (Edmiston et al., 2011; Hanson et al., 2012; Leonard et al., 2019; Lu et al., 2013; Vidal-Ribas et al., 2019), the associations between cortisol reactivity, brain structure, and executive function across childhood remain unclear.

Alterations in stress response systems have aversive effects on brain development, especially the prefrontal cortex (PFC; McEwen, 2007; McEwen and Gianaros, 2011, 2010; McEwen and Morrison, 2013; Teicher et al., 2003), the major region supporting cognition (Diamond, 2013, 2002).The hypothalamus-pituitary-adrenal (HPA) axis is one of the major systems regulating the stress response with the end product of cortisol (Smith and Vale, 2006). Short-term elevations in cortisol enable appropriate initiation of the HPA axis; but, chronic or cumulative activation of the stress-response system has been shown to have adverse effects on

the body and on the cortical systems (Lupien et al., 2009; McEwen, 2017; McEwen and Gianaros, 2010). Rodent research demonstrates that chronically high cortisol levels, cumulative stress, and trauma result in structural changes including reduced dendritic arborization and decreased growth of dendrites, increase dendritic shortening in regions regulating the HPA axis with a high density of glucocorticoid receptors and reduced dendritic arborization in the PFC (McEwen, 2007; McEwen and Gianaros, 2011, 2010; McEwen and Morrison, 2013; Teicher et al., 2003). The development of PFC grey matter follows an inverted U-shape pattern with increases throughout early childhood, peaks between 7-10 years, and then decreases through refining of connections into the mid-twenties (Giedd et al., 1999; Gogtay et al., 2004). The prolonged development of the PFC or "window of opportunity" can be beneficial for continued refining of brain development and connections; however, the protracted development is also a potential window of vulnerability for negative influences such as chronic high levels of stress (Andersen, 2003; Pechtel and Pizzagalli, 2011). The stress acceleration theory even suggests that early exposure to high levels of stress may offset normal brain maturation and accelerate the process leading to early maturation of the brain including the PFC (Callaghan and Tottenham, 2016). For example, compared to children raised in low stress environments, children exposed to high stress, low socioeconomic status (SES) environments demonstrate decreased PFC cortical thickness (Brito et al., 2017; Hanson et al., 2013; Lawson et al., 2013; Mackey et al., 2015; Noble et al., 2015; Luciane R. Piccolo et al., 2016), decreased PFC function during cognitive tasks (D'Angiulli et al., 2008; Finn et al., 2017; Kim et al., 2013; Kishiyama et al., 2009; Sheridan et al., 2012), and altered resting state connectivity within the PFC and between the PFC and the middle temporal lobe (Demir-Lira et al., 2016). Thus, the negative impact of stress is a

proposed mechanism for individual differences in PFC development and the PFC-dependent cognitive abilities such as executive function.

An abundant of studies demonstrated children exposed to high levels of stress, including poverty and trauma, have higher levels of cortisol (Lupien et al., 2000, 2009; Luciane Rosa Piccolo et al., 2016) and lower executive function (see for review Hackman et al., 2010; Lawson et al., 2018; Pakulak et al., 2018). According to Diamond (2013), executive function refers to higher level cognitive abilities necessary to adapt to demands and expectations of the environment composed of inhibitory control, working memory, and set shifting. Inhibitory control is the ability to override prepotent thoughts and responses to instead respond in the appropriate manner given the situation (Diamond, 2013).Working memory is the ability to hold and manipulate information in the mind (Baddeley and Hitch, 1994). Set shifting is the ability to switch rules or mindsets to correctly solve the task at hand (Diamond, 2013). Executive function begins to develop early in childhood and continue to develop into early adulthood with major peaks in adolescence (Zelazo et al., 2008). Executive function development coincides with the protracted PFC development, the major region supporting executive function (Diamond, 2013). Importantly, the specific regions of the brain associated with executive function, such as the PFC, are impacted by chronic levels of stress (Farah, 2017; Hackman et al., 2010; Johnson et al., 2016; Pakulak et al., 2018). At the physiological level, differences in cortisol responses are also shown to relate to executive function. In general, better executive function has been associated with an increase in cortisol in response to a stressor followed by a decrease and recovery to baseline, reflecting larger magnitudes of change in cortisol in response to a stressor (Lupien et al., 2000, 2009). On the other hand, lower executive function is associated with higher baseline cortisol levels and lower change in cortisol in response to a stressor, also referred to as a blunted

response (Blair et al., 2011, 2006). Together, these studies demonstrate the impact of high levels of cortisol on the brain may underlie the associations between cortisol responses and executive function.

Although the impact of stress on the prefrontal cortex is proposed to explain individual differences in executive function (Blair and Raver, 2012; Danese and McEwen, 2012; Johnson et al., 2016; Pakulak et al., 2018; Shonkoff, 2010), few studies have directly tested the associations in young children across childhood using physiological measures of stress. In adults, individuals with childhood trauma displayed increased cortisol awakening responses and decreased middle cingulate grey matter (Lu et al., 2013). Even further, prefrontal cortex grey matter volumes mediated the association between reports of cumulative life stress and working memory performance in adults (Hanson et al., 2012). Work in adolescents also support the association between stress and PFC grey matter as reported childhood adverse experiences in adolescents were negatively associated with PFC grey matter (Edmiston et al., 2011). Even fewer studies have examined this link in children. A recent study in 4-7-year-old children demonstrated reasoning, which requires working memory, was positively associated with the rostrolateral PFC thickness in the children from low SES backgrounds (Leonard et al., 2019). Another recent study in children showed higher reports of stressful life events at age 7 were associated with reduced function of the prefrontal cortex during a reward task at age 10 years which was associated with higher cortisol reactivity at 13 years old (Vidal-Ribas et al., 2019). Together these studies suggest toxic stress and altered stress responses are associated with PFC development and executive function. However, how cortisol responses relate to prefrontal cortex structure and executive function across childhood has yet to be examined within a longitudinal study.

The current study aimed to fill the gap in the literature by identifying whether children's cortisol reactivity at preschool-age and school-age is related to prefrontal cortex thickness and executive function at school-age. We examined associations between children's salivary cortisol reactivity to a laboratory stressor assessed at preschool-age (Time 1) and three years later at school-age (Time 2) along with the children's PFC cortical thickness and executive function at school-age. To further understand the associations, we examined whether PFC cortical thickness mediated the relation between preschool-age cortisol reactivity and school-age executive function. Based on previous research demonstrating high chronic stress is associated with lower executive function (Blair and Raver, 2012; Danese and McEwen, 2012; Farah, 2017; Hackman et al., 2010; Pakulak et al., 2018; Shonkoff, 2010) and higher executive function is associated with lower baseline cortisol and higher total change in cortisol in response to the stressor (Blair et al., 2006), we predicted that greater total cortisol release and lower cortisol change would be associated with children's lower executive function and reduced prefrontal cortex cortical thickness. In addition, we predicted that reduced PFC cortical thickness would mediate associations between greater cortisol reactivity and decreased executive function.

2. Materials and Methods

2.1 Procedure

Participants were recruited as part of a larger longitudinal study (*N*=175) examining neuroendocrine functioning in young children of parents with and without a lifetime history of depression (Dougherty et al., 2013; Kushner et al., 2016) assessed using the Structured Clinical Interview for DSM-IV (SCID, First et al., 2002). The present report includes data from baseline

(Time 1) and three years later (Time 2). At baseline, children were preschool-aged (3-5 years), and they completed a stress inducing laboratory task with salivary cortisol assessments. Three years later at school age (5-9 years), children completed a second laboratory-based cortisol reactivity assessment, behavioral measures of executive function, and a Magnetic Resonance Imaging (MRI) assessment. The study protocol was approved by the University of Maryland College Park's Institutional Review Board (IRB) including informed consent of the parents and written assent of children 7 years old or older.

2.2 Recruitment

The participants were recruited through flyers and a commercial mailing list from the Washington D.C. greater metropolitan area. Children were considered for the study if they were 3-5 years old, had an English-speaking biological parent with at least 50% custody, no parent-reported history of significant medical conditions or developmental disorders, and had biological parents without a history of bipolar or psychotic disorders. Children were excluded if the ability to comprehend English was not sufficient to complete the behavioral tasks in the laboratory.

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The sample size at baseline was 175 preschool-age children; 156 of the 175 children completed the cortisol reactivity assessment at Time 1. A total of 117 children returned for the Time 2 assessment; 104 children completed the cortisol reactivity assessment at Time 2. The current study focuses on the children that completed cortisol assessments at both Time 1 and Time 2 and executive function at Time 2 (n=95). At Time 2, 64 participants agreed to attend the MRI assessment, and 63 children completed the assessment (one child did not scan due to claustrophobia). All the 63 children who completed the MRI assessment also completed assessments of executive function, 61 of the children completed cortisol reactivity at Time 2, and 58 of the children completed cortisol reactivity assessment at Time 1.

2.3 Demographics

The demographics are reported on the children who completed the cortisol reactivity assessments and executive function measures (*n*=95) as well as the subset of those children that completed the MRI assessment (*n*=63) in Table 1. Demographics measures, cortisol reactivity, or executive function did not differ significantly between the full sample and the MRI subsample.

2.4 Cortisol Reactivity

At both timepoints, children completed developmentally-appropriate stress-inducing laboratory tasks and provided five saliva samples to assess cortisol levels before and after the stressors. At Time 1, children's cortisol levels were assessed during a stressor paradigm (Dougherty et al., 2013; Kryski et al., 2011; Leppert et al., 2016). The task involved children matching specific colored balls (blue/red) to the correct animals (bear/frog) during timed trials (total length of task: M = 8.11 min, SD = 1.96). On three consecutive trials, the experimenter manipulated the timer to end before the child finished the task to induce stress. The child was debriefed about the timer incorrectly functioning after three trials. At Time 2, the stress inducing task was an adapted version of the Trier Social Stress Task (TSST; Buske-Kirschbaum et al., 1997). During the TSTT the child was judged on their performance of telling a 4.5-minute story after a 30 second preparation period and completing an impossible puzzle in 3 minutes. After a five-minute period of the child waiting for the judges to decide their prize, the child was debriefed and told the puzzle was missing pieces (for a complete description of the tasks, see Leppert et al., 2016). The TSST has previously been shown to evoke a cortisol response in children (Gunnar et al., 2009; Leppert et al., 2016). Time of day is shown to impact cortisol levels; therefore, most of the tasks were completed during the afternoon (afternoon assessments at Time 1: 78.1% and Time 2: 93.8%).

At both Time 1 and Time 2, cortisol reactivity was assessed through the analysis of cortisol levels in the child's saliva (described in Dougherty et al., 2013; Leppert et al., 2016). Five samples of cortisol were collected from each child. A baseline sample was collected after a 30-minute play session with the child but prior to the stressor task. The four other samples were collected following the stress-inducing task at 20, 30, 40, and 50 minutes. The saliva was collected with cotton rolls using a tiny amount of Kool-Aid® (~0.025mg) that was chewed until saturated (~1 minute). The cotton roll was then put into a syringe and the saliva was extracted into a plastic vial. This method increases cooperation of young participants and is suggested to be beneficial for research with children when using a small consistent amount (Talge et al., 2005). To address prior research stating consumption of food and caffeine influence cortisol levels (Gunnar et al., 2009), parents were instructed not to give food to the child an hour prior the laboratory visit nor any caffeine to the child two hours prior to the visit.

After collection, cortisol vials were frozen at -20º Celsius until assayed 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-assay coefficient ranged from 7.1%–9.0% and intra-assay coefficient ranged between 4.0%–6.7% respectively. Of the 630 samples collected, two were missing and three were extreme values (exceeded 44 nmol/L). Extreme values were discarded, and the five missing data points were interpolated using the average of five multiple imputations (Rubin, 1987). Multiple imputations method is a valid and commonly used method for estimating missing data for cortisol values (e.g., (Little et al., 2014; Müller et al., 2015; Rotenberg and McGrath, 2014; Walker, 2010). Multiple imputations methods use the individual's data (i.e., the other four cortisol samples) and data from the entire sample to estimate the missing data points (Rubin,

1987). Previously used imputation methods, including mean substitution and regression imputation, leave no margin of error around the predicted missing value, artificially shrinking the standard error, and yielding biased estimates. In contrast, multiple imputations method involves computing a series of plausible estimates of what the missing values may have been, creating variability in the predicted estimates (Little et al., 2014). Given that there is debate as to whether extreme cortisol values should be discarded or winsorized (Adam and Kumari, 2009) we chose to discard the extreme samples and treat the values as missing. However, the results were similar when the three extreme values were winsorized.

Two measures of area under the curve (AUC) of the five cortisol samples were calculated based on the trapezoid formula (Pruessner et al., 2003), area under the curve with respect to ground (AUCg), a measure of the magnitude of total cortisol release, and with respect to increase (AUCi), a measure of change in cortisol secreted. These values were log-transformed and z-scored (Pruessner et al., 2003). The two AUC measures capture different aspects of the cortisol response to stress. Within the children that completed the MRI scan, the two measures but the measures were correlated at both timepoints (Supplemental Table 1). Within the larger study, little to no stability in cortisol reactivity measures was observed across this developmental period (see Leppert et al., 2016).

2.5 Executive Function

Children completed three executive function tasks at Time 2. Each task assessed one of the three components of executive function: working memory, inhibition, and set shifting. Color span, an adapted version of the digit span on the Wechsler Intelligence Scale for Children (WISC, Wechsler, 1949), was used to assess working memory. The Color Span task presents a series of colored triangles to the child one at a time. There were two sets of trials of forward and

backward. The child was instructed to repeat the colors in the order that was presented (forward trials) or the child was instructed to repeat the colors in the reverse order (backward trials). The number of triangles (1-8) the child had to remember increased with each correct set the child completed. The total color span score was calculated by summing the number of correct forward and backward trials. Higher scores represent better working memory.

The child completed the "Simon Says" task (Strommen, 1973) to assess inhibitory control. During the task, the child was instructed to perform the exercises when the experimenter says "Simon says" before the exercise and not to perform the exercise when the experimenter does not say "Simon says" before the exercise. There were 8 trials with the "Simon says" command and 8 trials without the command. The movement of the child was scored for each trial on a scale from 0-3. For the trials with the "Simon says" command, a score of 0 represented no movement and a score of 3 indicated full command movement. The reverse scale was used for the trials without "Simon says" command, a score of 0 indicated full commanded movement and 3 represented no movement. The scores of all the trials were summed to indicate a total Simon Says score higher values indicate higher inhibition. The scores of the trials were summed to calculate a total score with higher values indicating better inhibitory control.

The Trails Making Test (Reitan and Wolfson, 1985) was used to assess set shifting. The Trails Making Task is a neuropsychological test that includes two parts. In part A, the experimenter shows a child a piece of paper with dots filled with numbers and instructs the child to connect the dots in numerical order. In part B, the experimenter shows the child a second piece of paper of dots filled with numbers and letters. The child was instructed to connect the dots in numerical and alphabetical order switching between numbers and letters. The total

number of errors during Part B was summed to compute a total score. The total number of errors was reversed-scored; thus, higher scores represent better attention shifting.

An average executive function composite was calculated by computing an average of the Z scores of the total Color Span score, total Simon Says score, and the reverse-scored Trails total score. The higher composite executive function score indicates better executive function.

2.6 Structural Magnetic Resonance Imaging

Prior to completing the scan, children participated in a mock scanner session to acclimate the child to the scanner environment and provide feedback on motion. During the collection of the structural scan, the child watched a video of their choice as a way to limit motion during the scan. Structural scans were collected on a 3T Siemen's scanner with a 12-channel coil using a high resolution T1 magnetization-prepared rapid gradient-echo (MPRAGE) sequence. A total of 176 adjacent sagittal slices were collected with 1.0 x 1.0 x 1.0 voxel size, TR of 1900ms, TE of 2.52ms, Inversion time of 900ms, flip angle 9^{o}, and pixel matrix = 256 x 256. If motion artifacts were identified during the scan, the structural scan was repeated, pending the child's willingness and ability. A total of 15 children had their structural scan repeated: two scans (n=12), three scans (n=2) and four scans (n=1).

The structural scans were analyzed using Freesurfer (Version 5.1.0; surfer.nmr.mgh.harvard.edu). The automated segmentation package was used for preprocessing. The images were then checked for overall correct segmentation and manual edits were made if large errors were present (n=11). For the current analyses, Freesurfer regions were selected that best corresponded with *a priori* regions associated with executive function within the prefrontal cortex (ascertained from Dosenbach et al., 2007). The selected regions were bilateral middle frontal gyrus (rostral, caudal), inferior frontal gyrus (pars opercularis, pars triangularis, pars

orbitalis), and anterior cingulate (rostral, caudal) using the Desikan-Killiany parcellation scheme (Desikan et al., 2006). The cortical thickness of the PFC regions for left and right hemispheres was used in subsequent analyses.

2.7 Data Analysis

Multiple regressions were conducted to examine the three primary analyses: 1) Are preschool-age and concurrent school-age cortisol reactivity (total cortisol release/total cortisol change) related to executive function in school-age children? 2) Are preschool-age and concurrent school-age cortisol reactivity (total cortisol release/total cortisol change) related to school-age children's cortical thickness of the PFC? 3) Is there a relation between PFC cortical thickness and concurrent school-age executive function? Exploratory analyses were conducted to examine the specific associations of the individual executive function components with cortisol reactivity and prefrontal cortical thickness. Although the sample of the current study is relatively higher SES and was not initially recruited to examine SES differences, additional analyses were also conducted to examine the associations with family income and maternal education with executive function, cortisol reactivity, and prefrontal cortical thickness. These results are also presented in the supplemental material.

Nonparametric bootstrapping procedures were used to evaluate the indirect effect of PFC cortical thickness on the association between preschool-age cortisol reactivity and school-age executive function. Only the earlier timepoint of cortisol reactivity was included when conducting the mediation analyses to avoid the use of three variables collected at the same timepoint. Unlike hypothesis testing based on parametric statistics, bootstrapping procedures do not assume normality (Hayes, 2009; Preacher and Hayes, 2008). An indirect effect and corresponding confidence interval are calculated by examining the product of the path from the

independent variable to the mediator and from the mediator to the dependent variable. The independent variable was the Time 1 cortisol reactivity (total cortisol release/total cortisol change), the mediators were the PFC cortical thickness variables, and the dependent variable was executive function. A significant indirect effect would be indicated by a confidence interval that does not contain zero. We employed the PROCESS Macro version 3.0 in SPSS version 25 to conduct all mediation analyses with 5,000 bootstrapped samples as recommended by Hayes (2009) and Preacher and Hayes (2008). All analyses included Time 2, sex, and maternal depression as covariates. Time 1 age was an additional covariate in the analyses including Time 1 cortisol reactivity. Analyses were conducted in SPSS (Version 26) using $\alpha < 0.05$. Multiple comparison corrections using a Bonferroni correction accounting for 83 analyses were conducted using $\alpha < 0.0006$.

3. Results

The descriptive statistics for the independent and dependent variables are displayed in Tables 1 and 2. The bivariate correlations of the variables included in the analyses are shown in Supplementary Table 1.

3.1 Associations between cortisol reactivity and executive function

Greater Time 2 total cortisol release was significantly related to lower executive function (b = -0.50, SE = 0.21, pr = 0.02, $p < 0.001$; Figure 1a) after adjusting for age, sex, and maternal depression. The associations between cortisol reactivity (total cortisol release/total cortisol change) at Time 1 or total cortisol change at Time 2 and executive function were not significant.

3.2 Associations between cortisol reactivity and PFC cortical thickness

Greater Time 1 total cortisol release was significantly related to greater right caudal middle frontal cortical thickness (b = 0.33, *SE = 0.12, pr = 0.01,* p = 0.02; Figure 1b) after controlling for age, sex, and maternal depression. The associations between Time 1 preschool-age cortisol reactivity and other ROIs were not significant.

Greater Time 2 total cortisol change was associated with decreased right pars opercularis cortical thickness (b = -0.54, *SE* = 0.23, *pr = 0.02,* p = 0.06; Figure 1c), after adjusting for age, sex, and maternal depression although the overall regression model was only moderately significant after adjusting for age, sex, and maternal depression. The associations between Time 2 concurrent school-age cortisol reactivity and the other PFC regions cortical thickness were not significant.

3.3 Associations between PFC cortical thickness and executive function

None of the associations between PFC cortical thickness and executive function were significant.

3.4 Does PFC cortical thickness mediate these associations between preschool-age cortisol reactivity and school-age executive function?

We examined whether PFC cortical thickness mediated the association between Time 1 preschool-age cortisol reactivity and Time 2 school-age children's executive function. We focused only on regions that were significantly related to cortisol reactivity (right pars orbitalis/right caudal middle frontal). The independent variables were preschool-age cortisol (total cortisol release/total cortisol change) at Time 1 and the dependent variable was executive function at Time 2. Four separate meditations were conducted with one of the two PFC ROIs as the mediator. Analyses revealed a significant indirect effect (path ab, see Figure 1b,

Supplemental Table 2) of Time 1 total cortisol release on Time 2 executive function through the right caudal middle frontal cortical thickness (ab [5,000 bootstrapped samples] = -0.31, SE = 0.18, bias-corrected 95% CI [−0.78, −0.04]). More specifically, greater preschool-age total cortisol release was associated with greater school-age right caudal middle frontal cortical thickness which was in turn related to lower school-age executive function.

4. Discussion

Although cortisol reactivity is proposed to be a contributor to individual differences in brain development and executive function, the current study is the first to demonstrate an association between cortisol reactivity, prefrontal cortex thickness, and executive function across childhood. Out of the prefrontal regions examined, two were associated with cortisol reactivity: middle frontal cortex and inferior frontal cortex. More specifically, greater right caudal middle frontal cortical thickness mediated the association between greater preschool-age total cortisol release and lower school-age executive function. To the best of our knowledge this is the first time the PFC mediating the association between cortisol reactivity and executive function was observed in young children over time. These results provide support for individual differences in cortisol reactivity relating to differences in PFC structure and the PFC-dependent executive function. These findings are an important contribution to the understanding of the impact of stress and cortisol reactivity on prefrontal cortex structure and executive function throughout early childhood.

In support of our primary hypothesis, greater concurrent school-age total cortisol release was associated with poorer performance on executive function tasks. More specifically, school-age total cortisol release was related to set shifting, but not working memory or inhibitory control when the executive function components were examined separately. These results are consistent

with previous studies demonstrating high levels of cortisol are associated with lower cognition in rodents (McEwen and Sapolsky, 1995) and studies demonstrating that children raised in stressful environments, including trauma and poverty, have worse executive function than children from less stressful backgrounds (Hackman et al., 2010; Lawson et al., 2018; Noble et al., 2005; Pakulak et al., 2018). Similarly, previous studies have demonstrated that higher overall cortisol levels and less change in cortisol in response to a stressor are associated with worse executive function in children in poverty (Blair et al., 2011, 2006; Piccolo et al., 2014). One peculiar finding in the current study was that concurrent measures of cortisol reactivity at school-age, but not those at preschool-age, predicted executive function performance, suggesting cognitive functioning is related to more recent physiological responses. This more recent physiological responses may reflect the cumulative experience and development of the cortisol response over childhood. However, it is also possible that the different tasks used to elicit stress response at preschool-age and school-age assessments may have differences in sensitivity and reliability. Both tasks had similar stress-inducing components of social evaluation and inability to complete the task, which reliably evoke a cortisol response in children and adults (Gunnar et al., 2009); but it is possible that one of the assessments may have induced a larger or different cortisol response. Future studies should aim to replicate this finding and further examine the potential of school-age cortisol reactivity, rather than preschool-age cortisol reactivity, predicting executive function.

Although we predicted, based on previous findings, that greater total cortisol release and less total cortisol change in response to a stressor would be associated with lower PFC cortical thickness, the results revealed the opposite pattern. Greater cortisol release at preschool-age was related to increased right caudal middle frontal cortical thickness and greater change in cortisol at

school-age was associated with decreased right inferior frontal cortical thickness. This pattern highlights two important points, the timing of the associations between cortisol reactivity and PFC cortical thickness may differ by PFC region and these associations may vary across development. Within the PFC, multiple regions exist with distinct function and developmental rates resulting in putatively differential periods of vulnerability. For instance, grey matter volume reaches peak development first within the orbital frontal cortex, then the ventrolateral PFC, and finally the dorsolateral and rostrolateral PFC, which have similar developmental timing (Diamond, 2002; Giedd et al., 1999). Therefore, the timing of the window of vulnerability of the PFC may differ by region and cortisol reactivity could be associated with different regions of the PFC throughout different periods of development. Thus, the implication of these findings is that the relation between cortisol reactivity and PFC development may not be a simple negative association, rather the byproduct of chronic stress may change the non-linear dynamics of cortical development for areas like PFC.

According to the simple linear explanation, our cortisol findings may be capturing cortisol responses that are promoting the development of the PFC and potentially executive function. Rodent and human research suggest a small amount of stress or an acute stress can be beneficial for cognitive performance (Parker et al., 2005; Schwabe et al., 2012, 2010). However, given that higher cortisol release was associated with lower executive function performance, it appears unlikely that is the case. The negative linear relationship is consistent with the model from rodent literature in which intense or repeated stress is shown to alter the dendrites and function of the prefrontal cortex and impact cognition negatively (Arnsten, 2009; McEwen and Gianaros, 2010; McEwen and Morrison, 2013; Radley et al., 2004; Teicher et al., 2003). Previous studies in older children and adolescents show negative associations between self-

report cumulative life stress or reports of trauma and prefrontal cortex volumes (Edmiston et al., 2011; Hanson et al., 2012). Given the divergence in findings, a simple linear account of stress response and cortical development is unlikely and a more nuanced understanding is necessary.

An alternative explanation is that the increased prefrontal cortical thickness is less beneficial at this stage in development based upon the expected non-linear dynamics of development. This account challenges the simple linear assumption that greater cortical thickness is associated with greater cognitive development in this age group. The previous work is largely conducted in rodents or adults, and the children in the current study are younger than previous studies examining cortisol and prefrontal structure (Arnsten, 2009; McEwen and Morrison, 2013; Teicher et al., 2003). Given the protracted development of the prefrontal cortex, the association between cortisol and prefrontal cortex may reflect differential development with respect to the peaking of grey matter that occurs around the age range of the children in our study. Consistent with the stress acceleration theory (Callaghan and Tottenham, 2016), chronic or cumulative stress may lead to faster maturation of brain, in this case with increased grey matter development earlier, potentially shifting the inverted U-shape curve of PFC grey matter development. Previous studies demonstrate that age-related brain changes are curvilinear in children from low SES backgrounds and linear in children from higher SES backgrounds (Piccolo et al., 2016). More specifically, children from low SES backgrounds showed earlier peaks in brain development, followed by earlier thinning of the cortex (Piccolo et al., 2016). Therefore, it is possible that high levels of stress, such as cortisol, may shift the developmental trajectory of the prefrontal cortex leading to earlier peaks in grey matter thickness consistent with accelerated maturation, thus, impacting executive function.

In support of a non-linear association between cortisol reactivity and prefrontal cortex development, the right caudal middle frontal cortical thickness indirectly mediated the association between preschool-age total cortisol release and school-age executive function. More specifically, increased preschool-age total cortisol release was associated with increased right caudal middle frontal cortical thickness that was related to decreased school-age executive function. Our findings are consistent with a study in an older sample of individuals from 3-20 years old (mean 12 years) that demonstrated thinner grey matter was associated with better executive function and that SES moderated this association (Brito et al., 2017). Another relevant study demonstrated the right middle frontal cortex activation differed in 8-12 year old children from low SES backgrounds and the amount of change in cortisol during the scan related to the percent of activation in the right middle frontal cortex during a working memory task (Sheridan et al., 2012), supporting our findings of an association between cortisol and the right middle frontal cortex in school-age children. However, this is the first time this mediation with cortisol reactivity in demonstrated in young children over time. Together these results suggest, the role of greater preschool-age total cortisol release may be associated with increased middle frontal cortical thickness, which is less beneficial, resulting in lower executive function. Although, different regions within the PFC have different developmental trajectories and future studies should further examine the varying associations between development timing of cortisol reactivity and prefrontal cortex development as it relates to executive function.

Several limitations in the current study should be considered for future studies. First, the current study included one MRI assessment and executive function at school-age. To truly understand the developmental effects of how the environment impacts stress regulation, neural development, and executive function, future studies should include neural and executive function

measures at an earlier wave of data collection. Second, the sample size limited power to examine mediation pathways but our results provide initial steps to delineating mechanisms and require replication in larger samples. To truly determine if these associations and mediations are present within a larger model, future studies need to address these complex questions with larger sample sizes. Third, the two stress-inducing tasks share components that reliably evoke a cortisol response in children and adults including social evaluation and inability to complete the task (Gunnar et al., 2009); however, two different stress inducing tasks at the two timepoints. Therefore, the results may be capturing components of the task difference within the cortisol reactivity measurements that should be further examined and clarified in future studies. Fourth, multiple analyses were conducted and the findings were not significant using multiple-comparison corrections. Therefore, future studies should aim to replicate these associations.

5. Conclusions

To the best of our knowledge the current study is the first to demonstrate young children's cortisol reactivity is associated with individual differences in prefrontal cortical thickness and executive function using a longitudinal design. Preschool-age total cortisol release was associated with right middle frontal cortical thickness and concurrent school-age total cortisol change related to inferior frontal cortical thickness. Importantly, greater caudal middle frontal cortical thickness mediated the association between greater preschool-age total cortisol release and lower concurrent school-age executive function. Together the results suggest that variation in the prefrontal cortex associated with cortisol reactivity may explain individual differences in executive function in childhood. Understanding the role of cortisol reactivity in cognitive and brain development is crucial for early identification and preventative interventions

targeting stress regulation and coping. At the broader level, these findings inform the long-term implications of early environments and stress on brain and cognitive development in young children. Although we are just beginning to understand the long-term implications of stress on the brain in children, research furthering this line of work is crucial for understanding how the environment and stress impact child development.

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Tables

Table 1. Participant Demographics Comparing Full Sample at Timepoint 2 and Subset for MRI analyses

	Larger Sample at Timepoint 2	Subset for MRI Analyses
N	95	63*
% Male	50.8%	53.7%
Age in years at Time 1 mean (SD)	4.20 (0.84)	4.97 (0.84)
Age in years at Time 2 mean (SD)	7.29 (0.96)	7.51 (0.74)
% Maternal Depression	48 (50.5%)	38 (60.3%)
Race (W[a]/AA[b]/O[c])	46/29/18	33/15/11
Family Income	5 < $20,000	5 < $20,000
	7 $20,001-$40,000	4 $20,001-$40,000
	18 $40,001-$70,000	13 $40,001-$70,000
	29 $70,001-$100,000	15 $70,001-$100,000
	33 >$100,001	26 >$100,001
Maternal Education	1 < High School	1 <High School
	2 High School/GED	1 High School/GED
	27 Some College	21 Some College
	32 College Degree	15 College Degree
	25 Master's Degree	15 Master's Degree
	8 Doctoral Degree	4 Doctoral Degree
Cortisol Reactivity[d]		
Time 1 AUCg[e] (n=57), mean (SD)	1.05 (0.22)	1.06 (0.22)
Time 1 AUCi[f] (n=57), mean (SD)	1.88 (0.06)	1.88 (0.07)
Time 2 AUCg (n=61), mean (SD)	1.08 (0.26)	1.08 (0.28)
Time 2 AUCi (n=61), mean (SD)	1.30 (0.13)	1.31 (0.10)
Executive Function		
Trails B Total, mean (SD)	147.12 (40.81)	149.28 (39.47)
Simon Says, mean (SD)	23.52 (3.62)	23.18 (3.69)
Color Span Total, mean (SD)	8.93 (2.46)	9.12 (2.23)
Executive Function, mean (SD)	0.03 (0.61)	0.01

[*]N = 63 for MRI sample except where noted [a]White [b]African American [c]Multi/Other [d]Cortisol measured in nmol/L [e]Area under the curve in respect to ground [f]Area under the curve in respect to ground

Table 2: Descriptive Statistics for Cortical Thickness.

	n	Mean[a]	*SD*[a]
Right hemisphere			
Caudal Middle Frontal	63	3.05	0.25
Caudal Anterior Cingulate	63	3.03	0.26
Pars Triangularis	63	3.05	0.20
Pars Orbitalis	63	3.34	0.30
Pars Opercularis	63	3.13	0.19
Rostral Middle Frontal	63	2.82	0.23
Rostral Anterior Cingulate	63	3.48	0.21
Left hemisphere			
Caudal Middle Frontal	63	3.03	0.20
Caudal Anterior Cingulate	63	3.25	0.29
Pars Triangularis	63	3.03	0.18
Pars Orbitalis	63	3.40	0.31
Pars Opercularis	63	3.14	0.17
Rostral Middle Frontal	63	2.95	0.18
Rostral Anterior Cingulate	63	3.61	0.29

Volumes measured in mm^3

Figure Legends

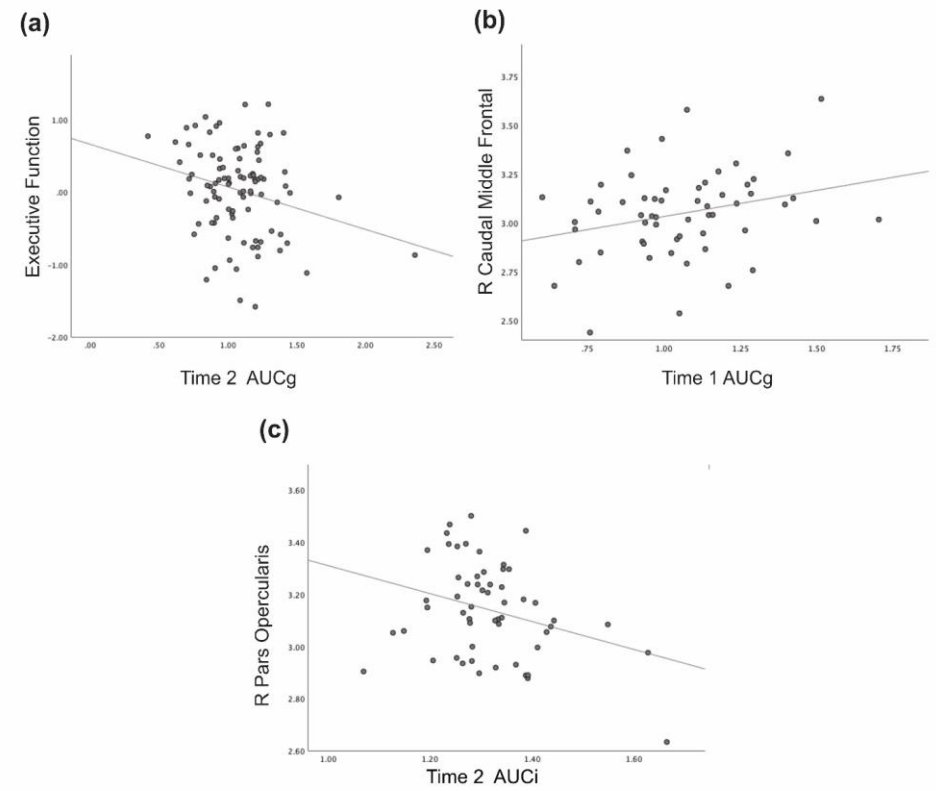

Figure 1: Significant associations between cortisol reactivity, executive function, and cortical thickness. a) The association between executive function and Time 2 AUCg. b) The association between right caudal middle frontal cortical thickness and the Time 1 AUCg. c) The association between right inferior frontal pars opercularis cortical thickness and Time 2 AUCi.

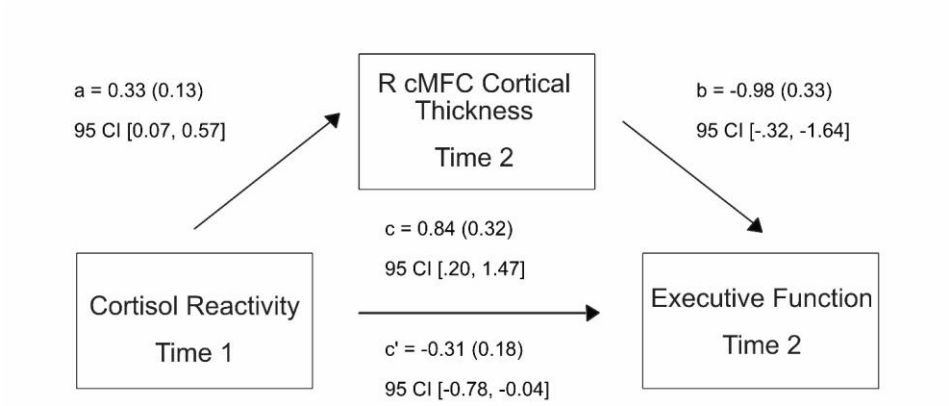

Figure 2: Mediation of the right caudal middle frontal cortical thickness on the association between Time 1 total magnitude of cortisol release (AUCg) and later executive function.