

ABSTRACT

Title of document: CORTISOL AWAKENING RESPONSE IN
PRESCHOOLERS AND DEPRESSION RISK:
RELATIONS WITH MATERNAL HISTORY OF
DEPRESSION AND CHILD TEMPERAMENT

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Increasing research suggests that elevations in the cortisol awakening response (CAR), the natural increase of cortisol 30 to 40 minutes after waking, may serve as a vulnerability marker for depression. However, existing studies have focused on adolescence and adulthood; very little is known about the CAR in early childhood and the factors that are associated with it. The current study aimed to examine the validity of the CAR as a potential early-emerging vulnerability marker for depression in a sample of preschool-age children. We examined associations between the CAR and two well-established risk factors for depression: maternal psychopathology and early child temperament (high negative emotionality (NE) and/or low positive emotionality (PE)).

The sample consisted of 146 preschool-age children, of whom 71 (49.3%) had a biological mother with a history of depression and 65 (45.5%) had a biological mother with a history of anxiety. To assess the CAR, salivary cortisol samples were collected from the child upon waking, 30 and 45 minutes post-waking on two weekdays. Children's CAR was examined as the total volume of cortisol secreted (AUC_g) and the total increase in cortisol (AUC_i) across waking. Evening cortisol was collected 30 minutes before bedtime. Child temperament was assessed using observational laboratory measures. Maternal depression and anxiety were assessed with clinical interviews. Associations with children's CAR, as indicated by AUC_g or AUC_i , appeared to be specific to maternal current psychopathology and symptoms of anhedonia. Additionally, we observed significant interactions for both maternal lifetime and current depression and anxiety, in combination with child NE and PE, on elevated evening cortisol levels and flattened diurnal cortisol rhythms, indicating altered patterns of basal cortisol activity in offspring. Our study contributes to the limited but growing knowledge on the development of the CAR in preschool age children and as a marker of early risk. Findings suggest that there is a complex interplay between familial risk, affective vulnerability, and their joint effects on neuroendocrine dysfunction in young children, and highlight the need for future research to examine which aspects of the early diurnal rhythm predict the emergence of later depressive illness.

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AND DEPRESSION RISK:
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AND CHILD TEMPERAMENT

By

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Dedication

To my parents, for a lifetime of sacrifices and support, which made possible this academic achievement. Thank you for instilling in me the values of hard work, discipline, perseverance, integrity, and a commitment to lifelong learning. All that I am and ever hope to be, I owe to you.

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Chapter 1: Introduction

Depression and the Hypothalamic-Pituitary-Adrenal (HPA) Axis

Depression is a major public health problem, with a lifetime prevalence of approximately 20.8% (Kessler et al., 2005). It is associated with serious impairment in physical, occupational, social, and academic functioning (Cuijpers, 2001), and is a leading cause of disability worldwide (Michaud, Murray, & Bloom, 2001). Moreover, a considerable body of research has established that depression is associated with greater risk of poor outcomes, including substance abuse and suicide (Gould, Greenberg, Veltin, & Shaffer, 2003), and increased morbidity and mortality (Cuipers & Smit, 2002). Importantly, depression is highly chronic and recurrent (Kessler, 2002), and is associated with considerable disease burden, with estimated direct and indirect annual costs of \$83.1 billion per year, in the United States alone (Greenberg et al., 2003). Given its profound personal and financial costs to individuals and society, there is a pressing need to obtain a better understanding of the etiology of depression. The identification of risk factors for depression in early childhood is particularly critical as it may provide a stronger foundation for more effective identification, prevention, and intervention efforts.

Abnormalities in the HPA axis are one of the most consistent and robust findings reported in depression research (Gold & Chrousos, 2002). Indeed, meta-analyses report that several indices of HPA axis functioning are disrupted in depressed adults (Burke, Davis, Otte, & Mohr, 2005) and youth (Lopez-Duran, Kovacs, & George, 2009). Given that life stress is a strong risk factor for the onset of depression (Hammen, 2005), and as the HPA axis plays a critical role in coordinating the body's stress response, theorists posit that changes in the HPA axis in response to stress may mediate the development of

depressive symptoms and recurrence of depression (Hammen, 2005). Increasing research supports this hypothesis; for example, childhood neglect, maltreatment, and trauma have been associated with early HPA axis dysfunction (Gunnar & Donzella, 2002), and subsequent development of depression (Nanni, Uher, & Danese, 2012). Theorists also hypothesize that pre-existing differences in HPA axis functioning may underlie the high sensitivity to stress observed in depression (Hammen, 2009; Holsboer, 2000; Silberg & Rutter, 2002). Thus, understanding individual differences in HPA axis functioning is important to understanding depression vulnerability.

Overview of the HPA Axis

The HPA axis, one of the body's major stress-response systems, adapts organisms to immediate internal or external challenges by inducing a complex set of physiological responses (Tsigos & Chrousos, 2002), thus representing an important pathway linking stress to physical health. In response to stress, a hormonal cascade is initiated in the hypothalamus, ultimately leading to an increase in cortisol (Tsigos & Chrousos, 2002). Cortisol is the major end product of the HPA axis and the primary stress hormone in humans, with widespread effects throughout the body and brain, including effects on metabolism, immune response, cardiovascular function, mood, and cognition (Jacobson, 2005; McEwen & Seeman, 1999). Although the HPA axis provides an adaptive biological response to acute stress critical to everyday functioning, chronic or prolonged stress is associated with dysregulation of cortisol and adverse effects on health (McEwen, 1998). Indeed, elevated cortisol secretion has been associated with depression, cognitive decline, and health problems such as obesity, hypertension, and cardiovascular disease (see Chrousos & Gold, 1998).

Cortisol levels follow a diurnal rhythm, seen initially in infancy and stabilizing to an adult-equivalent rhythm during the preschool-age period (Jessop & Turner-Cobb, 2008). This diurnal rhythm is typically characterized by high levels of cortisol levels upon waking, rapidly increasing by 50-75% to reach peak levels approximately 30 minutes after awakening (known as the cortisol awakening response (CAR); Pruessner et al., 1997), followed by a gradual decline throughout the day with lowest levels occurring around bedtime (Clow, Thorn, Evans, & Hucklebridge, 2004; Fries, Dettenborn, & Kirschbaum, 2009). The activity of cortisol levels throughout the day is widely viewed by researchers as an important indicator of HPA axis functioning. While a strong diurnal rhythm (i.e., high morning levels, steep slope, and low evening levels) is indicative of normative or healthy functioning (Stone et al., 2001), deviations from the typical rhythm of cortisol have been increasingly studied, as it may provide important information about the role of the HPA axis in risk for stress-related disorders, including depression (Chrousos & Gold, 1998; Gunnar & Vazquez, 2001; Heim et al., 2000). Moreover, growing evidence suggests that cortisol dysfunction may not merely be epiphenomena, but rather an endophenotype playing an important causal role in the pathophysiology of depression (Hasler, Drevets, Manji, & Charney, 2004).

Significance of the Cortisol Awakening Response

In recent years, the CAR has gained an increasing amount of attention from researchers for several reasons. First, the CAR reflects the rapid increase in cortisol levels which peak approximately 30 minutes after waking (Clow et al., 2004), and the development of salivary cortisol sampling procedures has allowed the collection of early morning cortisol samples in a home setting with relative ease. Second, the CAR is a

reliable index of HPA axis activity which demonstrates meaningful individual differences in its size as well as high intraindividual stability across time (Hellhammer et al., 2007; Wüst et al., 2000). Although its physiological function is not yet fully understood, research suggests that the CAR serves to mobilize energy necessary for the perceived demands of the day (e.g., Fries et al., 2009). Third, the CAR is regulated by separate mechanisms from underlying diurnal cortisol levels, and thus represents a distinct phenomenon superimposed on the diurnal cortisol rhythm (Clow et al., 2004). For example, research has documented that the CAR is under greater genetic influence than diurnal cortisol levels later in the day (Wüst et al., 2000). Fourth, increasing research has linked the CAR to various physical and psychological disorders, including depression. For example, evidence suggests that as the CAR occurs on top of already elevated levels of diurnal morning cortisol, it is likely to reach physiological levels that activate the low affinity central glucocorticoid receptor systems involved in the pathophysiology of depression (Adam et al., 2010). Thus, the CAR is an important indicator of risk to consider in understanding vulnerability for depression.

CAR and Depression Risk

A growing body of cross-sectional and prospective longitudinal research has linked elevated CAR to depression. With regard to cross-sectional studies, elevated cortisol has been observed among individuals with current and remitted depression. Bhagwager and colleagues (2005) collected multiple morning cortisol samples (at waking, and 15 minute intervals for an hour after waking) to measure the CAR in a sample of 20 acutely depressed adult patients and 40 healthy controls. Acutely depressed patients were found to evidence elevated CAR in comparison to controls. Bhagwager and

colleagues (2003) also examined the CAR in a sample of 31 recovered adult patients and 31 healthy controls, and found that recovered depressed patients evidenced elevated CAR in comparison to controls. Similar findings were reported in a large study of 701 adult participants with a current major depressive disorder (MDD) diagnosis, 579 adult participants with remitted MDD and 308 adult participants with no psychiatric diagnosis, in which the CAR was found to be elevated among remitted and current MDD participants, in comparison to controls (Vreeburg et al., 2009).

Several prospective studies have found elevated morning cortisol, a facet of the CAR, to precede and predict the onset of depression. Harris and colleagues (2000) examined whether early morning cortisol, measured at 8 am, prospectively predicted the onset of MDD, in a sample of 116 adult women. Elevated morning cortisol at 8 am was found to predict the onset of depression over the 13 month follow-up period. Studies in youths have documented similar findings. Goodyer and colleagues (2000) found that in a sample of 180 adolescents at risk for depression due to psychosocial adversity, high emotionality, or parental psychopathology, early morning cortisol (measured at 8 am across 4 days of sampling) predicted the onset of MDD over the subsequent 12 months. In addition, Halligan and colleagues (2004) found that elevated morning cortisol (measured at 8 a.m. across a period of 10 days) at age 13-years predicted elevated self-reported depressive symptoms at age 16, even after controlling for depressive symptoms at age 13. Moreover, in a follow-up study using the same sample, elevated morning cortisol at age 13 years was found to mediate the relation between maternal postnatal depression and self-reported depressive symptoms at age 16 (Halligan, Herbert, Goodyer, & Murray, 2007).

It is important to note that many of these past prospective studies were methodologically limited by the use of single morning samples collected at fixed-times, rather than the use of multi-morning samples collected at person-specific wake times which are required to assess the CAR. However, a growing body of methodologically rigorous research provides strong support for associations specifically between the CAR and increased risk for depression. Adam and colleagues (2010) measured cortisol at waking, 40 minutes post-waking, 3, 8 and 12 hours post waking, and at bedtime, in a sample of 230 older adolescents. Elevated CAR was found to predict the onset of MDD 1 year later. Interestingly, analyses indicated that it was specifically the CAR, in contrast to the diurnal slope and individual morning cortisol samples that prospectively predicted depression. This suggests that previous studies in youth utilizing fixed time samples likely captured a facet of the CAR, rather than the true valid CAR, and that it is the CAR specifically that is an indicator of risk for depression. In a follow-up study using the same but larger sample, Vrshek-Schallhorn and colleagues (2013) found that elevated CAR predicted the onset of MDD up to 2.5 years later. Although CAR did not significantly increase vulnerability to stressful life events, elevated CAR predicted the recurrence of MDD above and beyond first-onset of MDD. Nelemans and colleagues (2013) measured cortisol at waking, and 30 and 60 minutes post-waking, in a sample of 184 adolescents, annually for three consecutive years. Latent Class Growth Analysis revealed two groups: a high CAR group with increasing CAR over time, and a low CAR group with stable and low levels of CAR over time. Adolescents in the high CAR group showed significantly higher depressive symptoms over time, compared to adolescents in the low CAR group. Moreover, this relationship was specific to depressive symptoms, rather than anxiety

symptoms. Notably, one study reported no association between the CAR and later depression in youth. Carnegie and colleagues (2013) measured the CAR at waking, 30 minutes after waking, after school, and before bed, on three consecutive days in a sample of 841 15-year olds. No significant association was observed between the CAR at 15-years and subsequent depression at 18-years.

Notably, research has also linked depression to blunted CAR, particularly among less severely depressed patients and outpatient/community samples. For example, in cross-sectional studies using community samples of adults, blunted CAR has been observed among depressed individuals, compared to non-depressed controls (Peeters et al., 2004; Stetler & Miller, 2005). Prospective studies have also linked blunted CAR to depression. Indeed, in a sample of 55 remitted recurrently depressed adult patients, Bockting and colleagues (2012) found that lower mean morning cortisol levels (measured at 8:00 am) predicted earlier time to recurrence over 5.5 years. In addition, in a community sample of adults, Vreeburg and colleagues (2013) found that blunted CAR (measured at waking, 30, 45 and 60 minutes post-waking) predicted increased risk of a chronic trajectory of depression and/or anxiety disorders over two years; interestingly, this association was specific to the CAR, compared to other salivary cortisol measures.

In sum, both elevated and blunted CAR have been related to risk for depression. To date, it remains unclear why mixed findings are observed in the literature, yet it is notable that such findings are not uncommon in neuroendocrine research. Indeed, both elevated and blunted levels of cortisol have been linked to adverse health outcomes (Chida & Steptoe, 2008; Gunnar & Vazquez, 2001). Nevertheless, a growing body of methodologically rigorous research provides strong evidence that abnormalities in the

CAR are linked to increased risk for later depression. Moreover, given that abnormalities in the CAR have been observed in currently depressed and remitted-depressed adults, as well as the never depressed offspring of depressed parents (reviewed below), the literature suggests that the CAR is not merely a state marker of depression, but rather a trait marker/endophenotype that may play an important causal role in the pathophysiology of depression (Hasler et al., 2004; Oswald et al., 2006).

Gaps in the Literature

First, the majority of studies examining the CAR and depression risk in youth have assessed morning samples collected at fixed-times, which may potentially contribute to the inconclusive results in the literature (Knorr, Vinberg, Kessing, Wetterslev, 2010). Given that cortisol rhythms depend on person-specific sleep-wake schedules (Kudielka & Kirschbaum, 2003; Wilhelm, Born, Kudielka, Schlotz, & Wüst, 2008), fixed-time sampling is problematic as cortisol levels may differ as a function of time awake, rather than true individual differences in cortisol levels. Moreover, previous studies have largely assessed morning cortisol using single morning samples, rather than multiple samples collected across the morning, which are necessary to assess the CAR as well as other indicators of diurnal cortisol functioning. Further research incorporating multi-morning samples in relation to person-specific wake times is thus warranted. Such research would shed light on the CAR as a vulnerability marker for depression, and provide much needed comparison of the predictive value of the CAR to other indices of diurnal cortisol.

Second, the majority of studies examining the CAR and depression risk have used samples of adolescents and adults, many of whom have already developed significant

psychopathology and impairment. To examine the CAR as a vulnerability marker, it is critical to examine the CAR in early childhood, a period that is less likely to be confounded by concurrent depression. Moreover, research examining the CAR in early childhood would provide fundamental insight into our understanding of the development of the HPA axis, and into the origins of HPA axis dysregulation and its potential role in depression risk.

Third, although depressive and anxiety disorders share many features and are highly comorbid (Mineka, Watson & Clark, 1998), they are rarely examined together in relation to cortisol functioning. Interestingly, emerging cross-sectional and prospective research has begun to link the CAR to risk for anxiety disorders (Adam et al., 2014; Vreeburg et al., 2010). For example, Vreeburg and colleagues (2010) examined the CAR in a sample of adults and found that individuals with current anxiety disorders evidenced significantly elevated CAR compared to individuals with remitted anxiety disorder and healthy controls. Adam and colleagues (2014) measured cortisol at waking, 40 minutes post-waking, 3, 8 and 12 hours post waking, and at bedtime, in a sample of older adolescents. Elevated CAR was found to predict the onset of anxiety disorders over a 6 year period. Interestingly, analyses indicated that it was specifically the CAR, in contrast to the diurnal slope and individual morning cortisol samples that prospectively predicted anxiety. Moreover, associations remained even after controlling for depression. Collectively, the literature supports the possibility that the CAR is a vulnerability marker for the development of depressive *and* anxiety disorders. It is thus crucial to investigate the CAR in relation to depression with consideration to anxiety, in order to better

delineate developmental neuroendocrine pathways to the development of psychopathology.

Fourth, little is known about the factors that are associated with, or account for, an increased CAR in early childhood. Demonstrating associations between the CAR and better established vulnerability factors for depression such as parental depression, and child temperament, would provide increased evidence for the CAR as a vulnerability marker for depression. Below, we will review the literature on the association between parental history of depression and offspring HPA axis functioning, followed by a section on early child temperament and HPA axis functioning.

Parental Depression and Offspring HPA Axis Functioning

Parental psychopathology is a robust risk factor for youth depression (Weissman et al., 2006). The offspring of parents with a lifetime history of depression are at greatly increased risk for psychiatric problems, including depression, anxiety, and substance-use disorders. Moreover, the offspring of depressed parents have also been found to evidence greater levels of physical health problems and psychosocial impairment (Hammen, 2009; Weissman et al., 2006).

Several studies have found elevated cortisol among the adult offspring of depressed parents. For example, Mannie and colleagues (2007) conducted a study in which cortisol was measured at waking and twice afterwards at 15 minute intervals over a period of 2 days, in a sample of 49 asymptomatic young adults who either had a family history of depression ($n = 49$) or not ($n = 55$). Elevated morning cortisol was observed among individuals at familial risk for depression. Moreover, this association was not accounted for by differences in self-reported parenting relationships, life events, or

neuroticism (Mannie et al., 2007). Similarly, Vreeburg and colleagues (2010) examined the CAR in a sample of adults with and without a parental history of depression or anxiety. Cortisol was measured at waking, 30, 45, and 60 minutes post waking. Elevated CAR was observed among individuals with a parental history of depression or anxiety. Interestingly, the elevated CAR observed among offspring of depressed or anxious parents was similar in size to levels observed in the participants with depression or anxiety disorders.

Studies in children and adolescents have documented similar findings of elevated cortisol among offspring of depressed parents. For example, in a sample of school age children, Young and colleagues (2006) found that offspring of depressed parents demonstrated elevated morning cortisol in response to dexamethasone, compared to offspring of non-depressed parents; findings were strongest among offspring of currently depressed parents. In a sample of 13-year old adolescents who had ($n = 48$) and had not ($n=30$) been exposed to postnatal maternal depression, Halligan and colleagues (2004) assessed cortisol at 8 a.m. over a period of 10 days and found that maternal postnatal depression was associated with higher morning cortisol in offspring. In a sample of preschool age children, Dougherty and colleagues (2009) found that elevated early morning cortisol (measured 30 minutes post waking) was significantly associated with maternal history of depression. Interestingly, this relation was observed specifically for maternal melancholic depression, a subtype of depression primarily characterized by anhedonia, the diminished capacity to experience pleasure. This finding is noteworthy given research demonstrating associations between hypercortisolism and the melancholic subtype of depression (Stetler & Miller, 2011), and as melancholia has been found to

confer greater risk for the intergenerational transmission of internalizing and externalizing disorders than other depression subtypes (Shannon et al., 2007). Lastly, in one study the joint, interactive effects between maternal depression and child temperament have also been prospectively linked to elevated morning cortisol (measured at 30 minutes post-waking) three years later (Dougherty et al., 2013).

In sum, research in youth has documented associations between parental history of depression and elevated morning cortisol in offspring. Nevertheless, significant limitations in the literature remain. First, no studies in youth have assessed the CAR in the offspring of depressed parents. Instead, existing studies in youth have used single morning samples, largely collected at fixed-times, which preclude the ability to assess the CAR. Second, only two studies have examined exposure to maternal depression (Dougherty et al., 2013; Halligan et al., 2004). Increasing research highlights the importance of considering how timing of maternal stress or depression may impact children's neuroendocrine functioning (Ashman et al., 2002; Essex et al., 2002; Halligan et al., 2004; Young et al., 2006). Third, the majority of studies examining neuroendocrine functioning in the offspring of depressed parents have used samples of adolescents and adults, many of whom have already developed significant psychopathology and impairment. As depression is rare in preschool-age children (Egger & Angold, 2006), examining the CAR in this age group would provide the opportunity to examine HPA axis abnormalities in at-risk individuals *prior* to the onset of a subsequent disorder, and provide fundamental insight into the origins of HPA axis dysregulation and its role in depression risk. Therefore, further work examining associations between maternal history of depression and the CAR in early childhood is needed.

Temperamental Risk for Depression

Another important developmental risk factor for depression is early temperament. The role of temperament in the nature and origins of depressive disorders has been a topic of increasing interest in developmental psychopathology (see Bijttebier & Roeyers, 2009). Temperament refers to biologically-based individual differences in behavioral and emotional reactivity and regulation that are early emerging, and relatively stable over time (Rothbart & Bates, 2006). Two broad, higher-order dimensions featured centrally in major models of temperament are negative emotionality (NE) and positive emotionality (PE). NE includes sadness, fear, anxiety, and anger, whereas PE includes positive affect (PA) and enthusiasm; reward sensitivity and appetitive behavior; affiliation and sociability; and surgency and dominance (Shiner & Caspi, 2003).

Several influential models linking NE and PE to depression have been proposed (Shankman & Klein, 2003). The tripartite model (Clark & Watson, 1999) proposes that the predisposition for depression is associated with high NE and low PE. Similarly, other models, including the approach-withdrawal (Davidson, 1998) and valence-arousal models (Heller & Nitschke, 1998) propose that facets of PE, such as low hedonic capacity/deficits in the approach system of motivation and reward sensitivity, and facets of NE such as an overactivation of the withdrawal system characterize depression. Thus, across leading models, high negative emotionality is generally hypothesized to confer non-specific vulnerability to the development of psychopathology and is common to depression and anxiety disorders, whereas low positive emotionality is hypothesized to confer specific vulnerability to depression (see Shankman & Klein, 2003). Moreover, it is notable that

melancholic depression and anhedonia (a diminished capacity to experience pleasure) are characterized by low PE.

Cross-sectional studies provide support that depression is associated with high NE and low PE. For example, Anthony and colleagues (2002) found that high NE and low PE were associated with self-reported depressive symptoms in a community sample of youths. These findings have also been observed in studies using parent and peer reports (Phillips, Lonigan, Driscoll, & Hooe, 2002), and in clinical samples of youths (Joiner, Catanzaro, & Laurent, 1996; Lonigan, Carey, & Finch, 1994).

Prospective studies have also linked high NE and low PE to the development of depressive symptoms and disorders (e.g., Block, Gjerde, & Block, 1991; Kendler, Gatz, Gardner, & Pederson, 2006; Lonigan, Phillips, & Hooe, 2003; van Os, Jones, Lewis, Wadsworth, & Murray, 1997). For example, NE in early childhood has been found to prospectively predict parent-reported depressive symptoms at age 7 (Rende, 1993). Caspi and colleagues (1996; 2000) found that NE and PE-related behavioral ratings at age 3 significantly predicted higher levels of parent-reported internalizing symptoms at ages 13 and 15, and higher rates of depressive disorders and suicide attempts at age 21. Using maternal reports and laboratory and naturalistic home observations of temperament, Dougherty and colleagues (2010) found that low PE at age 3 significantly predicted depressive symptoms at age 10. Moreover, a significant interaction was observed between PE and NE at age 3 in predicting depressive symptoms at age 10, such that children with both lower PE and higher NE at age 3 exhibited the greatest increase in depressive symptoms at age 10.

Research also provides support that children at familial risk for depression demonstrate higher levels of NE and lower levels of PE than controls. In a community sample of preschool-age children, Durbin and colleagues (2005) compared the offspring of parents with a history of depression and the offspring of non-depressed controls on observational measures of PE, assessed using a standardized laboratory temperament paradigm. Low PE, but not NE, was associated with maternal history of depressive disorder. Olino and colleagues (2010) examined both the main and interactive effects of laboratory observation measures of PE and NE and their relation to parental depressive disorders, in a large community sample of preschool age children. Although no main effects for PE were observed, PE and NE interacted to predict parental depressive disorders; specifically, at low levels of NE, low PE was associated with higher rates of parental depressive disorder. In addition, at high levels of PE, high NE was associated with higher rates of parental depressive disorder. Moreover, a few studies have also documented longitudinal associations between NE, PE, and parental depression (e.g., Forbes, Cohn, Allen, & Lewinsohn, 2004; Olino et al., 2011). For example, Olino and colleagues (2011) used longitudinal, observational data collected from infancy to middle and late childhood to examine the developmental trajectories of NE and PE over time, in a sample of children at familial risk for the depressive disorders. Offspring of mothers with a history of depression evidenced significantly lower levels of PE over time, relative to controls.

Temperament and HPA Axis Functioning

A growing body of literature has documented associations between temperament and HPA axis functioning. Emerging evidence supports associations between affect and

health, and suggest that affect may involve the activation of neuroendocrine pathways (e.g., Dockray & Steptoe, 2010). Indeed, NE and NE-related constructs have been positively associated with higher average cortisol levels throughout the day in adults (Nater et al., 2010; Polk, Cohen, Doyle, Skoner, & Kirschbaum, 2005), increased morning cortisol in children (Kagan, Reznick, & Snidman, 1987), and elevated evening cortisol in adults (Gerritsen et al., 2009) and young children (Dougherty et al., 2013). In addition, elevated CAR has also been observed among individuals with high levels of neuroticism (Portella et al., 2005; Polk et al., 2005). However, results have been mixed in this area of research: studies have documented negative associations between NE-related constructs and diurnal cortisol slopes in adults (Doane et al., 2011), male adolescents (Hauner et al., 2008), and young children (Dettling et al., 1999; Watamura, Donzella, Alwin, & Gunnar, 2003), and no association between neuroticism and the CAR (Doane et al., 2011; Hill, Billington, Krägeloh, 2013).

In contrast to research on NE and cortisol, limited research has examined associations between PE, or PE-related constructs, and cortisol. In adults, low PE has been linked to higher cortisol output throughout the day (Steptoe, Wardle, & Marmot, 2005; Steptoe & Wardle, 2005) and elevated CAR (Steptoe, Gibson, Hamer, & Wardle, 2007). However, studies in adults have also documented no association between extraversion, which includes aspects of PE and high sociability, and the CAR (Munafò et al., 2006; van Santen et al., 2010).

To our knowledge, only two studies have examined associations between PE and cortisol in children. Dougherty and colleagues (2009) found a main effect of PE on morning cortisol such that low PE was associated with elevated waking cortisol. In a

subsequent study, Dougherty and colleagues (2013) found a significant interaction between maternal lifetime depression and child positive affectivity at age 3 in predicting offspring's morning cortisol (collected 30 minutes after waking) at age 6. Specifically, for the offspring of mothers with lifetime depression, higher positive affectivity at age 3 predicted lower morning cortisol at age 6. While low positive affectivity has been primarily linked to depression, these findings of high positive affectivity are noteworthy, as high positive affectivity has also been associated with risk and maladaptive outcomes including externalizing and internalizing problems in children (e.g., Degnan et al., 2011; Stifter et al., 2008). Thus, further examination of PE is needed to better elucidate its relation to morning cortisol and depression risk.

In sum, research suggests that there may be a relationship between NE, PE, and HPA axis functioning; however, the nature of the association remains unclear and significant limitations in the literature remain. First, the majority of studies have examined associations between temperament and diurnal HPA axis activity. To date, only a limited number have examined associations with the CAR, an important parameter to consider given its relation to depression. Second, the majority of studies have examined direct associations between temperament and cortisol. Failure to take joint, interactive effects into account could obscure important associations and pathways to depression. For example, high NE and/or low PE have been found among offspring at familial risk of depression (Durbin et al., 2005; Olino et al., 2011), it is possible that the combination of child temperament and familial risk may jointly impact neuroendocrine function, and increase risk for subsequent depression. Third, most studies of child temperament have assessed temperament traits through use of self- or parent-reports, which have been

shown to demonstrate poor convergent validity with teacher report and observational laboratory measures (Seifer, Sameroff, Barrett, & Krafchuk, 1994), and may be biased by the parent's own psychopathology or mood (Youngstrom, Izard, & Ackerman, 1999). Fourth, existing studies on neuroendocrine functioning have focused on NE. Limited work has examined PE, which is of critical importance given its fundamental relation to depression (Clark & Watson, 1999), and as anhedonia (which is characterized by low PE) is a cardinal feature of melancholic depression, which is strongly associated with hypercortisolemia (Stetler & Miller, 2011). Lastly, the majority of studies examining associations between temperament and the CAR have been conducted in adults. To our knowledge, no studies have examined associations with the CAR in young children. Given that temperament may be reliably assessed in early childhood (Caspi, & Shiner, 2006), the study of its associations with the CAR in early childhood would allow an assessment of the CAR as a vulnerability marker of depression in the absence of depression (Egger & Angold, 2006). Moreover, the identification of risk factors in early childhood would provide for an earlier window for intervention.

Current Study

Research suggests that the CAR is not merely a state marker of depression, but rather a trait marker that may represent an increased biological vulnerability for depression. Indeed, the CAR has been found to prospectively predict the onset of depression (Adam et al., 2010; Vrshek-Schallhorn et al., 2013). Moreover, the CAR has been found to predict poorer course (Vreeburg et al., 2013) and greater recurrence of depression (Bockting et al., 2012). To date, the majority of studies examining the CAR and depression risk have used samples of adolescents and adults, many of whom have

already developed significant psychopathology and impairment. To our knowledge, no study has examined the association between the CAR and depression risk in early childhood. Examining the CAR in early childhood, a period in which depression is rare, would shed greater light on the CAR as a vulnerability marker for depression and provide fundamental insight into the origins of HPA axis dysregulation and its role in depression risk. Furthermore, examining the CAR in early childhood is particularly vital given that early perturbations in HPA axis functioning have been found to demonstrate lasting impacts on the developing child (Heim et al., 2008; Meaney, 2001). Moreover, the identification of risk factors for depression in early childhood may provide a stronger foundation for early and potentially more effective identification, prevention, and intervention efforts. In addition, there is a need for research examining neuroendocrine functioning and depression risk to incorporate rigorous methodology including multi-morning samples, a comprehensive assessment of depression (e.g., clinical features, exposure), and comparison of the CAR to other indicators of HPA axis functioning (e.g., diurnal slope, evening cortisol) in its association with depression risk. Lastly, as little is known about the factors that are associated or account for dysregulated CAR, research examining associations between the CAR and better established vulnerability factors for depression such as maternal depression and child temperament is warranted and would provide increased evidence for the CAR as a vulnerability marker for depression.

The current study examined the CAR as a potential early-emerging vulnerability marker for depression, in a sample of preschool-age children. We examined associations between the CAR and two well-established risk factors for depression: maternal history of depression and early child temperament (high negative emotionality (NE) and low

positive emotionality (PE)) (Klein, Kotov, & Bufferd, 2011), using a rigorous methodological approach. Specifically, the current study included a comprehensive assessment of the CAR, utilizing multiple morning cortisol samples, which is critical given the rapid and dynamic nature of the CAR within 30 to 45 minutes after waking (Clow et al., 2004). Furthermore, various salivary measures were examined, including the diurnal cortisol slope and evening cortisol, to determine the specificity of associations to the CAR. We also used clinical interviews of maternal psychopathology and observational assessments of temperament. Lastly, given our focus on investigating the CAR as a potential early emerging vulnerability marker or trait marker, rather than a correlate of the disorder, any child with current depression was excluded from the study. In sum, the current study tested two specific aims:

Aim 1: Examine the concurrent associations between the CAR, and two well-established risk factors for depression in preschool-age children. We examined whether children's CAR is directly related to maternal depression and child temperamental NE and PE.

- a) Given previously observed relations of elevated CAR among adult offspring at familial risk for depression (Vreeburg et al., 2010), we hypothesized that preschool-age offspring of mothers with a history of depression would evidence elevated CAR compared to the offspring of mothers with no history of depression. As a few studies have also documented associations between the CAR and anxiety (e.g., Adam et al., 2014; Vreeburg et al., 2010; 2013), and as depression is highly comorbid with anxiety (Kessler et al., 2003), we also included maternal

history of anxiety in models, to determine the specificity of the relation of child CAR to depression.

- b) Although the literature remains limited, given previously observed associations between high NE, low PE, and the CAR in adults (Polk et al., 2005; Portella et al., 2005; Steptoe, Gibson, Hamer, & Wardle, 2007), and the relation between melancholic features of depression (e.g., symptoms of anhedonia, which is characterized by low PE) and hypercortisolemia (Stetler & Miller, 2011), we tentatively hypothesized that children's high NE and/or low PE will be associated with elevated CAR in children.

Aim 2: Examine the interactive effects between maternal depression and child temperament on children's CAR. It is possible that the familial or environmental stress of having a depressed mother may interact with certain temperamental styles to impact their neuroendocrine functioning and risk for depression. Thus, we examined how child temperament and familial risk jointly impact children's CAR. We hypothesized that offspring of mothers with a history of depression and who also exhibit high NE or low PE would evidence the greatest elevations in CAR.

Exploratory Aim: Examine whether the association between children's CAR and maternal depression is specific to children whose mothers had been depressed during the child's lifetime. As evidence suggests that exposure to maternal depression, especially during early development, may impact young children's stress physiology (Ashman et al., 2002; Dougherty, Tolep, Smith, & Rose, 2013; Essex et al., 2002; Halligan et al., 2004;

Young et al., 2006), we hypothesized that children exposed to maternal depression would evidence elevated CAR. We also hypothesized that the children of mothers exposed to maternal depression and with high NE and/or low PE would demonstrate the greatest elevations in CAR.

Exploratory aim: Examine how children's CAR is related to maternal anhedonia, a core feature of depression. Consistent with research documenting strong associations between melancholic features of depression, characterized by anhedonia, and hypercortisolemia (Stetler & Miller, 2011), we hypothesized that the offspring of mothers with greater symptoms of anhedonia would demonstrate elevated CAR. We also hypothesized that the children of mothers with greater anhedonia symptoms and with high NE and/or low PE would demonstrate the greatest elevations in CAR.

Chapter 2: Method

Participants

Participants consisted of 175 preschool-age children and their biological parents (Dougherty et al., 2013). Participants were recruited using print advertisements distributed throughout local schools, daycares, community centers, and health care providers in the greater Washington, DC area (73.1%), and a commercial mailing list (26.9%). A proportion of flyers specifically targeted parents with a history of depression. Families with no parental history of bipolar or psychotic disorder, with a child between three and five years of age without any significant medical conditions or developmental disabilities, who were not taking corticosteroids, and who lived with at least one English-speaking biological parent were eligible for the study.

Of the 175 children participating in the study, 156 children provided home cortisol samples. Of these 156 children, four children were excluded for providing samples with extreme cortisol values (i.e., > 3 standard deviation above the mean; Gunnar & White, 2001), and five children were excluded for taking corticosteroid ($n = 2$), stimulant ($n = 1$), analgesic ($n = 1$) medications, and/or because they were sick with a fever ($n = 1$), as these factors have been shown to impact cortisol levels (Granger, Hibel, Fortunato, & Kapelewski, 2009; Gunnar & Talge, 2007). No children met criteria for a current major depressive diagnosis based on the Preschool Age Psychiatric Assessment (PAPA; Egger, Ascher, & Angold, 1999). Participants were required to provide at least one valid cortisol sample, leaving a total of 146 children in the final sample.

The mean age of the children was 4.14 years ($SD = .81$; $Range = 3.00 - 5.92$ years); 71 (48.6%) were boys and 75 (51.4%) were girls. Children were of average

cognitive ability as measured by the Peabody Picture Vocabulary Test ($M = 110.57$, $SD = 15.49$, $Range = 68.00 - 148.00$) (PPVT; Dunn & Dunn, 1997). The mean age of mothers and fathers was 34.94 years ($SD = 6.26$) and 37.26 years ($SD = 6.69$) respectively.

Participating families identified themselves as White ($N = 71$; 49.3%), Black/African-American ($N = 49$; 34.0%), Asian ($N = 2$; 1.4%), or multi-racial or other race ($N = 22$; 15.3%); 25 (17.6%) children were of Hispanic/Latino descent. Approximately 68.5% children lived in two-parent households, and 70.5% of children had at least one parent with a 4-year college degree. Participating families reported a range of family incomes: less than \$20,000 (7.1%), \$20,000-\$40,000 (9.3%), \$40,000-\$70,000 (19.3%), \$70,001-\$100,000 (30.0%), and greater than \$100,000 (34.3%). Demographic characteristics of the study sample are presented in Table 1.

Overall Design

Children and their parents participated in two laboratory visits. During the first visit, children participated in a standardized temperament assessment battery. Parents completed a questionnaire on child and family demographic characteristics and received instructions and materials for their child's home salivary cortisol sampling. Between laboratory visits, clinical psychiatric interviews were conducted with children's biological mothers on the telephone. During the second visit, parents returned their child's salivary cortisol samples and materials and completed a psychiatric interview about their child's current emotional and behavioral problems. See Appendix A for a full list of the study's measures.

Study Implementation

Phone Screening: Trained research assistants conducted a preliminary phone screener to determine the study eligibility of potential participants. The phone screener consisted of questions regarding inclusion criteria. In addition, the phone screener served as a gross screen for parental lifetime bipolar disorder or psychosis; however, this information was later verified during the study using a psychiatric clinical interview. Parents who met criteria for these disorders were excluded from the study.

Visit One: Upon arrival to the laboratory, parents received a verbal overview of the study and gave informed consent. During the visit, children participated in a series of observational tasks designed to assess child temperament. Parents completed a questionnaire pertaining to child and family demographic characteristics. Parents were instructed to obtain salivary cortisol samples from their child at home at prescribed times on each of two consecutive weekdays: immediately after the child's waking, 30 and 45 minutes after the child's waking, and 30 minutes before the child's bedtime. Parents received all sampling materials in a kit. At the end of the visit, the experimenter demonstrated the sampling procedure to the parent by collecting a practice sample with the child. In addition, parents and co-parents were asked to participate in clinical psychiatric interviews over the telephone.

Visit 2: Upon the second visit to the research lab, which typically occurred within two weeks (+/- 1 week) after the first visit, parents returned their child's salivary cortisol samples and completed a psychiatric interview about their child's emotional and behavioral problems during the past three months.

Measures

Demographic and child characteristics. Parents completed a self-report questionnaire pertaining to child and family demographic characteristics, including race/ethnicity, marital status, household income and composition, and parental education (see Appendix B). Parents also completed the Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003) to screen for pervasive developmental disorders in preschool-age children. The SCQ is a 40-item parent-report measure of characteristic autistic behavior in preschool-age children and evidences good reliability and validity (Eaves, Wingert, Ho, & Michelson, 2006). To assess for gross cognitive impairment in children, children were administered the Peabody Picture Vocabulary Test (PPVT; Dunn & Dunn, 1997), which is a well-validated measure of receptive language.

Maternal psychopathology. The Structured Clinical Interview for DSM-IV, non-patient version (SCID-NP; First, Gibbon, Spitzer, & Williams, 1996) was used to assess the psychiatric histories of biological mothers. The SCID-NP is a widely used semi-structured diagnostic interview that has been documented to have excellent reliability and validity (Williams et al., 1992). All interviews were conducted by a master's level clinician with extensive training in the SCID. Interviews were conducted on the telephone, which yields similar results as face-to-face interviews (Rohde, Lewinsohn, & Seeley, 1997). Based on audiotapes of 16 SCID interviews, the interrater reliability (indexed by kappa) were 1.00 for lifetime depressive and anxiety disorders.

Of the 146 children who provided valid cortisol data, SCID interviews were obtained from 144 (98.6%) mothers. Major depressive disorder (MDD) and dysthymic disorder (DD) were collapsed into a single category reflecting depressive disorder. As

seen in Table 1, 71 (49.3%) mothers had a lifetime history of depressive disorder and 12 (8.4%) had a current depressive disorder. Sixty-five mothers (45.5%) had a lifetime anxiety disorder, and 29 (20.3%) had a current anxiety disorder.

If a child's mother had a lifetime depressive disorder based on the SCID, the onset and offset dates of all the episodes were recorded to determine whether they had a depressive disorder during the child's life. A life event calendar approach was used to aid recall (Belli, Shay, & Stafford, 2001). Of the 71 mothers with lifetime depression, 44 mothers (30.6%) had a depressive disorder during the child's life.

Maternal anhedonia symptoms. To assess maternal anhedonia symptoms, 137 mothers completed the Snaith-Hamilton Pleasure Scale (SHAPS; Snaith et al., 1995). The SHAPS is a 14-item self-report measure with established reliability and validity (Franken, Rassin, & Muris, 2007), which assesses hedonic capacity in a variety of situations. Higher scores indicate greater anhedonia severity. The SHAPS demonstrated good internal consistency ($\alpha = .92$).

Child temperament. All 146 children participated in the laboratory assessment of temperament, during which children interacted with a female experimenter in eight standardized tasks selected from the Laboratory Temperament Assessment Battery (Lab-TAB; Goldsmith, Reilly, Lemery, Longley & Prescott, 1995). The selected episodes were designed to elicit a range of emotions and temperament traits and ordered to prevent carry-over effects. Between episodes the child took brief play breaks with the experimenter to allow for return to a neutral state. Parents were present in the observation room for all episodes, with two exceptions noted below. When present in the room, parents were instructed to remain neutral and redirect the child to task when solicited for

help. Episodes are described in the order in which they were conducted. For a full description of the laboratory assessment of temperament refer to Appendix B.

Make that Car Go (positive affect [PA], interest). The child and experimenter raced two remote-controlled cars.

Transparent Box (persistence, interest, anger, sadness). The child selected a toy, which the experimenter then locked in a transparent box. The child was then left with a set of inoperable keys to attempt to open the box. After a few minutes, the experimenter returned to the child and told him/her that she had left the wrong set of keys. The child was then encouraged to use the new keys to open the box and allowed to play with the toy.

Exploring New Objects (fear). The child was presented with the opportunity to explore novel and ambiguous stimuli, including a tent, a small pet carrier, “gooey” toys, a remote-controlled spider, and a plastic head covered with a black cloth.

Pop-up Snakes (PA, fear). The experimenter showed the child what appeared to be a can of potato chips, which actually contained coiled spring snakes, without the parent in the observation room. The child was then encouraged to surprise his or her parent with the snakes.

Impossibly Perfect Green Circles (anger, sadness, persistence). The experimenter repeatedly asked the child to draw a circle on a large piece of paper. Each attempt was mildly criticized. After approximately two minutes, the experimenter praised the child for his/her efforts.

Popping Bubbles (PA, interest, activity). The child and experimenter played together with a bubble-shooting toy.

Snack Delay (anticipatory PA, inhibitory control). The child was instructed to wait for the experimenter to ring a bell before eating a small snack. The experimenter implemented a schedule of systematically increasing delays before ringing the bell.

Box Empty (anger, sadness, fear). The child was given a wrapped empty box to open, under the assumption that a prize was inside. After a brief delay in which the child was left alone to discover that the box was empty, the experimenter returned with several small toys for the child to keep, explaining that she had forgotten to place the toys inside the box.

Coding procedures followed those reported in previous studies (Dougherty et al., 2011; Durbin et al., 2005; Olino et al., 2010). All episodes were recorded for subsequent coding by trained undergraduate and graduate students. The coding system considered facial, bodily and vocal indicators of positive affect (PA), fear, sadness and anger. A single rating was made per episode, which was based on all behaviors that were relevant to each affective dimension during that episode. Ratings of PA were made with consideration of the qualitative and quantitative displays of joy and enthusiasm. Overall, PA ratings were computed as the average standardized weighted sum of instances of low, moderate, and high displays of facial, vocal, and bodily PA across all episodes. The same procedure was done to create aggregate scores for sadness, anger, and fear. The PA, sadness, and anger composite variables were calculated using ratings across all eight episodes. The fear composite variable was calculated using ratings based on three episodes only (Exploring New Objects, Pop-Up Snakes, and Box Empty).

Additionally, global ratings of child interest and sociability were made across episodes. Interest ratings were made based on the child's comments about the activity and

how engaged the child was in play. Sociability ratings were based on the quality and quantity of the child's attempts to engage and interact with the experimenter and, to a lesser degree, the parent. Both of these subscales were rated on a 4-point scale from 0 to 3 (higher scores indicating greater interest and sociability).

The following scales were used to create the study's aggregate temperament variables (described below), and internal consistency estimates, as measured by alpha, were adequate: PA ($\alpha = .89$), interest ($\alpha = .60$), sociability ($\alpha = .77$), sadness ($\alpha = .66$), anger ($\alpha = .76$), and fear ($\alpha = .80$). We calculated an aggregate PE variable, which consisted of the average of facial, bodily, and vocal PA, interest and sociability across all episodes. Additionally, we calculated an aggregate NE variable, which consisted of averaging ratings of facial, bodily, and vocal anger, sadness, and fear. Internal consistencies for PE and NE were .92 and .79, respectively. Interrater reliability, as indexed by the intraclass correlation (ICC) and based on a subsample of 15 cases, was adequate for the composite scales of PE ($ICC = .93$) and NE ($ICC = .83$).

Child depression. Children's current major depressive disorder (MDD) diagnoses were assessed using the PAPA Version 1.4, a structured psychiatric diagnostic interview with parents (Egger et al., 1999). The PAPA is a parent-reported interview that assesses emotional and behavioral problems in young children (between the ages of 2 and 6 years) during the past three months. The PAPA follows a required set of questions and probes, but symptoms are only endorsed when they meet the criteria, as outlined in the extensive glossary. The PAPA includes a broad set of diagnostic criteria adapted from the DSM-IV-TR (American Psychiatric Association, 2000), with the exclusion of certain items that are not relevant to preschool-age children (Egger & Angold, 2004). Satisfactory test-

retest reliability of the PAPA has been reported at levels similar to those found in psychiatric assessments of older children and adults (Egger et al., 2006). All interviews were conducted by trained graduate students who were unaware of all data on child and parental psychopathology and parenting. Primary caregivers (132 Mothers, 7 Fathers, 5 Both Parents) provided diagnostic information on 144 (98.6%) children. The PAPA was used to exclude any child with a major depressive disorder within the past three months. No child met criteria for MDD within the past three months.

Salivary cortisol. Parents were instructed to obtain salivary cortisol samples from their child immediately upon the child's waking, 30 and 45 minutes post-waking, and 30 minutes before bedtime on two consecutive days, for a total of 8 cortisol samples per child. Of the 1,156 cortisol samples collected, 50 samples (4.3%) were excluded due to extreme cortisol values (i.e., > 3 standard deviation above the mean; Gunnar & White, 2001), leaving a total of 1106 valid cortisol samples. Of the 146 children who provided valid cortisol samples, 122 children (82.2%) provided all 8 cortisol samples, 15 children (10.3%) provided 7 cortisol samples, 7 children provided 4 to 6 samples (6.4%), and 4 children (2.8%) provided 1 to 3 samples. Sampling times were selected to capture the cortisol rise in awakening and nadir cortisol levels at bedtime. Samples were collected on two days in order to reliably assess the CAR (Hellhammer et al., 2007), and on weekdays only as the type of day has been associated with cortisol levels (Kunz-Ebrecht, Kirschbaum, Marmot & Steptoe, 2004).

Parents received all sampling materials in a kit. Parents were instructed to refrain from sampling if their child was sick or taking antibiotics. In addition, parents were instructed to refrain from the following for the period prior to or during sampling: (1)

brushing their child's teeth (2) giving their child food and/or drink, and (3) giving their child caffeine and dairy products, as these factors have been found to influence cortisol levels (Gunnar & Talge, 2008).

To collect cortisol for analysis, parents were instructed to have their child chew on a cotton dental roll dipped in .025 g of cherry-flavored Kool-Aid® to stimulate saliva. A series of experiments conducted by Talge and colleagues (2005) showed that the use of cherry-flavored Kool-Aid® does not compromise the quality of cortisol data when used consistently and sparingly. When the cotton roll was saturated, parents were instructed to expel their child's saliva from the cotton roll into a vial using a needleless syringe. Parents were instructed to label and store samples in the refrigerator until their second visit to the laboratory, typically occurring within 2 weeks, upon which samples were stored at -20° C until assayed. Salivary cortisol samples were assayed at the University of Trier, Germany in duplicate with a time-resolved immunoassay with fluorometric end point detection (DELFI A). Inter- and intra-assay coefficients of variation ranged between 7.1%-9.0% and 4.0%-6.7%, respectively. For a description of the Salivary Cortisol Sampling protocol see Appendix C.

The following cortisol variables were included in analyses: cortisol values for each time point (waking, 30, 45 minutes post-waking, and evening), the CAR, and the diurnal cortisol slope (the rate of decline in cortisol levels from waking to evening). The CAR was quantified in two ways: the area under the curve with respect to ground (AUC_g; total cortisol secretion across the morning samples) and with respect to increase (AUC_i; measure of the dynamic of the CAR, as reflected by the *change* in morning cortisol levels over time) for the waking, 30 and 45 minute post-waking samples (Pruessner,

Kirschbaum, Meinlschmid, & Hellhammer, 2003). To assess the diurnal cortisol slope separately from the CAR, the diurnal cortisol slope was computed as the difference in waking and bedtime cortisol levels divided by the number of hours between the two samples (Adam & Kumari, 2009). Steeper slopes are represented by lower negative values and indicate more rapid declines in cortisol; flatter slopes are represented by higher values and indicate slower declines. Following Gunnar and Talge (2007), summary variables (i.e., AUC_g , AUC_i , and diurnal slope) were computed using untransformed values; the distributions of these summary variables were then inspected for normality. Cortisol values for each time point (waking, 30, 45 minutes post-waking, and bedtime) and the diurnal cortisol slope showed positive skew; thus, \log_{10} transformations were applied. As AUC variables were normally distributed, untransformed values were used in all analyses. For ease of interpretation, data presented in all tables and figures reflect untransformed values.

Parental compliance to cortisol sampling. Previous studies indicate that compliance with sampling times is important for valid and high quality cortisol data (Adam & Kumari, 2009; Broderick et al., 2004; Kudielka et al., 2003, 2007; Smith & Dougherty, 2014). To assess parental compliance to sampling times, all parents completed a diary measure in which parents recorded the child's time of waking, bedtime, and all sampling times (see Appendix D). In addition, for a random subsample of 80 (55.2%) children, objective parental compliance with the timing of cortisol sampling was assessed using electronic monitoring. Specifically, parents withdrew sampling cotton dental rolls from a bottle with a pressure-activated microcircuitry cap

that recorded the date and time of each bottle opening (MEMS Track Cap; Aardex Ltd., Zug, Switzerland).

Following prior studies (e.g., Bäumlér, Kirschbaum, Kliegel, Alexander & Stalder, 2013; Stalder, Bäumlér, Miller, Alexander, Kliegel, & Kirschbaum, 2013), information from parent-report and objective electronic monitoring was combined to impose strict criteria for compliance: when compliance data based on the electronic monitor was not available, compliance data based on the daily diary was used. Although it would be ideal to have compliance data based on the electronic monitor for the full sample, inclusion of this information for 55.2% of the sample provides an indication of the extent to which the results are robust to the effects of noncompliance (Adam, Hawkley, Kudielka, & Cacioppo, 2006).

Compliance was determined for each method at the sample-level and person-level. To determine compliance, sampling time window criteria was applied to samples. Consistent with previous literature (i.e., Broderick et al., 2004; Jacobs et al., 2005; Kudielka et al., 2003, 2007), a time window of ± 10 minutes was selected for samples that compose the CAR (i.e., waking, 30 and 45 minute samples), as cortisol levels change rapidly during the morning (Clow et al., 2004). As cortisol levels change more slowly during the evening (Fries et al., 2009), a time window of ± 1 hour was selected for the evening samples. Samples collected within these respective time windows were considered to be collected in compliance with the specified sampling time (0 = noncompliant, 1 = compliant).

To define compliance at the person-level, the CAR, diurnal slope, and evening cortisol of participants was dummy coded as compliant or non-compliant (0 = compliant,

1 = noncompliant). For the CAR, participants were coded as compliant if all morning samples (i.e., waking, 30 and 45 minute post-waking samples) were collected within their established time windows; i.e. one or more noncompliant morning samples resulted in the participant being considered as noncompliant (Kudielka et al., 2007). For the diurnal cortisol slope, participants were coded as compliant if both the ‘waking’ sample and the ‘evening’ sample were collected within their established time windows. For the evening sample, participants were coded as compliant if their evening sample was collected within the established time window.

Following Adam et al., (2006), dummy coded compliance variables indicating whether the participant was compliant or noncompliant for the respective cortisol variable were entered into each statistical model as a covariate.

Data Analysis Plan

To ensure that study associations were attributable to cortisol rather than other risk factors for maternal psychopathology or confounds related to cortisol, we first examined associations between cortisol and potential covariates, including demographic (child age, gender, race/ethnicity, maternal age, parental education), health (child health status), and lifestyle (time of waking dairy, caffeine, recent meal intake, parental compliance) variables (Adam & Kumari, 2009). Any significant covariates were included as covariates in all analyses.

Generalized estimating equations (GEE) were conducted to examine main and interactive effects of maternal psychopathology and child temperament on children’s CAR. GEE is a statistical approach that accounts for within-person correlations in time-course data (Liang & Zeger, 1986). Since cortisol was collected across two days, GEE

accounts for within-person correlation between repeated-cortisol measurements. Analyses were conducted in SPSS v. 22 (SPSS Inc., Chicago, IL) with a normal distribution, identity link function, and an unstructured correlation matrix specified. For each GEE model, maternal lifetime depression, maternal lifetime anxiety, child temperamental NE and PE, and their respective cross products (i.e., maternal lifetime depression \times NE, maternal lifetime depression \times PE, maternal lifetime anxiety \times NE, maternal lifetime anxiety \times PE) were entered as independent variables. To examine effects on the CAR, AUC_g and AUC_i were entered as dependent variables. To provide comparison of the CAR to other indicators of HPA axis functioning, cortisol levels at each sampling time (waking, 30, 45 minutes post-waking, and bedtime) and diurnal cortisol slopes were also examined as dependent variables. As exploratory analyses, we also conducted analyses examining the main and interactive effects of maternal current psychopathology, child exposure to maternal depression, and maternal anhedonia symptoms with child temperamental NE and PE on children's CAR to examine the specificity of associations between maternal psychopathology and children's CAR. Lastly, given our focus on investigating the CAR as a potential early emerging vulnerability marker or trait marker, all children included in analyses were free of current depression.

Significant interactions were probed using simple slopes analyses (Aiken & West, 1991). In addition, following Hayes and Matthews' guidelines (2009), parameter values derived from GEE models were used to test regions of significance according to the Johnson-Neyman technique. This approach was used to demonstrate the specific upper and lower values of child temperament (i.e., child NE or PE) at which specific

differences in children's cortisol emerge for children of mothers with and without depression or anxiety.

Chapter 3: Results

Descriptive analyses

Table 1 presents descriptive statistics for cortisol variables. Across the entire sample, cortisol levels showed the expected diurnal pattern: waking values ($M = 7.89$ nmol/L; $SD = 5.35$) increased approximately 29% to reach a peak 30 minutes post-waking ($M = 10.15$ nmol/L; $SD = 5.46$), $t(141) = 5.79$, $p < .001$; declining thereafter to reach lower levels at 45 minutes post waking ($M = 8.02$ nmol/L; $SD = 5.05$), $t(141) = -8.25$, $p < .001$; and lowest levels in the evening ($M = 2.33$ nmol/L; $SD = 4.85$), $t(142) = -24.99$, $p < .001$.

Based on the 1.5 nmol/L criteria for child CAR responder status (Bäumler et al., 2013; Kirschbaum et al., 2013), 101 (71.1%) children were classified as CAR responders on at least one of the two study days. Forty-six children (32.4%) were classified as responders on both sampling days. Fifty-four children (40.0%) changed responder status between both sampling days. On day 1 of sampling, 86 (61.9%) children showed a rise in cortisol levels between the waking and 30-minute post-waking sample; for 74 children, this increase in cortisol was at least 1.5 nmol/L. On day 2 of sampling, 89 children (64.5%) showed a rise in cortisol levels between the waking and 30-minute post-waking sample; for 74 children, this increase was at least 1.5 nmol/L.¹ Across both sampling days, the mean increase from waking to 30 minutes post-waking was 2.17 nmol/L ($SD =$

¹ Given multiple methods of determining CAR responder status, we also computed CAR responder status using the widely used 2.5 nmol/L criteria (derived from research in adults; Wüst et al., 2000b). Results were similar based on this approach: 67 children were classified as CAR responders on Day 1; 67 children were classified as CAR responders on Day 2; 95 (66.9%) were classified as CAR responders on at least one study day; 39 (27.5%) children were classified as responders on both sampling days; 54 (40.0%) children changed responder status between both sampling days.

5.94).

One-hundred-and-ten (79.1%) children showed a positive AUC_i on at least one day. Eighty children (59.7%) showed a positive AUC_i on Day 1; 85 children (64.9%) showed a positive AUC_i on Day 2. For 50 children (39.7%), the AUC_i changed signs between both sampling days. Fifty-five children (39.6%) showed a positive AUC_i on both sampling days. Across both sampling days, the mean AUC_i was 5.05 nmol/L (SD = 18.45).

Pearson correlations were conducted to assess the stability of cortisol levels across the two sampling days. The correlation between day 1 and day 2 waking, 30 and 45 minute post-waking, and evening cortisol were $r = .17$, $r = .45$, $r = .36$, and $r = .58$, respectively (all correlations were significant at $p < .05$). The correlation between day 1 and day 2 AUC_g was $r = .52$, $p < .001$; the correlation between day 1 and day 2 AUC_i was $r = .23$, $p = .01$. The correlation between day 1 and day 2 diurnal cortisol slopes was $r = .42$, $p < .001$. Overall, correlations ranged from $r = .17$ to $r = .58$, indicating moderate stability of cortisol levels across days. In addition, all cortisol variables (waking, 30 and 45 minute post-waking, evening, AUC_g , and AUC_i) were highly interrelated.

Associations among main covariates, independent variables, and dependent variables

Child age was significantly positively associated with child PE. Boys displayed greater levels of PE than girls. Children of parents without a 4-year college degree displayed lower levels of PE, demonstrated greater levels of noncompliant sampling, and were more likely to have a mother with a current depressive disorder or current anxiety disorder. Greater levels of noncompliant sampling were associated with current maternal anxiety disorder status. As expected, maternal depression and anxiety disorder diagnoses

were highly correlated. Maternal current depressive disorder was significantly negatively associated with child PE.

Associations between cortisol and a comprehensive set of potential covariates were also examined (see *Data Analysis Plan*). Cortisol was significantly associated with three variables: children with at least 1 parent with a 4-year college degree evidenced significantly lower evening cortisol on day 1, $t(139) = 3.64, p < .001$, significantly steeper diurnal slope on day 1, $t(134) = 4.35, p < .001$, and significantly higher 30 minute post-waking cortisol on day 2, $t(137) = -2.09, p = .04$. Time of waking was negatively associated with 45 minutes post-waking cortisol on day 1 ($r = -.21, p = .02$), and with 45 minutes post-waking cortisol on day 2 ($r = -.18, p = .04$). Parental noncompliance to cortisol sampling times (0 = compliant, 1 = noncompliant) was significantly positively associated with AUC_g on day 2 ($r = .20, p = .02$). Therefore, parental education, time of waking, and parental noncompliance were included as covariates in all subsequent analyses involving cortisol, with the exception of analyses involving evening cortisol in which time of waking was not included as a covariate.

Maternal depressive and anxiety disorders and child CAR

Maternal lifetime depressive and anxiety disorders and child CAR. No significant main effects of either maternal lifetime depressive or anxiety disorders were observed for waking, 30 and 45-minute post-waking cortisol levels, AUC_g, AUC_i, evening cortisol and the diurnal cortisol slope (Table 2).

Maternal current depressive and anxiety disorders and child CAR. Children of mothers with current depressive disorder (past month) evidenced lower waking cortisol, lower AUC_g, higher AUC_i, and a flatter diurnal cortisol slope than children of mothers

without a current depressive disorder. No significant main effects of current maternal anxiety (past month) were observed (Table 3).

Child temperament and child CAR

The main effects of child temperament on children's CAR are presented in Table 4. Child NE was not significantly associated with waking cortisol, 30 or 45-minute post-waking cortisol, AUC_g, AUC_i, evening cortisol, or the diurnal cortisol slope. Low child PE was significantly associated with elevated waking cortisol, but was not significantly associated with 30 or 45-minute post-waking cortisol, AUC_g, AUC_i, evening cortisol, or the diurnal cortisol slope. No significant interactions between child NE and PE were observed for waking, 30 and 45-minute post-waking cortisol, AUC_g, AUC_i, evening cortisol, or the diurnal cortisol slope.

Full Model I: Maternal lifetime depressive disorder, maternal lifetime anxiety disorder, child temperament, and child CAR

We examined the interactive effects between maternal lifetime depressive disorder, maternal lifetime anxiety disorder, child NE and PE on children's CAR (Table 5). For each GEE model, maternal lifetime depressive disorder, maternal lifetime anxiety disorder, child NE, child PE, and their cross-product were entered as independent variables, and cortisol values at each time point (waking, 30, and 45-minutes post-waking, evening), AUC_g, and AUC_i were included as dependent variables in separate models.

As seen in Table 5, there was a significant interaction between maternal lifetime depression and child NE on children's evening cortisol. Figure 1 shows that for children of mothers with lifetime depression, there was a significant positive association between

child NE and evening cortisol ($B = .26, SE = .08, p = .002$). In contrast, for children of mothers with no history of depression, there was no significant association between child NE and evening cortisol ($B = .02, SE = .05, p = .66$). To examine the degree of child NE at which significant group differences in evening cortisol emerge, Hayes and Matthes' (2009) guidelines were used for testing regions of significance according to the Johnson-Neyman technique. Regions of significance tests indicated that the effect of maternal lifetime depression on children's evening cortisol was significant at both high and low levels of child NE. Specifically, at levels of NE greater than 1.04 (standardized z-score), children of mothers with a lifetime history of depression demonstrated significantly elevated evening cortisol than children of mothers without a lifetime history of depression. In contrast, at levels of NE below $-.82$, children of mothers with a lifetime history of depression demonstrated significantly lower evening cortisol than children of mothers without a lifetime history of depression.

We also observed a significant interaction between maternal lifetime depression and child NE on the diurnal cortisol slope. Figure 2 shows that for children of mothers with a lifetime history of depression there was a significant positive relationship between child NE and the diurnal cortisol slope ($B = .01, SE = .006, p = .03$), whereas for children of mothers without a lifetime history of depression, child NE was not significantly associated with the diurnal cortisol slope ($B = .00, SE = .004, p = .89$). Regions of significance tests indicated that at levels of NE greater than 3.02 (standardized z-score), children of mothers with a lifetime history of depression demonstrated significantly flatter diurnal cortisol slope than children of mothers without a history of depression.² In

² This region of significance should be interpreted with caution given that there were only 2 children in the sample with NE values greater than 3.02.

contrast, at levels of NE below 3.02, children of mothers with or without a history of depression did not differ in diurnal cortisol slope. There were no other significant interactions between maternal lifetime depression and child NE or PE on cortisol levels at waking, 30 or 45-minute post-waking cortisol, AUC_g , or AUC_i .

As seen in Table 5, we also observed a significant interaction between maternal lifetime anxiety disorder and child PE on children's 45-minute post-waking cortisol levels. Figure 3 shows that for children of mothers with lifetime anxiety, there was a significant negative association between child PE and 45-minute post-waking cortisol ($B = -.11$, $SE = .03$, $p = .001$). In contrast, for children of mothers with no lifetime history of anxiety, there was no significant association between child PE and 45-minute post-waking cortisol ($B = -.01$, $SE = .03$, $p = .81$). Regions of significance tests indicated that the effect of maternal lifetime anxiety on children's 45-minute post-waking cortisol was significant at both high and low levels of PE. Specifically, at levels of PE greater than .36 (standardized z-score), children of mothers with a lifetime history of anxiety demonstrated significantly decreased 45-minute post-waking cortisol than children of mothers without a history of anxiety. In contrast, at levels of PE below -2.31 (standardized z-score), children of mothers with a lifetime history of anxiety demonstrated significantly elevated 45-minute post-waking cortisol than children of mothers without a lifetime history of anxiety.³

As seen in Table 5, we also observed a significant interaction between maternal lifetime anxiety and child PE on evening cortisol. Figure 4 shows that for children of mothers with a lifetime history of anxiety, there was a significant negative association

³ This latter region of significance should be interpreted with caution given that there were only 4 children in the sample with PE values greater than -2.31.

between child PE and evening cortisol ($B = -.13$, $SE = .07$, $p = .08$), whereas for children of mothers without a lifetime history of anxiety, child PE was not significantly associated with evening cortisol ($B = -.13$, $SE = .05$, $p = .01$). Regions of significance tests indicated that at levels of PE lower than -1.35 (standardized z-score), children of mothers with a lifetime history of anxiety demonstrated significantly elevated evening cortisol than children of mothers without a history of anxiety. In contrast, at levels of PE above -1.35 (standardized z-score), children of mothers with or without a history of anxiety did not differ in evening cortisol.

We also observed a significant interaction between maternal lifetime anxiety and child PE on the diurnal cortisol slope. Figure 5 shows that for children of mothers with a lifetime history of anxiety, there was a significant negative association between child PE and the diurnal cortisol slope ($B = -.01$, $SE = .0036$, $p = .01$), whereas for children of mothers without a lifetime history of anxiety, child PE was not significantly associated with the diurnal cortisol slope ($B = .01$, $SE = .003$, $p = .08$). Regions of significance tests indicated that at levels of PE lower than $-.42$ (standardized z-score), children of mothers with a lifetime history of anxiety demonstrated a significantly flatter diurnal cortisol slope than children of mothers without a history of anxiety. In contrast, at levels of PE above $-.42$ (standardized z-score), children of mothers with or without a history of anxiety did not differ in diurnal cortisol slope.

There were no other significant interactions between maternal lifetime anxiety and child NE or PE on cortisol levels at waking, 30 minutes post-waking cortisol, AUC_g , or AUC_i (Table 5).

Full Model II: Maternal current depressive disorder, maternal current anxiety disorder, child temperament, and child CAR

The interactive effects between maternal current depression, maternal current anxiety, child NE and PE on children's CAR are presented in Table 6. We observed a significant interaction between maternal current depression and child NE on children's 30-minute post-waking cortisol. Figure 6 shows that for children of mothers with current depression, there was a significant negative association between child NE and 30-minute post-waking cortisol ($B = -.10, SE = .03, p = .004$). In contrast, for children of mothers with no current depression, there was no significant association between child NE and 30-minute post-waking cortisol ($B = .03, SE = .02, p = .24$). Regions of significance tests revealed that at levels of NE greater than 1.57 (standardized z-score), children of mothers with current depression demonstrated significantly lower 30 minute-post waking cortisol than children of mothers without current depression. In contrast, at levels of NE below 1.57, children of mothers with or without current depression did not differ in 30-minute post-waking cortisol. There were no other significant interactions between current maternal depression and child NE or PE on cortisol levels at waking, 45-minute post-waking cortisol, AUC_g , AUC_i , evening cortisol or the diurnal cortisol slope.

As seen in Table 6, we also observed a significant interaction between maternal current anxiety and child NE on children's AUC_g . Figure 7 shows that for children of mothers with current anxiety, there was a significant trend-level negative association between child NE and AUC_g ($B = -6.56, SE = 3.77, p = .08$). In contrast, for children of mothers with no current anxiety, there was no significant association between child NE and AUC_g ($B = 3.19, SE = 2.44, p = .19$). Regions of significance tests indicated that the

effect of maternal current anxiety on children's AUC_g was significant at both high and low levels of NE. Specifically, at levels of NE greater than 2.81 (standardized z-score), children of mothers with current anxiety demonstrated significantly lower AUC_g than children of mothers without current anxiety. In contrast, at levels of NE below -2.54 (standardized z-score), children of mothers with current anxiety demonstrated significantly elevated AUC_g than children of mothers without current anxiety.

We also observed a significant interaction between maternal current anxiety and child NE on children's 45-minute post-waking cortisol. Figure 8 shows that for children of mothers with current anxiety, there was a significant negative association between child NE and 45-minute post-waking cortisol ($B = -.12, SE = .05, p = .02$). In contrast, for children of mothers with no current anxiety, there was no significant association between child NE and 45-minute post-waking cortisol ($B = .04, SE = .02, p = .11$). Regions of significance tests revealed that at levels of NE greater than .25 (standardized z-score), children of mothers with current anxiety demonstrated significantly lower 45-minute post-waking cortisol than children of mothers without current anxiety. In contrast, at levels of NE below .25, children of mothers with or without a current anxiety disorder did not differ in 45-minute post-waking cortisol.

As seen in Table 6, we also observed significant interactions between maternal current anxiety and child NE and between maternal current anxiety and child PE on children's evening cortisol levels. Figure 9 shows that for children of mothers with a current anxiety disorder, child NE was not significantly associated with evening cortisol ($B = -.07, SE = .08, p = .38$). In contrast, for children of mothers without a current anxiety disorder, there was a significant positive association between child NE and evening

cortisol ($B = .14, SE = .05, p = .01$). Regions of significance analyses indicated that the effect of no current maternal anxiety disorder on children's evening cortisol was significant at both high and low levels of NE. Specifically, at levels of NE greater than 2.89 (standardized z-score), children of mothers without a current anxiety disorder demonstrated significantly elevated evening cortisol than children of mothers with a current anxiety disorder. In contrast, at levels of NE less than -1.24 (standardized z-score), children of mothers without a current anxiety disorder demonstrated significantly lower evening cortisol than children of mothers with a current anxiety disorder.

In addition, Figure 10 shows that for children of mothers with a current anxiety disorder, there was a significant negative relationship between child PE and evening cortisol ($B = -.20, SE = .09, p = .02$). In contrast, for children of mothers without a current anxiety disorder, there was no significant association between child PE and evening cortisol ($B = -.002, SE = .03, p = .96$).

Regions of significance analyses indicated that at levels of child PE less than -.71 (standardized z-score), children of mothers with a current anxiety disorder demonstrated significantly elevated evening cortisol than children of mothers without a current anxiety disorder. In contrast, at levels of PE above -.71, children of mothers with or without a current anxiety disorder did not differ in evening cortisol. No other significant interactions between current maternal anxiety and child NE or PE were observed for cortisol levels at waking, 30-minute post-waking cortisol, AUC_i , or the diurnal cortisol slope (Table 6).

Maternal depression exposure, child temperament, and child CAR

We examined the effects of maternal depression exposure on child CAR.

Maternal depression exposure was dummy coded into two groups: 0 = no child exposure to maternal depression and 1 = maternal depression occurring during the child's life. As seen in Table 7, maternal depression exposure was significantly associated with children's elevated evening cortisol and flatter diurnal cortisol slopes.

We next examined the interactive effects between maternal depression exposure and child temperament on children's CAR. As seen in Table 8, there was a significant interaction between maternal depression exposure and child NE on children's evening cortisol. Figure 11 shows that for children who were exposed to maternal depression, child NE was significantly positively associated with evening cortisol ($B = .18$, $SE = .08$, $p = .02$). In contrast, there was no significant association between child NE and children's evening cortisol for children who had no exposure to maternal depression ($B = .01$, $SE = .05$, $p = .85$). Regions of significance tests revealed that at levels of NE greater than .43 (standardized z-score), children who were exposed to depression demonstrated significantly elevated evening cortisol than children who were not exposed to maternal depression. In contrast, at levels of NE below .42, children of mothers who were or were not exposed to depression did not differ in evening cortisol.

There were no other significant interactions between maternal depression exposure and child NE or PE on cortisol levels at waking, 30- or 45-minute post waking cortisol, the diurnal cortisol slope, AUC_g , or AUC_i .

Maternal anhedonia symptoms, child temperament, and child CAR

No significant main effects of maternal anhedonia symptoms were observed for waking, 30 and 45-minute post-waking cortisol, AUC_g , AUC_i , evening cortisol, or the diurnal cortisol slope.

We examined interaction effects between maternal anhedonia symptoms and child temperament on children's CAR. As seen in Table 9, we found a significant interaction between maternal anhedonia symptoms and child PE on AUC_g . Figure 12 shows that for children of mothers with greater anhedonia symptoms, child PE was negatively associated with AUC_g ($B = -4.06$, $SE = 1.44$, $p = .01$), whereas for children of mothers with lower anhedonia symptoms, child PE was not significantly associated with AUC_g ($B = 1.53$, $SE = 1.71$, $p = .37$). Regions of significance tests indicated that the effect of maternal anhedonia symptoms on children's AUC_g was significant at both high and low levels of child PE. Specifically, at levels of child PE greater than .94 (standardized z-score), children of mothers with greater anhedonia symptoms demonstrated significantly lower AUC_g than children of mothers with less anhedonia symptoms. In contrast, at levels of child PE less than -2.37 (standardized z-score), children of mothers with greater anhedonia symptoms demonstrated significantly elevated AUC_g than children of mothers with less anhedonia symptoms.

We also observed a significant interaction between maternal anhedonia symptoms and child PE on AUC_i . Figure 13 shows that for children of mothers with lower levels of anhedonia, child PE was positively associated with AUC_i ($B = 5.13$, $SE = 1.43$, $p < .0001$), whereas for children of mothers with higher levels of anhedonia, child PE was not significantly associated with AUC_i ($B = -.72$, $SE = 1.71$, $p = .67$). Regions of significance

tests indicated that the effect of maternal anhedonia symptoms on children's AUC_i was significant at both high and low levels of child PE. Specifically, at levels of child PE greater than .57 (standardized z-score), children of mothers with less anhedonia symptoms demonstrated significantly increased AUC_i than children of mothers with greater anhedonia symptoms. In contrast, at levels of child PE lower than -1.39 (standardized z-score), children of mothers with less anhedonia symptoms demonstrated significantly decreased AUC_i than children of mothers with greater anhedonia symptoms.

Next, we examined the interaction effects between maternal anhedonia symptoms and child PE on cortisol levels at each sampling time. As seen in Table 9, there were significant interactions between maternal anhedonia symptoms and child PE on both 30 minute and 45-minute post-waking cortisol. Figures 14 and 15 respectively show that for children of mothers with greater anhedonia symptoms, there was a negative relationship between child PE and both 30 minute ($B = -.06, SE = .03, p = .04$) and 45 minute post-waking cortisol ($B = -.05, SE = .03, p = .03$), whereas for children of mothers with lower levels of anhedonia, child PE was not significantly associated with either 30 minute ($B = .04, SE = .03, p = .15$) or 45 minute ($B = .02, SE = .03, p = .62$) post-waking cortisol.

Regions of significance tests indicated that the effect of maternal anhedonia symptoms on children's 30 minute post-waking cortisol was significant at both high and low levels of PE. Specifically, at levels of child PE greater than .74 (standardized z-score), children of mothers with greater anhedonia symptoms demonstrated significantly lower levels of children's 30-minute post-waking cortisol than children of mothers with less anhedonia symptoms. In contrast, at levels of child PE less than -2.43 (standardized z-score), children of mothers with greater anhedonia symptoms demonstrated

significantly increased levels of children's 30-minute post-waking cortisol than children of mothers with less anhedonia symptoms.

Region of significance tests also indicated that at levels of child PE greater than 1.47 (standardized z-score), children of mothers with greater anhedonia symptoms demonstrated significantly lower levels of 45-minute post-waking cortisol than children of mothers with less anhedonia symptoms. In contrast, at levels of PE below 1.47, children did not differ in 45-minute post-waking cortisol.

There were no other significant interactions between maternal anhedonia symptoms and child NE or PE on cortisol levels at waking, evening cortisol, or the diurnal cortisol slope.

Chapter 4: Discussion

We examined the main and interactive effects between two prominent risk factors for depression, maternal psychopathology and early child temperamental emotionality, on children's cortisol awakening response (CAR; examined as AUC_g (the total volume of cortisol secreted across waking) or AUC_i (the total increase in cortisol across waking)). We found evidence for the presence of a CAR in children ranging from age 3 to 5 years. No main or interactive effects were observed for maternal lifetime psychopathology or child temperamental high NE and/or low PE on children's CAR, as indicated by AUC_g or AUC_i . Instead, associations with children's CAR appeared to be specific to maternal current psychopathology and symptoms of anhedonia (as indicated by AUC_g or AUC_i). Specifically, maternal current depression was associated with offspring's higher AUC_i and lower AUC_g . Moreover, the combination of maternal anhedonia and child temperamental PE was associated with abnormalities in children's AUC_g and AUC_i . We

also observed significant interactions between maternal lifetime and current depression and anxiety disorders and child temperamental emotionality on elevated evening cortisol levels and flattened diurnal cortisol rhythms, indicating altered patterns of basal cortisol activity in offspring. Interestingly, across analyses, effects of maternal psychopathology on children's HPA axis functioning were associated with high *and* low levels of child temperamental emotionality, highlighting the importance of a dimensional approach to developmental psychopathology. To our knowledge, this is the first study to investigate the association between the CAR and depression risk in early childhood, a period with important implications for prevention and intervention. Moreover, as limited work to date has examined the CAR specifically in preschool age children, our study contributes to the limited but growing knowledge on the development of the CAR in preschool age children and as a marker of early risk. Overall, our findings suggest that there is a complex interplay between familial risk, affective vulnerability, and neuroendocrine dysfunction in young children, and highlight the need for future research to examine which aspects of the early diurnal rhythm predict the emergence of later depressive illness.

Children's cortisol awakening response

Using a rigorous methodological approach including repeated cortisol samples over two days, person-specific wake times, and stringent controls for noncompliance, we observed the presence of a CAR in children ranging from age 3 to 5 years. Studies examining the CAR in preschool age children are rare (e.g., Bäumlner et al., 2013; Gribben, Watamura, Cairns, Harsh, & LeBourgeois, 2012), and our study is among the first to demonstrate its existence in this age range. To facilitate comparison with previous studies, we not only examined the total increase in cortisol *across* waking (i.e, AUC_i), but

also the change in cortisol from waking to 30-minutes post-waking, a frequently used measure of the CAR. We observed a positive AUC_i on at least one of the two study days in 110 (79.1%) children. In addition, we observed a significant increase of at least 1.5 nmol/L (CAR “responder” classification; Bäumlner et al., 2013; Miller, Plessow, Kirschbaum, & Stalder, 2013), from waking to 30-minutes post-waking on at least one of the two study days in 101 (71.1%) children. Our findings are consistent with previous literature documenting the presence of the CAR in preschool age children (Bäumlner et al., 2013; Gribben et al., 2012). These findings are also consistent with emerging literature documenting the presence of the CAR in infants and toddlers (Bäumlner et al., 2013; Stalder et al., 2013) and provide support that the CAR is present very early in human development, continuing throughout middle childhood (e.g., Michels et al., 2011; Rosmalen et al., 2005) into adulthood.

Previous research in adults has documented relatively consistent mean increases from waking to 30 minutes post-waking between studies (9.3 nmol/L; for a review see Clow et al., 2004). In our sample of 3 to 5 year old children, we found that the mean increase from waking to 30 minutes post-waking was 2.17 nmol/L (SD = 5.94). Overall, our findings are consistent with previous evidence suggesting that the CAR is smaller in children than in adults (e.g., Gribben et al., 2012; Pruessner et al., 1997; Rosmalen et al., 2005). However, values of the CAR (as assessed by the increase from waking to 30 minutes post-waking) documented in the child literature vary widely. For example, Rosmalen and colleagues (2005) reported a mean value of 3.8 nmol/L in a sample of 10-12 year old children. Gribben and colleagues (2012) reported a mean value of 5.0 nmol/L in a sample of 2 to 4 year old children. Bäumlner and colleagues (2012) reported a mean

value of 9.6 nmol/L in 3 to 4 year old children, and 7.90 nmol/L in 4 to 5 year old children. These inconsistent findings underscore the importance of future research examining developmental differences in the CAR, and the potential age or methodological factors that may contribute to such differences.

Consistent with other studies examining the CAR in children (e.g., Rosmalen et al., 2005), 33 (22.6%) children in our sample were classified as CAR “non-responders” (<1.5 nmol/L) on both days of sampling. On one hand, it is possible that there are true CAR nonresponders. For example, Dockray and colleagues (2008) observed a small proportion of adult participants who demonstrated compliance with the sampling procedure, yet showed no increase in cortisol over 30 minutes after waking. On the other hand, it is possible that CAR nonresponders result from noncompliant sampling. This may be particularly heightened in studies of young children, as they are not yet old enough to collect their own samples. For example, parents may not adhere to the cortisol sampling time protocol or may be inaccurate reporters of sampling time. Children may also wake up before their parents or experience transient awakenings making it difficult to determine wake time, thus impacting CAR measurement. We incorporated electronic monitoring devices in the sample to address this methodological issue. Nevertheless, such devices are not without limitation. Given that little work to date has examined CAR non-responsiveness, it will be important for future work to investigate the factors that contribute to the lack of a CAR.

Main effects on children’s CAR

We examined the main effects of maternal psychopathology and child temperament on children’s stress system functioning. Maternal lifetime depressive or

anxiety disorders were not significantly associated with children's cortisol. However, children of mothers with current depression demonstrated elevated CAR, as indicated by children's lower cortisol levels at waking, a lower total volume of cortisol (AUC_g), and a greater and more dynamic *increase* in cortisol (AUC_i), as well as flatter diurnal cortisol slopes, in comparison to children of mothers with no current depression. Furthermore, the relation between maternal current depression and children's HPA axis functioning persisted after controlling for maternal current anxiety. These findings are consistent with prior research linking elevated average cortisol, elevated bedtime cortisol, the slope of the diurnal rhythm (Dahl et al., 1991) and the CAR (Vreeburg et al., 2009) to individuals with current depression. Our findings also extend previous research documenting elevated morning cortisol levels among the offspring of currently depressed parents (Young et al., 2006), and support the significance of contextual factors (e.g., exposure to chronic stress, or the environmental stress of having a depressed parent) on children's developing stress physiology. However, our findings are the first, to our knowledge, to show that maternal current depression is associated specifically with elevated CAR and flattened diurnal cortisol slopes in offspring. This is particularly noteworthy, as rigorous methodological research provides compelling evidence that the CAR is a strong prospective predictor of major depressive disorder (Adam et al., 2010; Vrshek-Schallhorn et al., 2013) and anxiety (Adam et al., 2014), and as flatter diurnal cortisol slopes have been linked to extreme or chronic stress exposure (Gunnar & Vazquez, 2001) and negative health outcomes in adults, including cardiovascular risk (Rosmond & Bjorntorp, 2000) and mortality (Kumari, Shipley, Stafford, & Kivimaki, 2011). The observed differences in children's CAR and diurnal slopes may reflect early-emerging

vulnerability for depression; these results point to the importance of a better understanding of the mechanisms by which alteration in these indices confer risk for future psychopathology.

We also found that child PE was uniquely associated with children's waking cortisol. We did not find significant main effects for child NE on children's cortisol. Child low PE was significantly associated with elevated waking cortisol, but was not significantly associated with any other cortisol indices. Furthermore, the relation between low PE and waking cortisol persisted even after controlling for child temperamental NE, time of waking, parental education, and noncompliance. Our findings are consistent with prior research demonstrating associations between PE and morning cortisol in children prior to any depressive illness (Dougherty et al., 2009; 2013), and extend the broader literature linking low PE to other established indices of depression risk (Durbin et al., 2005; Hayden, Klein, Durbin, & Olino, 2006; Shankman et al., 2005). Our findings are also noteworthy as elevated waking cortisol has been found to predict the onset of depression (Goodyer et al., 2000; Halligan et al., 2004, 2007; Harris et al., 2000). Taken together, while our hypothesis that child low PE would be uniquely associated with elevated CAR was not supported by the data, our findings nonetheless suggest that the link between low PE and depression risk may be partially mediated by abnormalities in children's stress physiology and regulation. Specifically, children with lower levels of PE may experience increased stress sensitivity and reduced reward, rendering them more susceptible to the depressogenic effects of stress, which in turn may influence their neuroendocrine functioning and contribute to the development of depression over time. Alternatively, low PE may interact with the genetic or environmental stress of having a

depressed parent, to impact neuroendocrine functioning and risk for depression, as discussed in the sections below.

Interactive effects of maternal lifetime depression and child temperament on children's CAR

Contrary to hypotheses, we did not observe significant associations between maternal lifetime depression and child NE or PE on children's CAR, as indicated by AUC_g or AUC_i . However, we observed a significant interaction between maternal lifetime depression and child NE on children's evening cortisol. Interestingly, the effect of maternal lifetime depression on children's evening cortisol was significant at both high and low levels of child NE. Specifically, children of mothers with a lifetime history of depression and who exhibited *lower* levels of NE demonstrated lower evening cortisol. In contrast, children of mothers with a lifetime history of depression and who exhibited *higher* levels of NE demonstrated elevated evening cortisol levels, in turn contributing to a flattened diurnal slope. These findings are consistent with prior research in adults (Adam et al., 2006; DeSantis, Adam, Doane, Mineka, Zinbarg, & Craske, 2007) and young children (Dougherty et al., 2013), and are particularly striking as abnormalities in evening cortisol have been associated with current depression in youths (Goodyer et al., 1996) and have been found to predict a more chronic course of depression in adolescents (Goodyer, Park, & Herbert, 2001). Moreover, flattening of the expected diurnal rhythm has been linked to exposure to adverse and challenging conditions and to poor health outcomes in children (Gunnar & Vazquez, 2001).

Our findings suggest that increasing levels of child NE are related to increasing evening cortisol levels in the offspring of depressed mothers. Prior studies have linked

same-day higher levels of negative emotional states such as tension and anger to same-day elevated evening cortisol and flattened diurnal slopes in adults (Adam et al., 2006). Moreover, evening cortisol is highly influenced by socio-environmental contributions and state-dependent factors (Bartels, de Geus, Kirschbaum, Sluyter, & Boomsma, 2003), in contrast to the CAR, which has a greater genetic component (Wüst et al., 2000a). Thus, it is possible that children with higher levels of negative emotionality may experience greater difficulty developing adaptive ways to regulate stress responses and are consequently more susceptible to negative emotion and the stress and challenges of the day (such as those associated with having a depressed parent) (Goodman & Gotlib, 1999), contributing to elevated bedtime cortisol and a flatter diurnal rhythm.

We had hypothesized that maternal lifetime depression would interact with child low PE. However, we did not observe any significant interactions between maternal lifetime depression and child PE; instead, interactive effects were specific to child NE. Our findings are in contrast to previous studies reporting significant interactions between maternal lifetime depression and child PE on morning cortisol (Dougherty et al., 2013) and cortisol reactivity (Mackrell et al., 2014) in children. It is possible that our nonsignificant findings are related to differences in sampling (e.g., clinical versus community sample), composition of the sample (e.g., age, race/ethnicity), in comparison to previous studies. Alternatively, our differences in findings may be due in part to the interactions involving maternal anhedonia (a key feature of depression), described in the section below.

Indeed, increasing research highlights the importance of considering specific features of depression with regard to neuroendocrine function (e.g., Cizza et al., 2012;

Gold & Chrousos, 2002; Gold & Chrousos, 2013; Lamers et al., 2013). Moreover, there has been increased scientific attention to reducing the etiological and pathophysiological complexity of depression into endophenotypes (i.e. an internal, intermediate phenotype that represents more direct expressions of underlying genes) (Gottesman & Gould, 2003), as a strategy to better understand the genetic and neurobiological basis of depression. One of the most promising endophenotypes of depression is anhedonia, a key feature of depression involving the diminished capacity to experience pleasure. Importantly, anhedonia has been particularly linked to abnormalities in cortisol functioning (Stetler & Miller, 2011), even among depressed preschool-age children (Luby, Mrakotsky, Heffelfinger, Brown, & Spitznagel, 2004). For example, research has found that up to 80% of individuals with melancholic features of depression, characterized by anhedonia, demonstrated hypercortisolemia and disruptions in stress system functioning (Contreras et al., 2007). Thus, the current literature highlights the importance of examining anhedonia in relation to neuroendocrine functioning and underscores the importance of future developmental neuroendocrine studies incorporating endophenotypic features of depression.

Interactive effects of maternal lifetime anxiety and child temperament on children's CAR

Although depressive and anxiety disorders share many features and are highly comorbid (Mineka, Watson & Clark, 1998), they are rarely examined together in relation to cortisol, as we have done in the current study. We examined the interactive effects of maternal lifetime anxiety and child temperamental negative and positive emotionality on children's CAR. Controlling for maternal depression, we did not observe a significant association between maternal lifetime anxiety and child NE or PE on children's CAR.

However, we found that the children of mothers with a lifetime history of anxiety who also exhibited *lower* levels of PE demonstrated a flatter diurnal cortisol slope, driven by elevated evening cortisol levels. In contrast, we found that children of mothers with a lifetime history of anxiety who also exhibited *higher* levels of PE demonstrated lower 45-minute post-waking cortisol. These effects remained even after controlling for child NE, suggesting that PE may have distinctive effects on cortisol.

Interestingly, low levels of PE have been linked to social anxiety disorder (Brown, Chorpita, & Barlow, 1998; Mineka et al., 1998). For example, Brown and colleagues (1998) found that low PE uniquely characterized both depression and social anxiety disorder, distinguishing them from other anxiety disorders. Theorists posit that these relations may reflect the interpersonal nature of PE (Clark, Watson & Mineka, 1994). Indeed, low PE is characterized by low levels of social interactions and appetitive/motivated behavior when interacting with people and stimuli in the environment (Laptook et al., 2008). Importantly, low PE is closely related to high behavioral inhibition (BI), which is characterized by wary, hesitant, fearful behavior in unfamiliar contexts and withdrawal from social interaction (Kagan, 1997), and which is strongly associated with risk for anxiety disorders (Degnan, Almas, & Fox, 2010). Although low PE and high BI are distinct constructs, both are characterized by low approach in novel situations (Laptook et al., 2008; Laptook, Klein, Olino, Dyson, & Carlson, 2010). As our measure of low PE included sociability, it is possible that it tapped underlying aspects of shyness and withdrawal-related behavior that are linked to BI and risk for anxiety. Unfortunately, BI was not assessed in this study. Further work is

needed examining the main and interactive effects of PE and BI on children's neuroendocrine functioning and risk for the later development of anxiety disorders.

Taken together, as we also observed that children of mothers with a lifetime history of anxiety who also exhibited *higher* levels of PE demonstrated lower morning cortisol, our data suggest that higher levels of PE can protect children from elevated cortisol levels and neuroendocrine dysfunction. Our findings are consistent with previous research highlighting the role of positive emotions in buffering against the adverse effects of stress. Higher levels of positive emotions have been found to attenuate/diminish stress-induced cardiovascular reactivity (Fredrickson & Levenson, 1998), and have also been linked to generally lower levels of cortisol output over the day, independent of gender, age, socioeconomic status, body mass index (BMI), and psychological distress (Steptoe et al., 2005; Steptow & Wardle, 2005). Thus, it appears that the *decreased* ability to generate positive emotions may contribute to loss of resilience, in turn contributing to greater stress sensitivity and increasing risk for psychopathology. Indeed, lower levels of PE have been linked to increased cortisol reactivity among children of depressed mothers (Mackrell et al., 2014).

To our knowledge, this study provides the first evidence of the moderating role of child PE on associations between maternal anxiety and child stress physiology, and underscores the importance of examining maternal anxiety in addition to maternal depression history. Our data are consistent with emerging research supporting associations between maternal anxiety and offspring HPA axis abnormalities. For example, a few studies have linked prenatal maternal anxiety to flatter diurnal cortisol slopes in adolescents (O'Donnell et al., 2013; Van den Bergh, Van Calster, Smits, Van

Huffel, & Lagae, 2008). Importantly, diurnal cortisol slopes have also been found to mediate the relationship between prenatal maternal anxiety and depressive symptoms in female adolescents (Van den Bergh et al., 2008). Nevertheless, research remains limited and inconsistent, given that other studies have also linked maternal anxiety to elevated waking cortisol (O'Connor et al., 2005; Dougherty et al., 2013) and lower CAR (O'Donnell et al., 2013) in adolescent offspring, and to elevated CAR in adult offspring (Vreeburg et al., 2010), and as no previous studies have examined moderating factors. Thus, further work examining the mechanisms by which maternal anxiety is related to abnormalities in offspring stress physiology and regulation is warranted, as mechanisms likely involve a complex, transactional interplay between parent, child, genetic, and socio-environmental factors.

Interactive effects of current maternal psychopathology and child temperament on children's CAR

Few studies have examined whether abnormalities in HPA axis functioning in children of depressed mothers result from a familial risk for depression or exposure to maternal depression and its associated effects on environmental stress and parenting (e.g., Dougherty et al., 2013; Halligan et al., 2004; Mannie et al., 2007). Thus, we examined the interactive effects of current maternal psychopathology and child temperament on children's stress physiology. In addition, we examined the interactive effects of timing of children's exposure to maternal depression (see section below).

We found that the combination of maternal current depression and child temperamental high NE was associated with lower morning cortisol. Specifically, for children of mothers with current depression, child high NE was associated with lower 30-

minute post-waking cortisol, whereas for children of mothers with no current depression, there was no significant association between child NE and 30-minute post-waking cortisol. Similarly, we also observed a significant interaction between maternal current anxiety and child high NE to predict lower 45 minute post-waking cortisol. While we did not observe interactive effects between exposure to current maternal depression and child high NE or low PE specific to children's CAR as we had hypothesized, these findings are nonetheless of interest as lower/blunted levels of morning cortisol have been associated with risk, including externalizing problems in children (for a review see Alink et al., 2008), stress-related disorders (e.g., fibromyalgia, chronic fatigue) (Heim, Ehlert, & Hellhammer, 2000) and depression in adults (Bockting et al., 2012), as well as conditions of chronic exposure to stress (for a review, see Gunnar & Vazquez, 2001). Children high in NE have been found to be prone to anger and behavior problems (Eisenburg et al., 2009). Thus, parenting a child high in NE may be challenging, particularly to mothers with current depression or anxiety, potentially contributing to negative, harsh, or overcontrolling parenting strategies, in turn worsening conflict in the parent-child relationship. These interactions may perpetuate heightened family stress and a chronically stressful environment, contributing over time to blunted cortisol levels and HPA axis dysfunction.

Interestingly, although literature remains limited, our findings are broadly consistent with previous studies suggesting similar patterns of association for both maternal current depression and anxiety on children's morning cortisol. O'Donnell and colleagues (2013) found that the offspring of mothers with a prenatal history of anxiety evidenced a significantly elevated CAR and flatter diurnal slope in adolescence, and

similar effects on offspring cortisol were observed for prenatal maternal depression. Vreeburg and colleagues (2010) found that the adult offspring of parents with either a history of depression or anxiety demonstrated a significantly elevated CAR compared to the offspring of healthy controls. It is possible that the lower levels of morning cortisol we observed among children of mothers with depression *and* anxiety reflect a shared genetic liability or underlying pathophysiological mechanism, or are due to exposure to maternal depression or anxiety and common associated disruptions in parenting and life stress (Feldman et al., 2009; Spence et al., 2002). Child temperamental emotionality, genetic risk, parenting, and child neuroendocrine functioning may therefore reflect interrelated risk factors, all of which contribute to increased risk for depression or anxiety; further investigation into the mechanisms underlying the transmission of risk are needed.

We also found that the combination of maternal current anxiety and child low PE was associated with elevated evening cortisol levels; this effect was not present for children of mothers without current anxiety. Interestingly, we also observed that children of mothers *without* current anxiety exhibited elevated evening cortisol among children with high NE, and lower evening cortisol among children with low NE. Collectively, these findings suggest that associations between environmental stressors or demands and evening cortisol may be closely tied to temperamental emotionality, particularly low PE and high NE, though it appears that for low PE it is the *combination* with maternal current anxiety that impacts offspring evening cortisol. Given that higher levels of positive emotionality help to buffer against the negative effects of stress (Tugade & Fredrickson, 2004), our findings suggest that children *low* in temperamental PE may

experience greater difficulty coping to the stress associated with having an anxious parent, the impact of which may increase risk for HPA axis dysfunction and contribute to elevated evening cortisol, particularly as evening cortisol is highly influenced by socio-environmental context (Bartels et al., 2003).

Interactive effects of exposure to maternal depression and child temperament on children's CAR

We examined whether associations we observed between children's cortisol functioning and maternal lifetime depression varied specifically as a function of exposure to maternal depression. In testing the specificity of these associations, we distinguished between children with no exposure to maternal depression (i.e., no history of maternal depression or maternal history of depression prior to the child's lifetime) and mothers with a history of depression during the child's lifetime. We found that the moderating effect of maternal lifetime depression on the association between child high NE and elevated evening cortisol (see previous section) was specific to maternal depression occurring during the child's life. In contrast, no significant associations were observed for children with no exposure to maternal depression. Our findings are consistent with previous literature linking exposure to maternal depression during the child's life to increased cortisol reactivity (Dougherty et al., 2011, 2013; Ashman et al., 2002), and elevated basal cortisol (Halligan et al., 2004) in offspring. Our results also highlight the significance of the contextual, environmental adversity associated with having a depressed parent, such as problematic parenting, on children's developing stress physiology and regulation. Indeed, maternal depression has been associated with hostile parenting behaviors (Lovejoy, Graczyk, O'Hare, & Neuman, 2000), which in turn have

been strongly associated with disturbances in children's neuroendocrine functioning (Gunnar & Vazquez 2006). Interestingly, there is also considerable evidence of the critical and enduring nature of early environmental experiences on children's neuroendocrine function. For example, research in the animal literature has documented strong epigenetic effects of maternal caregiving behavior occurring during the neonatal period on subsequent stress physiology and regulation in offspring (Meaney, 2001). Ultimately, as our study was cross-sectional, we cannot test the causality or directionality of the associations observed; several possible explanations may exist, though it is notable that effects may likely be bidirectional. For example, children who are prone to negative emotions may experience significant difficulty coping with the stress associated with having a depressed parent, which in turn may impact their neuroendocrine functioning; alternatively, the environmental stress of having a depressed parent may also *evoke* children's negative emotions, further contributing to an environment of heightened family stress, leading to neuroendocrine disturbances as evidenced by elevated evening cortisol. Taken together, it appears that children who are exposed to maternal depression *and* who are temperamentally prone to negative affect, are at greatest risk for HPA axis dysfunction, and possibly in turn, depression.

Interactive effects of maternal anhedonia and child temperament on children's CAR

We found that that the combination of maternal anhedonia and child temperamental PE was associated with children's CAR. Moreover, the effect of maternal anhedonia on children's CAR emerged at both high and low levels of child PE: at lower levels of child PE, children of mothers with high anhedonia demonstrated elevated CAR, as indicated by elevated total cortisol output (AUC_g), and elevated levels of 30 and 45-

minute post-waking cortisol; in contrast, at higher levels of child PE, children of mothers with high anhedonia demonstrated lower CAR, as indicated by lower AUC_g, and lower levels of 30 and 45-minute post-waking cortisol. Our findings are consistent with previous research demonstrating associations between maternal melancholic depression, characterized by anhedonia, and low child PE on children's morning cortisol (Dougherty et al., 2009). However, to our knowledge, this study is the first to demonstrate associations between maternal anhedonia and child PE on the CAR, which is considered to be a more sensitive predictor of depression (Adam et al., 2010) and anxiety (Adam et al., 2014).

Our findings are noteworthy as anhedonia is characterized by low PE and is a cardinal feature of depression. Moreover, anhedonia has been associated with reduced reward experience, hypercortisolemia, and increased sensitivity to stress (for a review see Pizzagalli, 2014). Thus, as genetic factors have been found to influence the CAR (Wüst et al., 2000a), it is also possible that mothers with high levels of anhedonia transmit genetic predisposition towards heightened stress sensitivity and deficits in reward sensitivity and the experience of pleasure, as evidenced by the relation we observed between child low PE and elevated CAR. Alternatively, as evidence suggests that positive emotions buffer against the negative effects of stress (Tugade & Fredrickson, 2004), it is possible that children low in temperamental PE demonstrate less resilience and greater sensitivity to the depressogenic effects of stress, as evidenced by elevated CAR. Conversely, higher levels of temperamental PE may serve a protective function against depression risk, as evidenced by lower CAR.

Surprisingly, we also observed a significant association between child PE and elevated CAR for children of mothers with low anhedonia, significant at both high and low levels of child PE: at higher levels of child PE, children of mothers with low anhedonia demonstrated elevated CAR, as indicated by elevated AUC_i . In contrast, at lower levels of child PE, children of mothers with low anhedonia demonstrated decreased CAR as indicated by lower AUC_i . These data were unexpected, but are in line with research documenting complex effects of depressive severity on cortisol levels. For example, a few studies have documented “inverted U-shaped” associations between dimensional measures of anhedonic depression (i.e., a more severe melancholic depression) and the CAR in adults, with low and high levels of anhedonia linked to blunted CAR, and intermediate levels of anhedonia linked to elevated CAR (Veen et al., 2011, Wardenaar et al., 2011). Moreover, differences in cortisol were only observed when examining dimensional measures of anhedonic depression and were not observed when examining DSM-IV categorical diagnoses. Previous studies have also documented an inverted U-shaped association between cortisol and depressive symptoms in samples of elderly adults, with low and high levels of depressive symptoms linked to hypocortisolism and intermediate levels linked to hypercortisolism (Bremmer et al., 2007; Penninx et al., 2007). These particular studies suggest that within a subset of depressive individuals, there may be initial hyperactivity of the HPA axis in response to depressive or anhedonic symptoms, with increasing severity and disease progression leading to downregulation of the HPA axis and blunted cortisol levels over time (Heim et al., 2000). Although we did not observe a U-shaped association between maternal anhedonia and children’s CAR, our data similarly demonstrated complex effects of

dimensional constructs of maternal anhedonia and child PE on levels of cortisol in children, underscoring the importance of a dimensional approach to examining depression. Furthermore, it is possible that if the severity of maternal anhedonia and levels of child PE remain low and stable over time, that we would observe a blunted CAR later in development as the HPA axis is downregulated. Future studies of this nature incorporating longitudinal designs and examining how risk for psychopathology covaries with depression severity or abnormalities/extremes in positive emotion are needed.

Specificity of children's CAR

Overall, although both maternal depression and anxiety were associated with abnormalities in indices of offspring diurnal cortisol, associations with the CAR, as indicated by AUC_g or AUC_i , appeared to be specific to depression risk. Nevertheless, it is noteworthy that we also observed an interactive effect between maternal current anxiety and child NE on AUC_g , which was significant at the trend-level. Moreover, this association remained even after controlling for maternal current depression. It is possible that the limited number of mothers with current psychopathology reduced power in this analysis. Our findings are nonetheless noteworthy given emerging research in older adolescents and adults providing evidence that the CAR may be a vulnerability marker for the development of depressive *and* anxiety disorders (Adam et al., 2014; Vreeburg et al., 2010; Vrshek-Schallhorn et al., 2013). It will be important for future studies to utilize prospective, longitudinal designs to examine the mechanisms and predictors of the CAR in relation to risk for depression or anxiety in order to better delineate developmental neuroendocrine pathways to the development of psychopathology.

It is also noteworthy that we observed significant associations between prominent, well-established risk factors for depression (i.e., maternal psychopathology and child temperament) and *other* indices of diurnal cortisol (i.e., the diurnal slope and evening cortisol levels). In other words, associations with depression risk did not appear to be specific to the CAR. This finding is in contrast to previous studies in adults in which the CAR, but not other aspects of diurnal cortisol, predicted depressive (Adam et al., 2010; Vrshek-Schallhorn et al., 2013) and anxiety disorders (Adam et al., 2014). However, it is noteworthy that the current study examined the CAR in a sample of young children, whereas previous studies examined the CAR in samples of older adolescents and adults. Perhaps the CAR is a non-specific vulnerability marker in early childhood, developing over time to a more specific marker of risk in adolescents and adults. Future studies tracing the development of the CAR are needed to gain a better understanding of the developmental pathways to depression and may facilitate much needed early intervention and prevention efforts.

Study strengths and limitations

This study had several strengths. First, we collected multiple morning cortisol samples yoked to person-specific wake times on each day, over two days, allowing us to specifically examine the cortisol awakening response, and increasing the reliability of measurement. Second, we also included several cortisol indices including the diurnal cortisol slope and evening cortisol, allowing us to better determine specificity of associations to the CAR. Third, we excluded any child with a current depressive disorder from the sample and controlled for demographic and sampling related covariates, including compliance to sampling time. Fourth, child temperament was assessed using a

comprehensive, standardized laboratory observation measure. Fifth, maternal psychiatric history was assessed using a well-validated semi-structured diagnostic interview by an expert diagnostician, with attention as to whether or not the child was exposed to maternal depression.

Sixth, we included an examination of both maternal depression and anxiety in our study, allowing us to examine their specific associations with offspring cortisol. Seventh, our measures of maternal psychopathology, child temperament, and stress system functioning were incorporated from multiple methods, including semi-structured diagnostic interviews, laboratory observations, and salivary cortisol, respectively, minimizing bias from shared method variance. Eighth, we had sufficient power to examine maternal psychopathology by child temperament interactions on children's CAR, which has not been done in previous research. Ninth, the children in our sample were preschool-age, which is prior to the risk period for depression, allowing us to examine HPA axis functioning in at-risk individuals likely *prior* to the onset of a subsequent disorder. Tenth, consistent with recent reviews highlighting the benefits of examining endophenotypes and dimensional concepts of psychopathology (for reviews, see Goldstein & Klein, 2014; Miller & Rockstroh, 2013) and in line with research domain criteria (RDoC), we incorporated continuous of maternal anhedonia, a key feature of depression, and child temperamental emotionality.

This study also had limitations. First, the design was cross-sectional; thus, we are unable to infer causality or directionality. Long-term follow up is necessary to determine whether maternal psychopathology, child temperament and changes in HPA axis functioning predict depression across development. Second, laboratory observations of

child temperament were limited to a single occasion and setting, which preclude the assessment of traits across different contexts and time. Nevertheless, evidence suggests that laboratory observations of temperament are associated with home observations of temperament and demonstrate moderate stability over time (Durbin et al., 2007). Future studies should incorporate multi-method assessments of temperament including multiple informant reports and observational measures. Third, there is evidence to support complex interactive relations between children's negative and positive emotionality (Olinio et al., 2010); however, we did not examine any three-way interactions between child NE, PE and maternal psychopathology due to a lack of statistical power. Similarly, although there is evidence to support the influence of socio-contextual factors such as parenting and life stress on children's stress system functioning (Gunnar & Donzella, 2002), we did not examine how these factors may interact with maternal psychopathology or child temperamental vulnerabilities. Future studies examining the complex and dynamic interplay of these factors on the stability and change in children's HPA axis abnormalities are needed. Fourth, we used an outdated version of the PPVT (PPVT-III; Dunn & Dunn, 1997). Fifth, we focused our analyses exclusively on mothers, as our data on fathers was limited. Maternal depression is more strongly associated with child internalizing problems than paternal depression, especially in samples of younger children (Connell & Goodman, 2002). Nevertheless, future studies incorporating fathers are needed as it would allow the examination of not only dyadic processes (i.e., mother-child, father-child), but also triadic processes (i.e., mother-father-child) to determine whether parent psychopathology differentially moderates children's stress system functioning. For example, while fathers with depression may compound an adverse

rearing environment, healthy fathers may conversely provide a protective functioning in the context of a depressed mother (Goodman et al., 2011). Sixth, we did not assess timing of maternal anxiety in our study. Given that evidence has linked exposure to maternal anxiety to disruptions in offspring morning cortisol (e.g., O'Connor et al., 2005; O'Donnell et al., 2013), future studies examining the influence of exposure to maternal anxiety on children's CAR are needed.

Conclusion

In closing, we found that the CAR is related to well-established, prominent vulnerability markers for depression in preschool-age children, suggesting that it may be an early emerging vulnerability marker for depression, though the factors and underlying processes involved are likely complex. With regard to implications for future research, further studies with attention to course, severity, features, and timing of exposure to maternal depression and anxiety, are needed to better delineate neuroendocrine pathways to depression. Our findings also suggest that abnormalities in children's neuroendocrine functioning may be linked to risk for both depression and anxiety. These shared abnormalities in offspring cortisol may be due to common disruptions in parenting, exposure to stressful environments, or may reflect shared etiological vulnerability such as genetic factors (Kendler, Gardner, Gatz, & Pedersen, 2007); these factors may differentially impact HPA axis functioning, and potentially contribute to current inconsistencies in the literature. Future research incorporating prospective designs and examining the effects of both maternal depression and anxiety and potential moderating and mediating influences on offspring cortisol over the course of development is needed.

Our findings also hold implications for prevention and intervention. For instance, stress-reduction programs aimed at youth may decrease risk for depression or anxiety. Indeed, Lupien and colleagues (2013) implemented a school-based stress reduction program, finding it to be effective at reducing cortisol levels and depressive symptomatology in adolescents. Continued intervention research incorporating assessment of psychiatric outcomes and home cortisol collection (e.g., Dozier, Peloso, Lewis, Laurenceau, & Levine, 2008; Fisher, Gunnar, Dozier, Bruce, & Pears, 2006) would help test the feasibility of modifying the CAR through behavioral interventions. Notably, Adam and colleagues (2008) suggest that the reduction of CAR or cortisol should be pursued with caution, as hypocortisolism has been associated with increased risk for physical and mental health disorder, such as burnout (Pruessner, Hellhammer, & Kirschbaum, 1999), chronic pain (Geiss, Varadi, Steinbach, Bauer, & Anton, 1997), sleep disturbances (Backhaus, Junghanns, & Hohagen, 2004), and chronic fatigue syndrome (Roberts, Wessely, Chalder, Papadopoulos, & Cleare, 2004). Thus, interventions that reduce the negative consequences of elevated CAR while still maintaining its adaptive function to daily challenges would be ideal. Lastly, as the level at which cortisol increases risk for depression or anxiety is currently unknown, future research should examine this issue to help shed light into the clinical utility of the CAR as a vulnerability marker.

Table 1.

Demographic and clinical characteristics of the study sample (N = 146)

Demographic Variable	% (N)	M (SD)	Min	Max
Child age: years		4.14 (.81)	3.00	5.92
Mother age: years		34.94 (6.26)	21.00	50.00
Father age: years		37.26 (6.69)	20.00	54.00
Child gender: female	51.4 (75)			
Child race				
White/ European-American	49.3 (71)			
Black/African American	34.0 (49)			
Asian	1.4 (2)			
Other	15.3 (22)			
Child Ethnicity				
Hispanic/Latino descent	17.6 (25)			
Parents' marital status				
Married	68.5 (100)			
Divorced, separated, or widowed	8.2 (12)			
Never married	23.3 (34)			
Parental education: ≥ 1 parent college graduate	70.5 (103)			
Income				
< \$20,000	7.1 (10)			
\$20,001 – \$40,000	9.3 (13)			
\$40,001 – \$70,000	19.3 (27)			
\$70,001 – \$100,000	30.0 (42)			
>\$100,000	34.3 (48)			
<i>Maternal psychopathology</i>				
Maternal lifetime depressive disorder	49.3 (71)			

Maternal current depressive disorder	8.4 (12)		
Maternal depression exposure	30.6 (44)		
Maternal anhedonia symptoms		48.58 (7.00)	14.00 56.00
Maternal lifetime anxiety disorder	45.5 (65)		
Maternal current anxiety disorder	20.3 (29)		
<i>Child temperament</i>			
Negative emotionality		0.00 (1.00)	-1.44 3.09
Positive emotionality		0.00 (1.00)	-2.52 2.57
<i>Salivary cortisol indicators</i>			
Time of waking (h), Day 1		7:25 (0:59)	4:28 12:45
Time of waking (h), Day 2		7:24 (1:05)	4:46 11:48
Bedtime (h), Day 1		20:45 (2:44)	19:00 0:00
Bedtime (h), Day 2		20:43 (2:45)	19:00 1:00
Waking cortisol values (nmol/L), Day 1		7.72 (5.18)	.12 31.57
Waking cortisol values (nmol/L), Day 2		8.06 (5.54)	.22 32.36
30 min post-waking cortisol values (nmol/L), Day 1		10.12 (5.79)	.88 31.02
30 min post-waking cortisol values (nmol/L), Day 2		10.18 (5.12)	.46 25.72
45 min post-waking cortisol values (nmol/L), Day 1		8.10 (5.14)	.15 32.36
45 min post-waking cortisol values (nmol/L), Day 2		7.94 (4.96)	.38 32.90
Evening cortisol values (nmol/L), Day 1		2.14 (4.33)	.14 26.59
Evening cortisol values (nmol/L), Day 2		2.53 (5.34)	.13 31.04
Diurnal cortisol slope (nmol/L per hour), Day 1		-46 (.42)	-2.12 1.38
Diurnal cortisol slope (nmol/L per hour), Day 2		-.44 (.50)	-1.83 1.98
AUC _g (nmol/L), Day 1		40.72	6.78 108.0
		(19.49)	2
AUC _g (nmol/L), Day 2		43.74	1.70 150.6
		(23.48)	6
AUC _i (nmol/L), Day 1		5.20 (18.90)	-44.53 58.30

AUC _i (nmol/L), Day 2	4.91 (18.01)	-43.76	63.38
AUC _i positive, Day 1	59.7 (80)		
AUC _i positive, Day 2	64.9 (85)		

Note. Categorical variables are presented as frequency and percentage; continuous variables are presented as mean and standard deviation. Temperament variables are presented as z-scores. Cortisol values reflect raw values for ease of interpretation and are presented in nmol/L. Area under the curve (AUC) was measured with respect to ground (AUC_g) and increase (AUC_i).

Table 2.

Generalized estimating equations: Main effects of maternal lifetime depression and maternal lifetime anxiety on child salivary cortisol

	Waking cortisol		30 min post-waking cortisol		45 min post-waking cortisol		Evening cortisol		Diurnal cortisol slope		AUC _g		AUC _i	
	<i>n</i> = 139	<i>n</i> = 138	<i>n</i> = 139	<i>n</i> = 138	<i>n</i> = 139	<i>n</i> = 138	<i>n</i> = 139	<i>n</i> = 138	<i>n</i> = 139	<i>n</i> = 137	<i>n</i> = 137	<i>n</i> = 139	<i>n</i> = 137	<i>n</i> = 137
	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>
Parental Education	.06 (.04)	.15	.07 (.05)	.18	.02 (.05)	.75	-.19 (.08)	.02*	-.02 (.01)	.001**	2.61 (3.72)	.48	-2.76 (3.05)	.37
Time of Waking	-.02 (.02)	.47	-.04 (.02)	.09	-.07 (.02)	.001	—	—	-.004 (.03)	.15	-1.02 (1.99)	.61	-2.63 (1.45)	.07
Parental Noncompliance	.10 (.05)	.03*	.001 (.05)	.99	-.02 (.04)	.65	.05 (.10)	.63	-.01 (.01)	.45	6.03 (3.18)	.06	-2.00 (2.73)	.46
Maternal Lifetime Depression	-.05 (.05)	.25	-.04 (.04)	.39	.03 (.05)	.54	.04 (.09)	.63	.01 (.01)	.46	-3.63 (3.43)	.29	.79 (2.69)	.77
Maternal Lifetime Anxiety	-.02 (.05)	.71	-.02 (.04)	.70	-.08 (.05)	.09	.04 (.09)	.62	.01 (.01)	.44	-2.21 (3.42)	.52	-.39 (2.60)	.88

Note. **p* < .05; ***p* < .01. AUC_g = area under the curve with respect to ground; AUC_i = area under the curve with respect to increase.

Table 3.

Generalized estimating equations: Main effects of maternal current depression and maternal current anxiety on child salivary cortisol

	Waking cortisol <i>n</i> = 139		30 min post-waking cortisol <i>n</i> = 138		45 min post-waking cortisol <i>n</i> = 139		Evening cortisol <i>n</i> = 138		Diurnal cortisol slope <i>n</i> = 139		AUC _g <i>n</i> = 137		AUC _i <i>n</i> = 137	
	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>
Parental Education	.05 (.05)	.31	.07 (.05)	.20	.002 (.05)	.97	-.15 (.08)	.06	-.02 (.01)	.01*	2.21 (3.76)	.56	-1.85 (3.05)	.54
Time of Waking	-.01 (.02)	.51	-.04 (.02)	.09	-.07 (.02)	.001**	—	—	-.004 (.003)	.12	-.10 (1.97)	.65	-2/70 (1.42)	.06
Parental Noncompliance	.10 (.05)	.04*	.001 (.05)	.98	-.02 (.04)	.56	.04 (.10)	.73	-.01 (.01)	.40	6.03 (3.17)	.06	-2.25 (2.76)	.41
Maternal Current Depression	-.13 (.06)	.03*	-.01 (.05)	.77	-.10 (.08)	.90	.22 (.13)	.09	.02 (.01)	.02*	-9.20 (3.61)	.01*	5.68 (2.77)	.04*
Maternal Current Anxiety	-.04 (.06)	.55	-.02 (.05)	.77	-.08 (.06)	.21	.12 (.10)	.24	.01 (.01)	.12	.52 (4.50)	.91	2.30 (3.20)	.47

Note. **p* < .05; ***p* < .01. AUC_g = area under the curve with respect to ground; AUC_i = area under the curve with respect to increase.

Table 4.

Generalized estimating equations: Main and interactive effects of child temperament on child salivary cortisol

Dependent Variable	NE		PE		NE × PE	
	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>
<i>Salivary cortisol</i>						
Waking	-.02 (.04)	.60	-.04 (.02)	.03*	-.01 (.02)	.77
30 min post-waking	-.01 (.03)	.83	-.01 (.02)	.64	.01 (.02)	.56
45 min post-waking	-.01 (.04)	.83	-.02 (.03)	.39	-.01 (.02)	.79
Evening	.11 (.07)	.13	-.04 (.04)	.36	.02 (.04)	.63
Diurnal cortisol slope	.01 (.01)	.11	.002 (.003)	.49	.002 (.003)	.15
AUC _g	.54 (3.18)	.87	-1.08 (1.50)	.47	.01 (1.27)	.99
AUC _i	-.97 (1.86)	.60	2.24 (1.56)	.15	-.22 (1.56)	.89

Note. * $p < .05$. All generalized estimating equation models controlled for parental education, time of waking, and parental compliance, with the exception of analyses involving evening cortisol in which time of waking was not included as a covariate. NE = Negative Emotionality; PE = Positive Emotionality. AUC_g = area under the curve with respect to ground; AUC_i = area under the curve with respect to increase.

Table 5.

Generalized estimating equations: The interactive effects of maternal lifetime depression, maternal lifetime anxiety, and child temperament on child salivary cortisol

	Waking cortisol		30 min post-waking cortisol		45 min post-waking cortisol		Evening cortisol		Diurnal cortisol slope		AUC _g		AUC _i	
	<i>n</i> = 139		<i>n</i> = 138		<i>n</i> = 139		<i>n</i> = 138		<i>n</i> = 139		<i>n</i> = 137		<i>n</i> = 137	
	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>
Parental Education	.07 (.05)	.18	.08 (.06)	.13	.03 (.06)	.62	-.18 (.08)	.03*	-.02 (.01)	.003**	3.10 (4.14)	.45	-3.88 (3.67)	.29
Time of Waking	-.01 (.02)	.56	-.04 (.02)	.12	-.06 (.02)	.005**	—	—	-.003	.31	-.95 (2.05)	.64	-1.85 (1.39)	.18
Parental Noncompliance	.10 (.05)	.05	-.002 (.05)	.97	-.02 (.04)	.54	.04 (.11)	.72	-.004 (.01)	.53	5.88 (3.20)	.07	-2.10 (2.66)	.43
Maternal Lifetime Depression	-.07 (.04)	.09	-.05 (.04)	.22	-.01 (.04)	.90	-.01 (.08)	.93	.003 (.006)	.58	-4.72 (3.29)	.15	.22 (2.63)	.93
Maternal Lifetime Anxiety	-.01 (.04)	.89	-.01 (.04)	.87	-.05 (.04)	.22	.09 (.08)	.29	.01 (.01)	.25	-1.23 (3.29)	.73	-.02 (2.58)	.99
Child NE	-.002 (.03)	.96	.03 (.03)	.24	.02 (.03)	.61	.02 (.05)	.73	.001 (.004)	.89	.33 (2.39)	.15	1.19 (2.40)	.62
Child PE	-.03 (.02)	.14	-.01 (.03)	.63	-.01 (.03)	.81	.02 (.05)	.70	.01 (.003)	.08	-2.09 (1.83)	.25	1.81 (2.03)	.37
Maternal Lifetime Depression × Child NE	.03 (.06)	.61	-.03 (.05)	.61	.05 (.05)	.27	.24 (.08)	.004*	.01 (.006)	.046*	3.79 (4.63)	.41	.44 (2.37)	.85
Maternal Lifetime Depression × Child PE	-.03 (.04)	.46	.05 (.03)	.18	.07 (.04)	.07	-.01 (.07)	.91	.003 (.01)	.65	1.74 (2.49)	.49	3.46 (2.46)	.16
Maternal Lifetime Anxiety × Child NE	-.05 (.06)	.43	-.05 (.05)	.34	-.09 (.05)	.07	-.10 (.08)	.25	-.001 (.01)	.88	-2.64 (4.26)	.54	-4.43 (2.51)	.08

Maternal Lifetime	.00 (.04)	.99	-.05 (.05)	.34	-.11 (.06)	.01*	-.15 (.07)	.04*	-.01 (.01)	.02*	.85 (2.51)	.74	-2.81 (2.46)	.25
Anxiety × Child PE														

Note. * $p < .05$; ** $p < .01$. AUC_g = area under the curve with respect to ground; AUC_i = area under the curve with respect to increase.

Table 6.

Generalized estimating equations: The interactive effects of maternal current depression, maternal current anxiety, and child temperament on child salivary cortisol

	Waking cortisol <i>n</i> = 139		30 min post-waking cortisol <i>n</i> = 138		45 min post-waking cortisol <i>n</i> = 139		Evening cortisol <i>n</i> = 138		Diurnal cortisol slope <i>n</i> = 139		AUC _g <i>n</i> = 137		AUC _i <i>n</i> = 137	
	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>
Parental Education	.05 (.05)	.27	.08 (.05)	.17	.01 (.05)	.83	-.13 (.08)	.11	-.02 (.01)	.56	2.68 (3.90)	.49	-3.08 (3.40)	.37
Time of Waking	-.01 (.02)	.56	-.04 (.02)	.14	-.07 (.02)	.004	—	—	-.01 (.003)	.06	-.73 (2.07)	.73	-2.14 (1.34)	.11
Parental Noncompliance	.10 (.05)	.04*	.08 (.05)	.17	-.03 (.04)	.42	.04 (.10)	.73	-.004 (.01)	.56	5.29 (3.11)	.09	-2.56 (2.70)	.34
Maternal Current Depression	-.13 (.10)	.19	-.03 (.06)	.61	-.10 (.16)	.53	.42 (.19)	.03*	.04 (.01)	.003	-12.71 (4.88)	.01	4.63 (4.57)	.31
Maternal Current Anxiety	-.03 (.05)	.54	-.003 (.05)	.95	-.07 (.06)	.24	.09 (.10)	.36	.01 (.008)	.17	1.67 (4.72)	.72	3.16 (3.47)	.36
Child NE	.01 (.02)	.59	.03 (.02)	.24	.04 (.02)	.11	.14 (.05)	.01*	.01 (.003)	.02*	3.19 (2.44)	.19	.52 (1.60)	.75
Child PE	-.04 (.02)	.04*	-.01 (.02)	.72	-.01 (.02)	.81	-.002 (.03)	.96	.004 (.003)	.10	-2.01 (1.29)	.12	2.73 (1.55)	.08
Maternal Current Depression × Child NE	.01 (.09)	.90	-.10 (.05)	.046	-.15 (.10)	.12	-.10 (.16)	.54	-.01 (.01)	.29	-7.19 (5.34)	.18	-4.29 (3.39)	.21
Maternal Current Depression × Child PE	.04 (.07)	.58	-.004 (.06)	.95	-.10 (.05)	.02	.30 (.19)	.11	.02 (.01)	.15	-2.24 (4.87)	.65	-2.38 (4.52)	.60

Maternal Current Anxiety × Child NE	-.11 (.07)	.11	-.10 (.06)	.10	-.13 (.06)	.02*	-.21 (.09)	.03*	-.01 (.01)	.61	-9.04 (4.30)	.04*	-3.56 (2.64)	.18
Maternal Current Anxiety × Child PE	-.05 (.05)	.29	-.04 (.04)	.43	-.09 (.05)	.07	-.20 (.09)	.03*	-.01 (.01)	.10	1.10 (3.90)	.49	-1.21 (3.10)	.70

Note. * $p < .05$; ** $p < .01$. AUC_g = area under the curve with respect to ground; AUC_i = area under the curve with respect to increase.

Table 7.

Generalized estimating equations: Main effects of maternal depression exposure and maternal anhedonia symptoms on child salivary cortisol

Dependent Variable	Maternal Depression Exposure		Maternal Anhedonia Symptoms	
	B (SE)	<i>p</i>	B (SE)	<i>p</i>
<i>Salivary cortisol</i>				
Waking	-.03 (.04)	.51	.0001 (.002)	.80
30 min post-waking cortisol	-.05 (.05)	.27	-.004 (.003)	.12
45 min post-waking cortisol	-.02 (.04)	.73	-.002 (.003)	.40
Evening	.22 (.09)	.02*	.01 (.01)	.07
Diurnal cortisol slope	.02 (.07)	.03*	.001 (.0004)	.07
AUC _g	-3.29 (3.84)	.39	-.15 (.22)	.50
AUC _i	-1.12 (2.60)	.67	-.16 (.16)	.31

Note. **p* < .05. All generalized estimating equation models controlled for parental education, time of waking, and parental compliance, with the exception of analyses involving evening cortisol in which time of waking was not included as a covariate. Maternal Depression Exposure = maternal depression occurring during the child's life (*n*=44); AUC_g = area under the curve with respect to ground; AUC_i = area under the curve with respect to increase.

Table 8.

Generalized estimating equations: The interactive effects between maternal depression exposure and child temperament on child salivary cortisol

	Waking Cortisol <i>n</i> = 140		30 min post-waking cortisol <i>n</i> = 139		45 min post-waking cortisol <i>n</i> = 140		Evening cortisol <i>n</i> = 139		Diurnal cortisol slope <i>n</i> = 140		AUC _g <i>n</i> = 137		AUC _i <i>n</i> = 137	
	B (SE)	<i>P</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>
Parental Education	.08 (.05)	.09	.09 (.05)	.10	.03 (.05)	.62	-.15 (.08)	.06	-.02 (.003)	.20	3.65 (4.02)	.36	-3.81 (3.44)	.27
Time of Waking	-.02 (.02)	.34	-.04 (.02)	.05	-.07 (.02)	.001**	—	—	-.004 (.003)	.20	-1.29 (2.03)	.52	-2.42 (1.34)	.07
Parental Noncompliance	.09 (.05)	.04*	-.01 (.05)	.80	-.03 (.04)	.40	.02 (.10)	.82	-.003 (.01)	.59	5.85 (3.22)	.07	-2.22 (2.74)	.42
Maternal Depression Exposure	-.05 (.04)	.26	-.04 (.05)	.44	-.03 (.05)	.55	.15 (.09)	.10	.1 (.01)	.09	-4.72 (3.74)	.21	.99 (2.86)	.73
Child NE	-.01 (.03)	.69	.02 (.02)	.38	-.01 (.03)	.71	.01 (.05)	.85	.002 (.004)	.69	.27 (1.82)	.88	-.32 (1.67)	.85
Child PE	-.05 (.02)	.01*	-.03 (.02)	.17	-.03 (.03)	.22	-.03 (.04)	.54	.003 (.003)	.34	-2.53 (1.35)	.06	1.76 (1.62)	.28
Maternal Dep Exposure × Child NE	.02 (.07)	.81	-.06 (.06)	.35	.04 (.06)	.55	.18 (.09)	.048*	.01 (.007)	.12	2.61 (5.72)	.65	-1.65 (2.41)	.50
Maternal Dep Exposure × Child PE	.02 (.04)	.59	.06 (.04)	.19	.02 (.05)	.71	-.02 (.04)	.54	-.001 (.007)	.85	1.82 (3.40)	.59	1.98 (2.85)	.49

Note. **p* < .05; ***p* < .01. AUC_g = area under the curve with respect to ground; AUC_i = area under the curve with respect to increase.

Table 9.

Generalized estimating equations: The interactive effects between maternal anhedonia symptoms and child temperament on child salivary cortisol

	Waking cortisol <i>n</i> = 137		30 min post-waking cortisol <i>n</i> = 136		45 min post-waking cortisol <i>n</i> = 137		Evening cortisol <i>n</i> = 136		Diurnal cortisol slope <i>n</i> = 137		AUC _g <i>n</i> = 134		AUC _i <i>n</i> = 134	
	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>	B (SE)	<i>p</i>
Parental Education	.07 (.05)	.17	.06 (.05)	.25	.02 (.05)	.78	-.14 (.09)	.12	-.02 (.01)	.01*	2.77 (4.09)	.50	-4.22 (3.32)	.20
Time of Waking	-.01 (.02)	.65	-.03 (.02)	.14	-.07 (.02)	.001	—	—	-.01 (.003)	.07	-.68 (2.08)	.74	-2.34 (1.30)	.07
Parental Noncompliance	.09 (.05)	.05	-.01 (.05)	.81	-.03 (.04)	.44	.02 (.10)	.86	-.01 (.01)	.43	6.65 (3.29)	.04*	-1.44 (2.83)	.61
Maternal Anhedonia	.004 (.02)	.84	-.02 (.02)	.28	-.01 (.02)	.57	.05 (.04)	.15	.01 (.002)	.10	-.74 (1.53)	.63	-.46 (1.09)	.67
Child NE	-.02 (.03)	.53	-.004 (.02)	.88	.001 (.03)	.98	.08 (.05)	.12	.01 (.003)	.07	.51 (2.28)	.82	-.36 (1.25)	.77
Child PE	-.04 (.02)	.02*	-.01 (.02)	.61	-.02 (.02)	.37	-.04 (.03)	.26	.001 (.003)	.58	-1.26 (1.22)	.30	2.20 (1.32)	.10
Maternal Anhedonia × Child NE	.01 (.02)	.82	-.01 (.02)	.77	.01 (.02)	.62	-.04 (.05)	.48	-.003 (.00)	.39	.38 (2.27)	.87	.61 (1.39)	.66
Maternal Anhedonia × Child PE	-.01 (.01)	.64	-.05 (.02)	.01*	-.04 (.02)	.047*	-.01 (.03)	.71	.00 (.00)	.88	-2.80 (1.01)	.01*	-2.93 (.86)	.001**

Note. **p* < .05; ***p* < .01. Moderator = Maternal Anhedonia. AUC_g = area under the curve with respect to ground; AUC_i = area under the curve with respect to increase.

Figure Captions

Figure 1. Children's Evening Cortisol as a Function of Maternal Lifetime

Depression and Child Negative Emotionality. The line on the X axis at 1.04, indicates the value of negative emotionality (standardized z-score) at and above which offspring of mothers with and without lifetime depression differ significantly in terms of evening cortisol levels. The line on the X axis at -.82, indicates the value of negative emotionality (standardized z-score) at and below which offspring of mothers with and without lifetime depression differ significantly in terms of evening cortisol levels. Bars reflect standard errors of measurement.

Figure 2. Children's Diurnal Cortisol Slope as a Function of Maternal Lifetime

Depression and Child Negative Emotionality. The line on the X axis at 3.02, indicates the value of negative emotionality (standardized z-score) at and above which offspring of mothers with and without lifetime depression differ significantly in terms of the diurnal cortisol slope. Bars reflect standard errors of measurement.

Figure 3. Children's 45-Minute Post-Waking Cortisol as a Function of Maternal

Lifetime Anxiety and Child Positive Emotionality. The line on the X axis at .36, indicates the value of positive emotionality (standardized z-score) at and above which offspring of mothers with and without lifetime anxiety differ significantly in terms of 45-minute post-waking cortisol levels. The line on the X axis at -2.31, indicates the value of positive emotionality (standardized z-score) at and below which offspring of mothers with and without lifetime anxiety differ significantly in terms of 45-minute post-waking cortisol levels. Bars reflect standard errors of measurement.

Figure 4. Children's Evening Cortisol as a Function of Maternal Lifetime Anxiety and Child Positive Emotionality. The line on the X axis at -1.35, indicates the value of positive emotionality (standardized z-score) at and below which offspring of mothers with and without lifetime anxiety differ significantly in terms of evening cortisol levels. Bars reflect standard errors of measurement.

Figure 5. Children's Diurnal Cortisol Slope as a Function of Maternal Lifetime Anxiety and Child Positive Emotionality. The line on the X axis at -.42, indicates the value of positive emotionality (standardized z-score) at and below which offspring of mothers with and without lifetime anxiety differ significantly in terms of evening cortisol levels. Bars reflect standard errors of measurement.

Figure 6. Children's 30-Minute Post-Waking Cortisol as a Function of Maternal Current Depression and Child Negative Emotionality. The line on the X axis at 1.57, indicates the value of negative emotionality (standardized z-score) at and above which offspring of mothers with and without current depression differ significantly in terms of 30-minute post-waking cortisol. Bars reflect standard errors of measurement.

Figure 7. Children's Total Cortisol Output as a Function of Maternal Current Anxiety and Child Negative Emotionality. The line on the X axis at 2.81 indicates the value of positive emotionality (standardized z-score) at and above which offspring of mothers with and without current anxiety differ significantly at the trend-level in terms of total cortisol output (AUC_g). The line on the X axis at -2.54 indicates the value of positive emotionality (standardized z-score) at and below which offspring of mothers with and

without current anxiety differ significantly at the trend-level in terms of total cortisol output (AUC_g). Bars reflect standard errors of measurement.

Figure 8. Children's 45-Minute Post-Waking Cortisol as a Function of Maternal Current Anxiety and Child Negative Emotionality. The line on the X axis at .25, indicates the value of negative emotionality (standardized z-score) at and above which offspring of mothers with and without current anxiety differ significantly in terms of 45-minute post-waking cortisol. Bars reflect standard errors of measurement.

Figure 9. Children's Evening Cortisol as a Function of Maternal Current Anxiety and Child Negative Emotionality. The line on the X axis at 2.89 indicates the value of negative emotionality (standardized z-score) at and above which offspring of mothers with and without current anxiety differ significantly in terms of evening cortisol. The line on the X axis at -1.24 indicates the value of positive emotionality (standardized z-score) at and below which offspring of mothers with and without current anxiety differ significantly in terms of evening cortisol. Bars reflect standard errors of measurement.

Figure 10. Children's Evening Cortisol as a Function of Maternal Current Anxiety and Child Positive Emotionality. The line on the X axis at -.71, indicates the value of positive emotionality (standardized z-score) at and below which offspring of mothers with and without current anxiety differ significantly in terms of evening cortisol levels. Bars reflect standard errors of measurement.

Figure 11. Children's Evening Cortisol as a Function of Maternal Depression Exposure and Child Negative Emotionality. Bars reflect standard errors of measurement.

Figure 12. Children's Total Cortisol Output as a Function of Maternal Anhedonia and Child Positive Emotionality. The line on the X axis at .94 indicates the value of

positive emotionality (standardized z-score) at and above which offspring of mothers with high or low anhedonia differ significantly in terms of total cortisol output (AUC_g). The line on the X axis at -2.37, indicates the value of positive emotionality (standardized z-score) at and below which offspring of mothers with high or low anhedonia differ significantly in terms of total cortisol output (AUC_g). Bars reflect standard errors of measurement.

Figure 13. Children's Total Change in Cortisol as a Function of Maternal Anhedonia and Child Positive Emotionality. The line on the X axis at .57 indicates the value of positive emotionality (standardized z-score) at and above which offspring of mothers with high or low anhedonia differ significantly in terms of total increase in cortisol (AUC_i). The line on the X axis at -1.39, indicates the value of positive emotionality (standardized z-score) at and below which offspring of mothers with high or low anhedonia differ significantly in terms of total increase in cortisol (AUC_i). Bars reflect standard errors of measurement.

Figure 14. Children's 30-Minute Post-Waking Cortisol as a Function of Maternal Anhedonia and Child Positive Emotionality. The line on the X axis at .74 indicates the value of positive emotionality (standardized z-score) at and above which offspring of mothers with high or low anhedonia differ significantly in terms of 30-minute post-waking cortisol. The line on the X axis at -2.43, indicates the value of positive emotionality (standardized z-score) at and below which offspring of mothers with high or low anhedonia differ significantly in terms of 30-minute post-waking cortisol. Bars reflect standard errors of measurement.

Figure 15. Children's 45-Minute Post-Waking Cortisol as a Function of Maternal Anhedonia and Child Positive Emotionality. The line on the *X* axis at 1.47 indicates the value of positive emotionality (standardized *z*-score) at and above which offspring of mothers with high or low anhedonia differ significantly in terms of 45-minute post-waking cortisol. Bars reflect standard errors of measurement.

Figure 1.

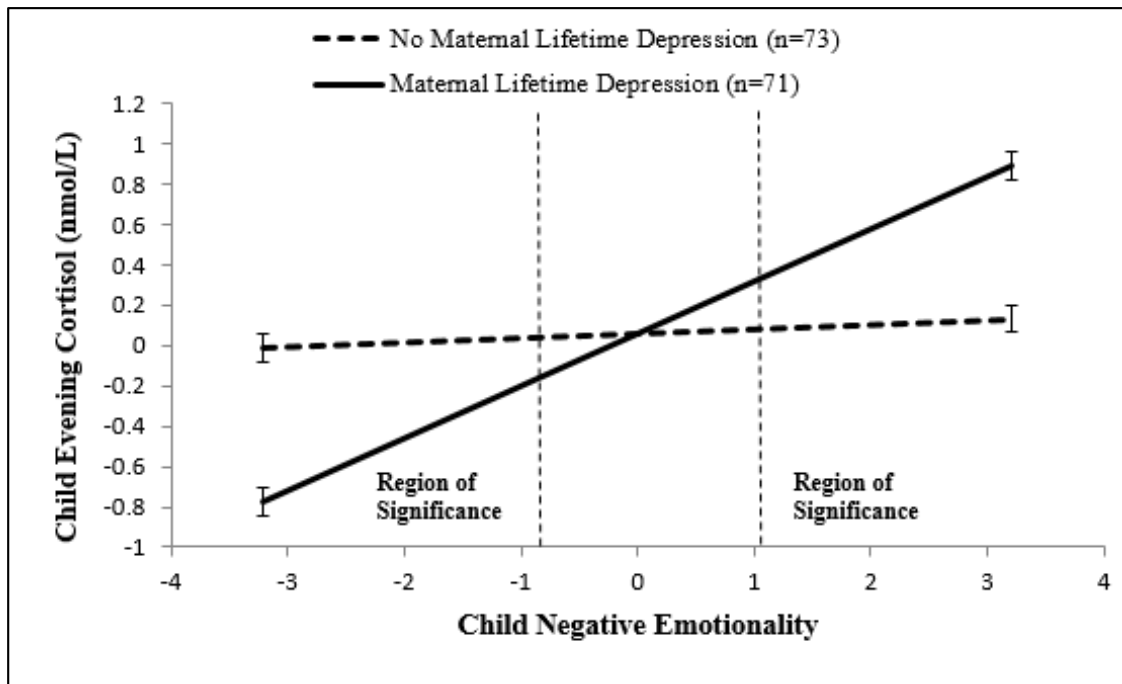


Figure 2.

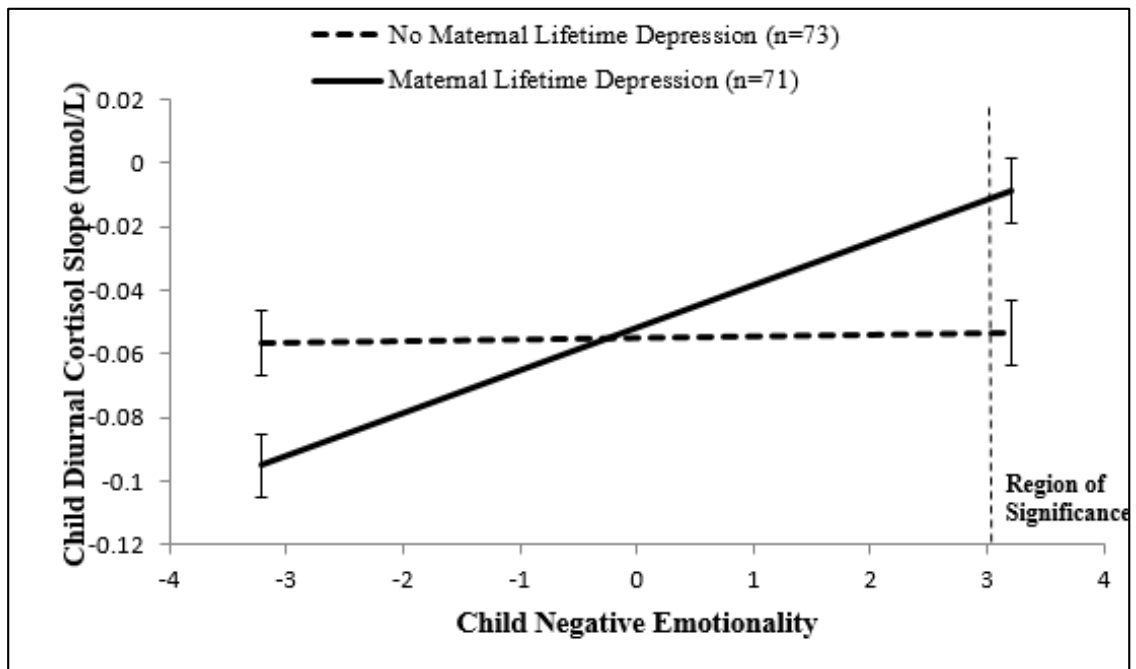


Figure 3.

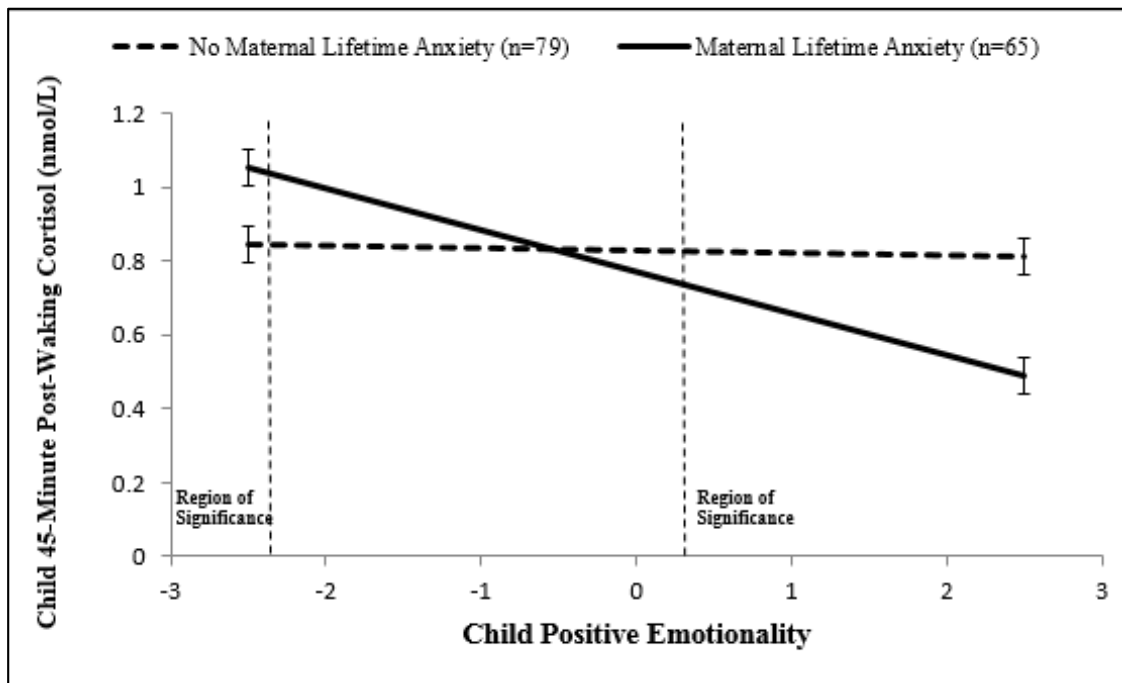


Figure 4.

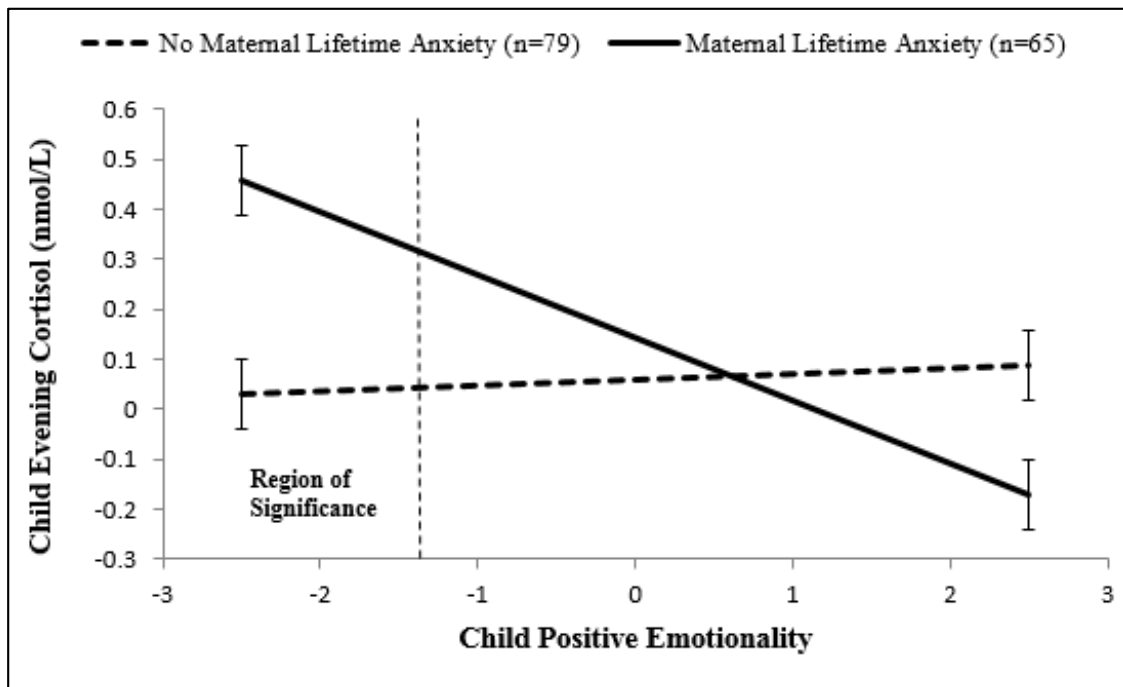


Figure 5.

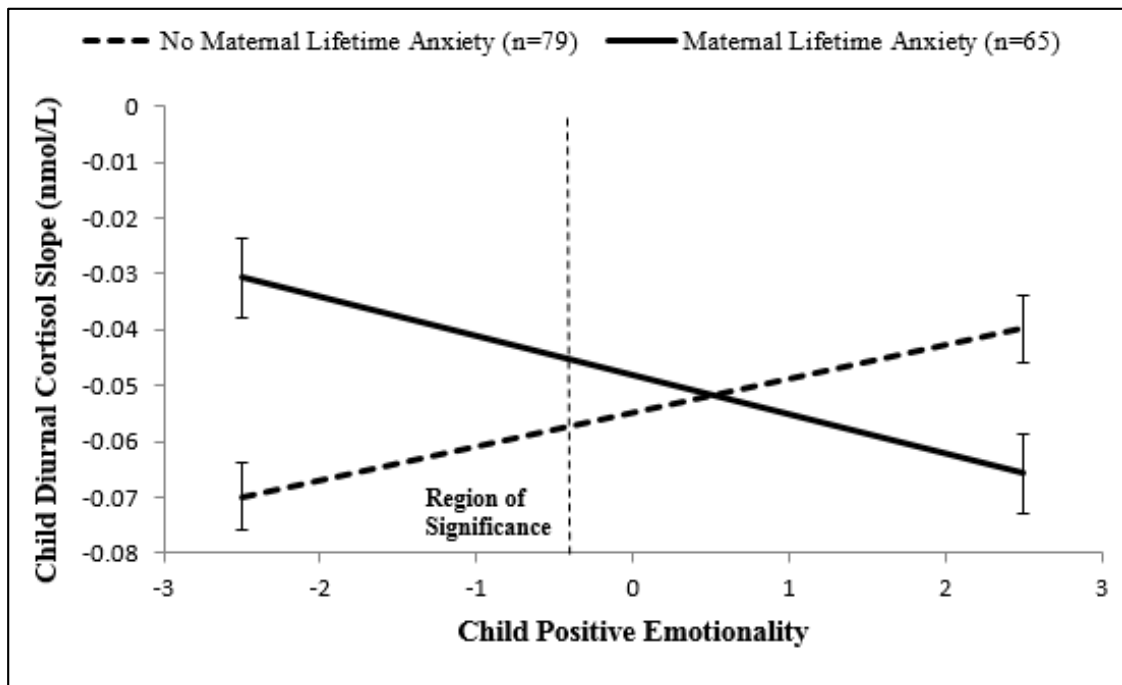


Figure 6.

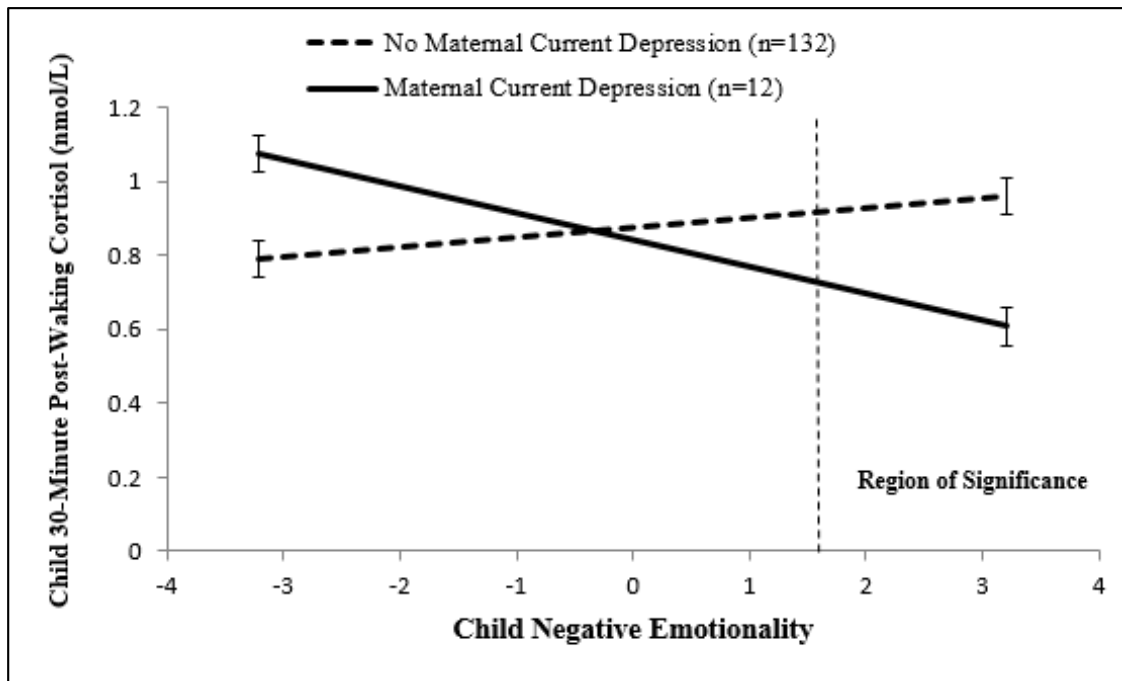


Figure 7.

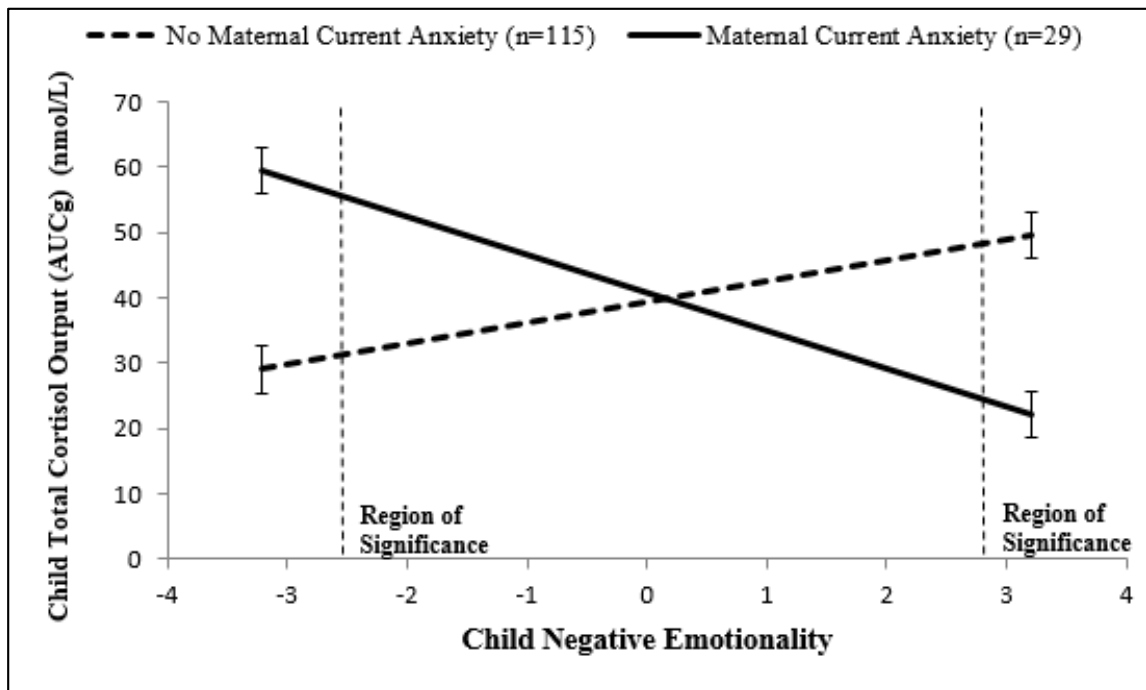


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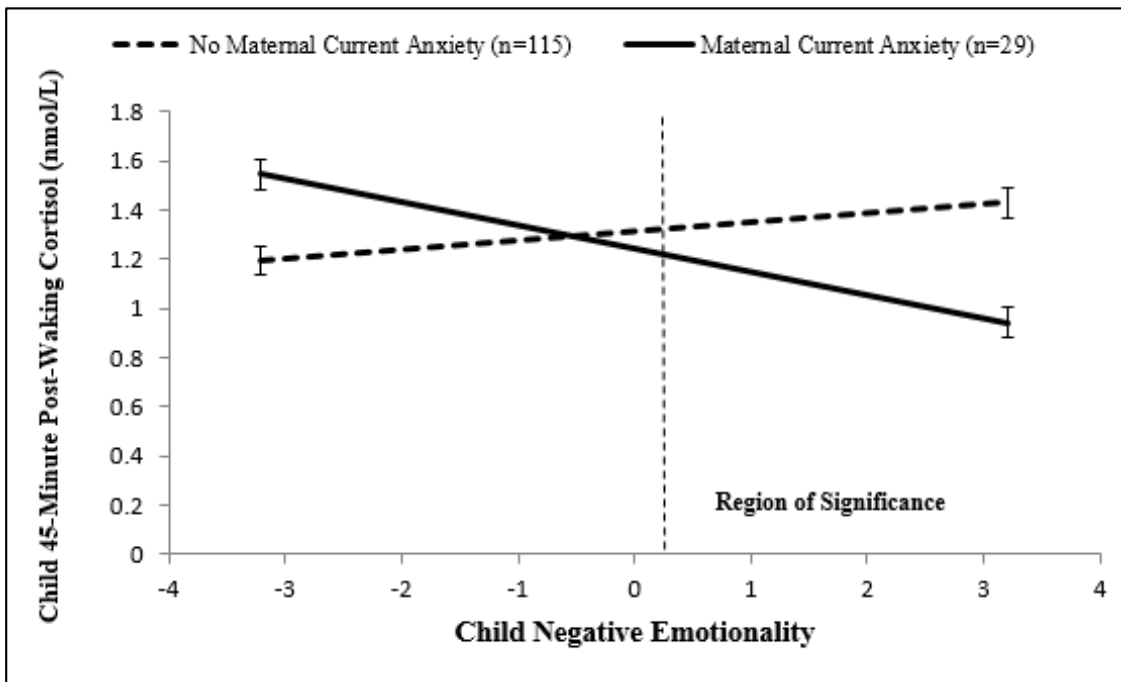


Figure 9.

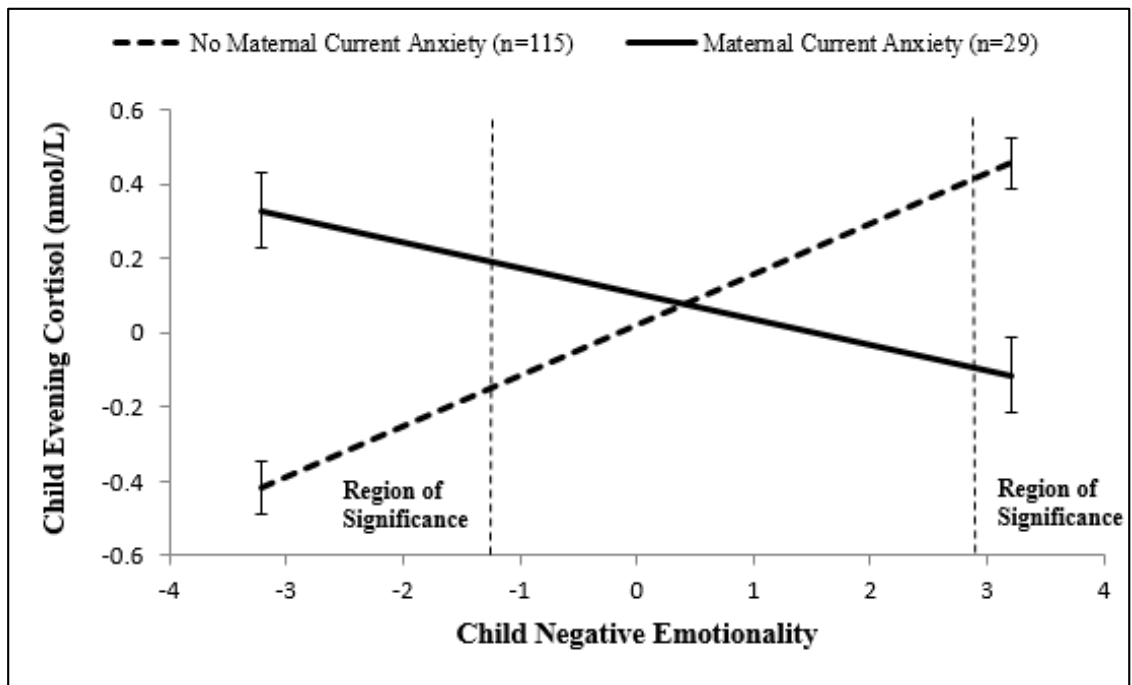


Figure 10.

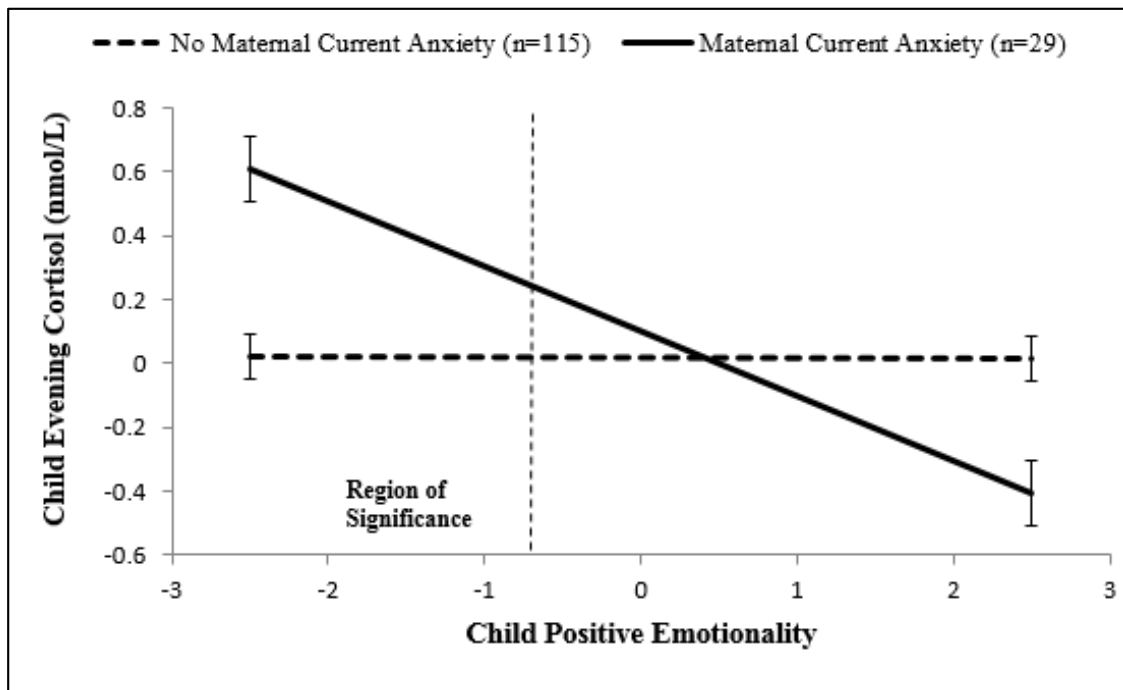


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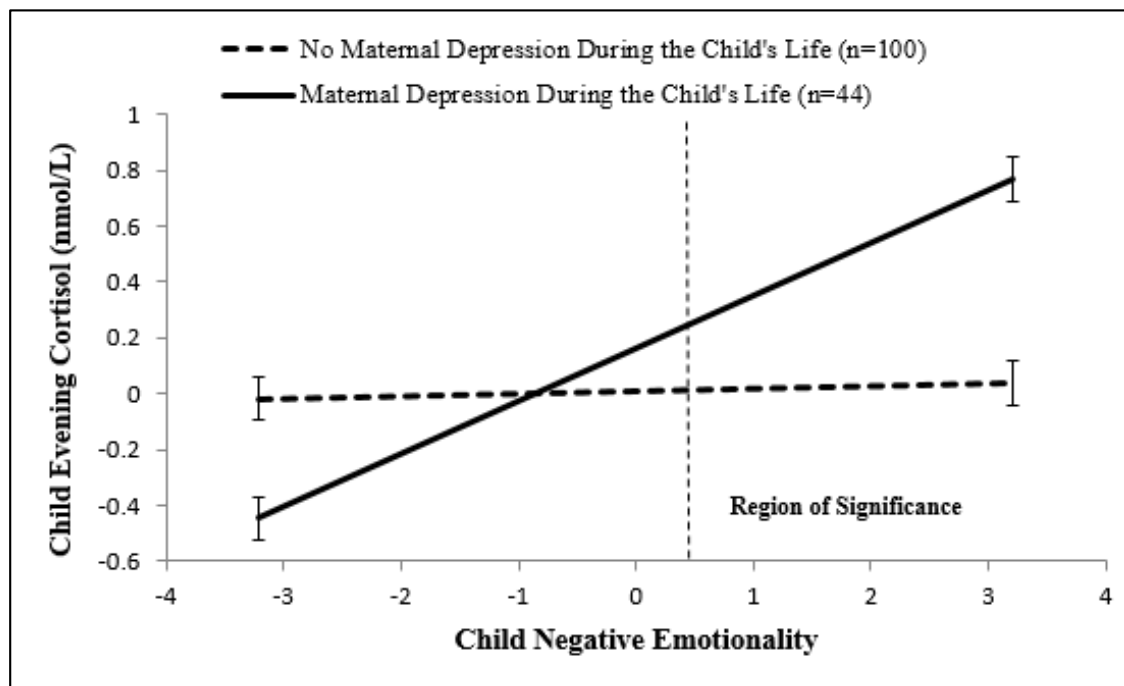


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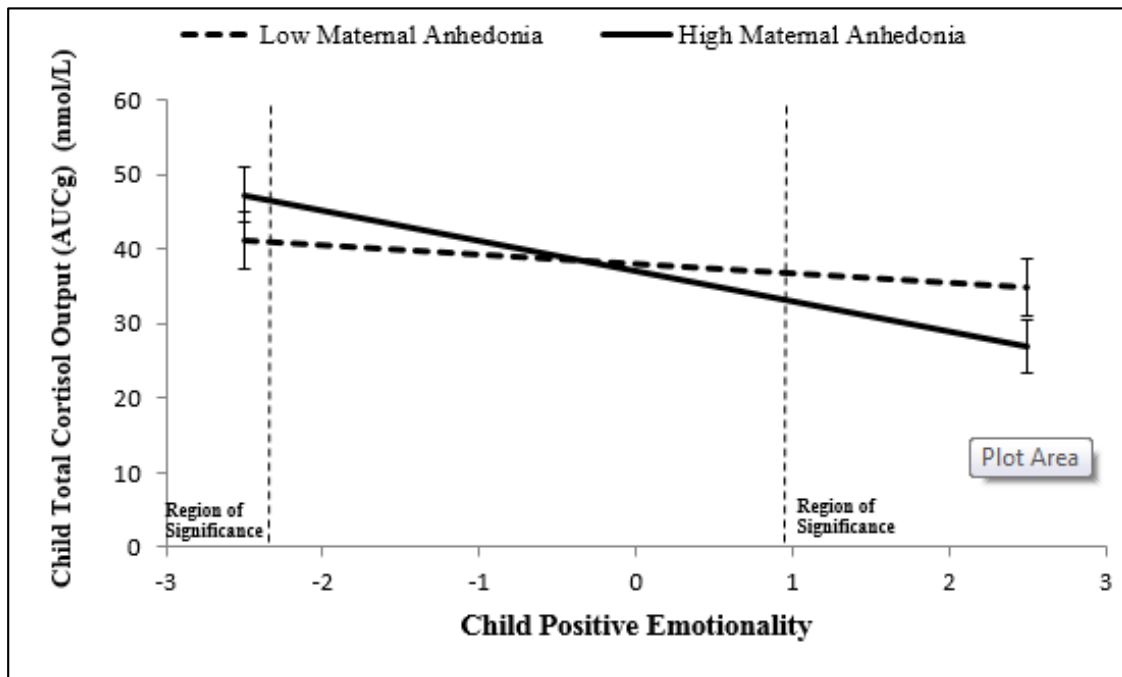


Figure 13.

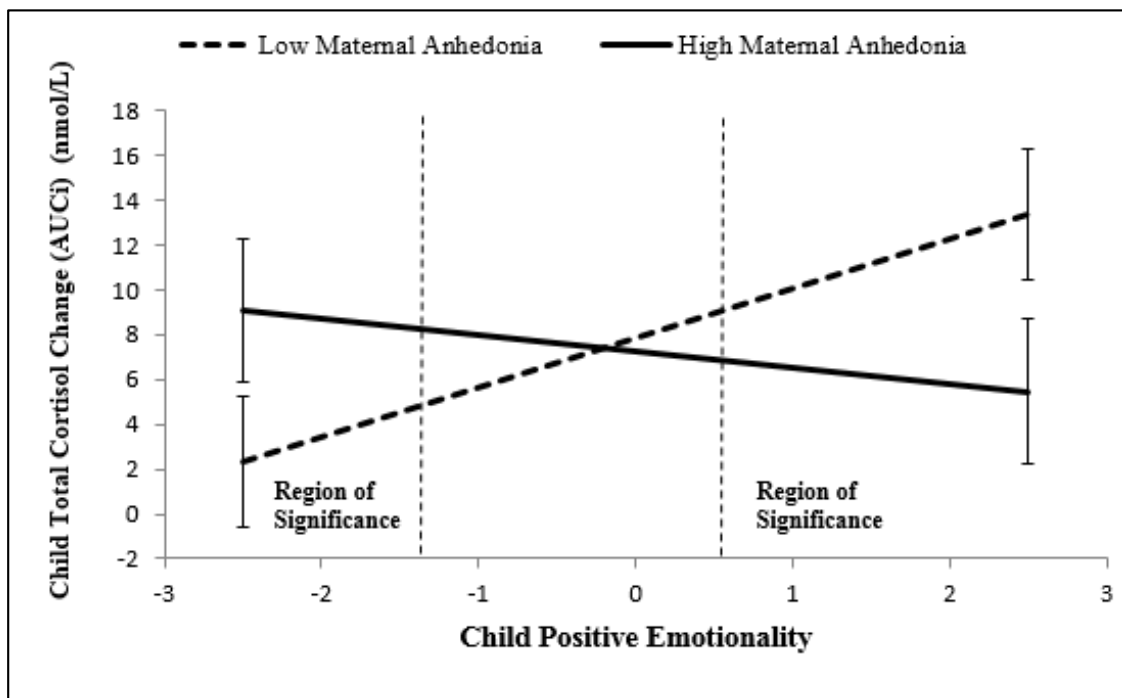


Figure 14.

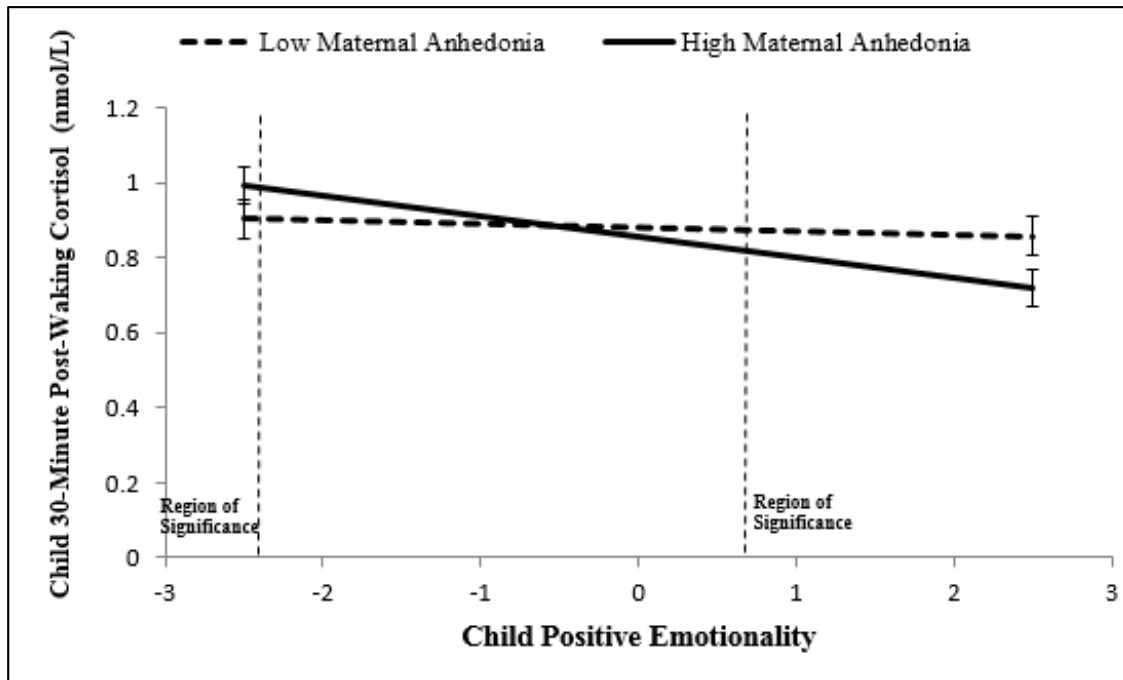
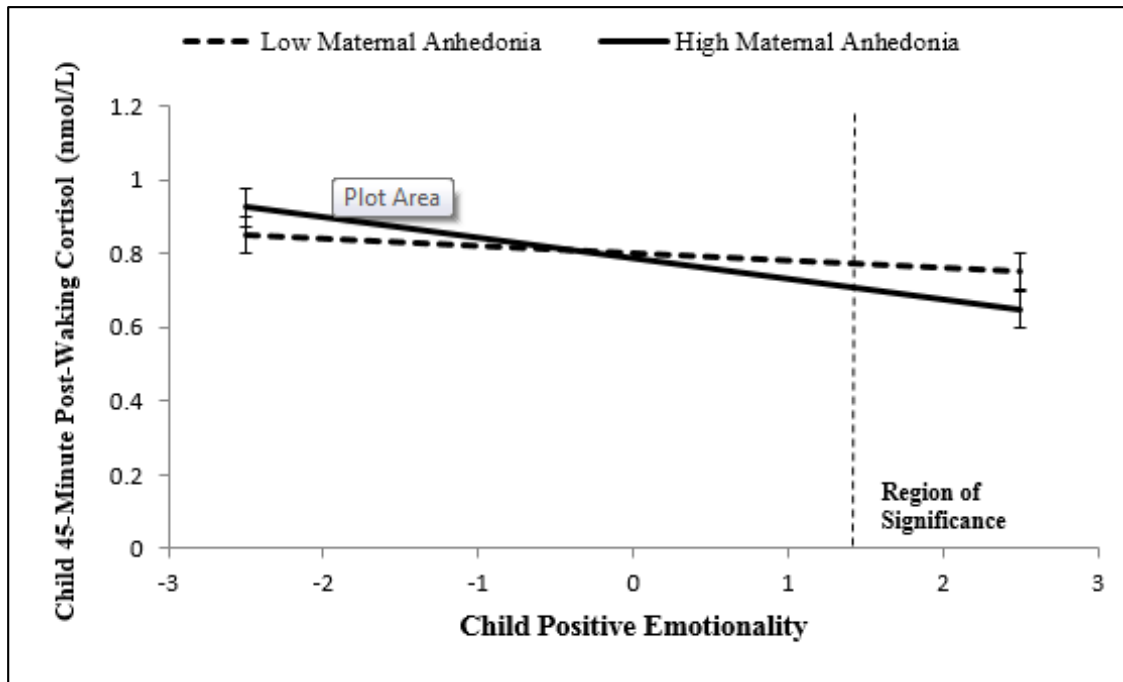


Figure 15.



Appendix A. List and schedule of measures and informants.

Measure	Informant	Schedule
Demographic Questionnaire	Parent	Visit 1
SCQ: Pervasive Developmental Disorder Screener	Parent	Visit 1
PPVT: Cognitive Impairment Screener	Child	Visit 1
SCID: Parental Depression History	Parent	Between Visits 1 & 2
Lab-TAB: Temperament	Observational coding	Visit 1
Child Depression	Parent	Visit 2
Salivary Cortisol	Child	Between Visits 1 & 2
Daily Diary Questionnaire	Parent	Between Visits 1 & 2

Note. SCQ = Social Communication Questionnaire; PPVT = Peabody Picture Vocabulary Test; Lab-TAB = Laboratory Temperament Assessment Battery; SCID = Structured Clinical Interview for Diagnostic Statistical Manual of Mental Disorders (DSM-IV-TR).

Appendix B. Demographic Questionnaire

Demographic Data

Child's Age: _____ years

Child's date of birth: _____ MM/ _____ DD/ _____ YYYY

Your relationship to child: Mother Father Other; Specify _____

Child's Ethnicity: White African American Asian Other

Is child of Hispanic descent? Yes No

With which adults does the child currently live? *(Check all that apply)*

- Biological mother
- Biological father
- Adoptive mother
- Adoptive father
- Step-mother or father's companion
- Step-father or mother's companion
- Other relative(s)
- Other non-relative(s)

Marital Status of child's biological parents:

- Married
- Living together
- Mother deceased
- Father deceased
- Father remarried
- Separated
- Divorced
- Never married
- Mother remarried

Please list the child's siblings in order of birth. *(Please indicate first names)*

First Name	Sex	Age	Living at Home
	<input type="radio"/> Male <input type="radio"/> Female		<input type="radio"/> Yes <input type="radio"/> No
	<input type="radio"/> Male <input type="radio"/> Female		<input type="radio"/> Yes <input type="radio"/> No
	<input type="radio"/> Male <input type="radio"/> Female		<input type="radio"/> Yes <input type="radio"/> No
	<input type="radio"/> Male <input type="radio"/> Female		<input type="radio"/> Yes <input type="radio"/> No
	<input type="radio"/> Male <input type="radio"/> Female		<input type="radio"/> Yes <input type="radio"/> No
	<input type="radio"/> Male <input type="radio"/> Female		<input type="radio"/> Yes <input type="radio"/> No

PARENT INFORMATION: *(Please complete for biological parents if known)*

Mother: Age: _____ Mother's date of birth:
_____MM/_____DD/_____YYYY

Mother's present occupation: _____

Father: Age: _____ Father's date of birth:
_____MM/_____DD/_____YYYY

Father's present occupation: _____

Education of Mother:

- 8th Grade or Less
- Some High School
- High School Graduate (or GED)
- Some College (or 2 Year Degree)
- 4 Year College Degree
- Master's Degree
- Doctoral Degree

Education of Father:

- 8th Grade or Less
- Some High School
- High School Graduate (or GED)
- Some College (or 2 Year Degree)
- 4 Year College Degree
- Master's Degree
- Doctoral Degree

Yearly Family Income:

<\$20,000 \$20,001 - \$40,000 \$40,001 - \$70,000 \$70,001 - \$100,000 > \$100,000

COMPLETE THIS SECTION IF ADULT(S) CARING FOR CHILD IS/ARE NOT BIOLOGICAL PARENTS:

A. Relationship to child: Adoptive parent Other relative Age: _____

_____ Step parent Other non-relative
B. Relationship to child: Adoptive parent Other Relative Age: _____

_____ Step parent Other non-relative

Highest level of education for non-biological caretaker: (See above)

Caretaker A (above):

- 8th Grade or Less
- Some High School
- High School Graduate (or GED)
- Some College (or 2 Year Degree)
- 4 Year College Degree
- Master's Degree
- Doctoral Degree

Caretaker B (above):

- 8th Grade or Less
- Some High School
- High School Graduate (or GED)
- Some College (or 2 Year Degree)
- 4 Year College Degree
- Master's Degree
- Doctoral Degree

Yearly family income of non-biological caretaker:

<\$20,000 \$20,001 - \$40,000 \$40,001 - \$70,000 \$70,001 - \$100,000 > \$100,000

CHILD'S MEDICAL HISTORY:

Does child have any illnesses or disabilities (either physical or mental)? Yes No

If yes, please describe:

Please mark the circle next to any medical conditions your child has ever had.

- Epilepsy/seizures/convulsions
- Head injuries or lacerations leading to loss of consciousness
- Seizures with high temperatures
- Unconscious (other)
- Birth abnormalities
- Anemia

- Heart disease
- Asthma
- Food sensitivities
- Allergies (describe)

- Lead poisoning
- Meningitis
- Encephalitis
- Mumps

-
- Chicken pox
 - German measles
 - Whooping cough
 - Problems with vision
 - Problems with hearing

-
- Emergency room visit
 - Poisoning, medicines
 - Poisoning, cleaning agent
 - Poisoning, non-food item
 - Physical handicaps (describe below)

Serious accident (describe below)

Other diseases (describe below)

Fever over 104, unknown cause

Is child taking medications for any conditions above? Yes No
 Medication (specify) _____

Has your child ever been hospitalized for a medical problem? Yes No

If yes, please specify:

a) Number of times _____

c) Reason(s)? _____

CHILDHOOD HISTORY:

How many pregnancies did mother have before the pregnancy with this child?

(Including those not carried to term)

_____ # pregnancies

Check any of the following that occurred during the pregnancy with this child:

(Check all that apply)

- | | |
|--|---|
| <input type="radio"/> Severe nausea and vomiting | <input type="radio"/> Toxemia |
| <input type="radio"/> High blood pressure | <input type="radio"/> Rubella, Mumps |
| <input type="radio"/> Incompatible Rh factor | <input type="radio"/> Diabetes |
| <input type="radio"/> Anemia | |
| <input type="radio"/> Bleeding 1st 3 months | <input type="radio"/> Bleeding 2nd 3 months |
| | <input type="radio"/> Bleeding 3rd 3 months |

Medications during pregnancy: No Yes

Please specify medications (include antidepressants, name of drug, dosage, and duration of use)

(1) _____

(2) _____

(3) _____

(4) _____

(5)

Check any of the following if they occurred at or following the delivery of the child:

(Check all that apply)

- Premature delivery Infant required oxygen
Specify weeks of gestation at birth: _____
 Cesarean section Infant required blood transfusion
 Breech delivery (feet or buttocks first) Infant was placed in an incubator
 Infant had cord around neck Infant was blue at birth
 Other problems (specify) _____
-

Child's weight at birth: _____ pounds _____ ounces

Did your child stay in the hospital after mother left? Yes No

If yes, please specify number of days _____

During the first year of life, did your child have difficulties in any of the following areas?

(Check all that apply)

- | | |
|--|--|
| <input type="checkbox"/> Sleep problems | <input type="checkbox"/> Excessive crying |
| <input type="checkbox"/> Feeding problems | <input type="checkbox"/> Difficult to comfort |
| <input type="checkbox"/> Resisted being held | <input type="checkbox"/> Sluggish, nonresponsive |
| <input type="checkbox"/> Overly active | <input type="checkbox"/> Fussy much of the time |
| <input type="checkbox"/> Under active | |

Was child breast-fed? Yes No **If yes, for how long?** _____ months

Age child started walking without assistance: _____ months

Age child spoke first words: _____ months

Age child dressed without supervision: _____ months

Did your child have difficulties with the development of speech? *(Check all that apply)*

- | | |
|---|---|
| <input type="checkbox"/> No difficulties | <input type="checkbox"/> Did not use "I" or "me" |
| <input type="checkbox"/> Delayed speech | <input type="checkbox"/> Often repeated other's words |
| <input type="checkbox"/> Stammering | <input type="checkbox"/> Talked excessively about one topic |
| <input type="checkbox"/> Hard to understand | <input type="checkbox"/> Other |

Child's primary caregiver(s) are:

(check all that apply)

- Mother Father Grandparent Live-in nanny/sitter
Other _____

How many hours per week does your child spend in the following:

_____ School _____ Daycare _____ Other childcare setting

Does mother work outside of the home? Yes No

If yes, how many hours per week? _____

Does father work outside of the home? Yes No

If yes, how many hours per week? _____

About how many close friends does your child have? (Do not include brothers and sisters)

- None 1 2 or 3 4 or more

About how many times a week does your child do things with any friends outside of regular school hours? (Do not include brothers and sisters)

- Less than 1 1 or 2 3 or more

Compared to others of his/her age, how well does your child:

- | | Worse | Average | Better | |
|---|-----------------------|-----------------------|-----------------------|------------------------------|
| a) Get along with his/her brothers and sisters?
siblings | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> Has no |
| b) Get along with other kids? | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | |
| c) Behave with his/her parents? | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | |
| d) Play and work alone? | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | |

Does your child receive special education or remedial services or attend a special class or special school?

- No Yes

If yes, please describe the kind of services, class or school

Has your child repeated any grades? No Yes

If yes, please describe the grades and reasons

Has your child had any academic or other problems in school?

- No Yes

If yes, please

describe.

Please describe the best things about the child:

Appendix C. Laboratory Assessment of Temperament Protocol

Episode 1: Car Go!

5 min

Look at these neat cars! (The experimenter will kneel next to the cars.) Do you want to see how they work? (Show how to operate – “You just push this up and it goes forward, just like that”) Let’s have a race! Which one do you want to be? See that finish line? Let’s have a race to that line! When you say “Ready, get set, go”, we’ll start the race.

(Race 4 times with cars, Let child win every time, can comment “Look at you, you are so fast, you’ve won every time, I’m gonna get you”)

Let me show you this other neat car! Do you want to try?(Make sure to switch off other cars)

Alright, do you want to play with some play dough? If the child does not want to leave the cars, play one more race and then say you have to leave so the toy people can come in the room.

Episode 2: Transparent Box

4 – 5 min

Have a seat and I will show you what’s inside the box. Which one is your favorite? (Put unselected toys on floor) Ok, we’re going to play a game with this toy. I’m going to put this toy in the box and then I’m going to put this lock on it. See these keys? You can use these keys to open the lock, and when you open the lock, you can open the box and play with the toy. Do you know how to use keys? Just put the key in the hole and turn it.

While I am gone, you can try all these keys. Ok, I’ll be back in a few minutes. (Take other toys out of room, leave room 2.5-3min, set stopwatch on belt)

Hi X! You know what, I made a big mistake. I gave you the wrong keys. I’m really sorry about that. These are the right keys, let’s try these and open the box up so you can play with the toy. (Play 1 min with toy). Alright, I’ve got another game for us to play!

Episode 3: Exploring New Objects

7 min

There’s a lot of interesting things in this room for you to look at and touch. While I’m gone, see if you can find out what is inside each of these things, you can look inside and underneath all the things in this room. I’ll be back in a few minutes. (Leave room 5min, set stopwatch on belt. Put play dough away and set up blocks)

Hi, X! Did you find out what all these things are? (Stand behind each object and ask the child to touch/play with object) Go to each of the items in the room and have the child touch and examine object: tent, spider, squishy box, pretend skull, pet box. *Be careful not to block camera

If necessary, prompt: Show me what this is... Good job! If child refuses: I’m going to see what it is, do you want me to look with you?

Wow, you did a really good job exploring all those things. Let's go play a game in the other room with some blocks!

Episode 4: Pop-Up Snakes

3 min

Have a seat and let's see what we have here. See this can? It says there are potato chips inside here. I'm pretty hungry, would you like some chips? (Struggle with lid, loosen slightly) I'm having a hard time opening this. Do you think you can help me? Here you try and open it. (Let child open can) 5 sec pause

Did that surprise you? Those aren't chips are they? They're pretend snakes!

You know what we can do with the pretend snakes, we can put them back inside the can and then we can surprise your mom! Would you like to surprise your mom? Ok, you wait right here and I will get your mom so you can surprise her.

30 sec pause. (Return with mom) I think X has something to show you. Go ahead and show your mom. (Wait for X to open can) Did you surprise your mom? Good job! Let's go back across the hall and play a game, while the toy people put new toys out for us.

Episode 5: Green Circles

3 min

Have a seat at the table; I need you to do me a favor. I need you to draw me the PERFECT green circle on this paper. (Offer criticism: too big, small, extra tail, etc.) After every 2 circles drawn, (3X) say: I need the PERFECT green circle. *(2.5min)
Draw 8-10 circles.

You know what? That one is a really good circle. Circles are really hard to draw aren't they? I asked you to draw so many! I think all of your circles are really good! Do you want to make one into a smiley face and give it your mom? Good job! Let's go play with some more play dough!

Episode 6: Popping Bubbles

4 - 5 min

You know what this is/Guess what I have? It's a bubble toy! Have you ever played with a bubble toy like this one? It's really fun!! Let me show you how this works. Let child try out the toy first.

Now let's play a game with the bubble toy. You stand here against the wall. I'm going to blow bubbles so you can pop them.

Can you show me where your hands are? I want to see how many bubbles you can pop using only your hands. (Blow 3 sets) Great job!!

Now can you show me where your feet are? I want you to pop as many bubbles with your feet. (Blow 3 sets) Good job!!

Okay, show me where your elbows are. I want to see how many bubbles you can pop using only your elbows now. (Blow 3 sets) Great!

Now, I'm going to blow some bubbles and I want you to run and catch as many as you can before they fall. Are you ready? (Blow 2 sets)

Now you can blow the bubbles for me and I will catch them before they fall.

You did such a great job! How about we go across the hall and wipe our hands off, and the toy people bring us some new toys. If necessary, say "It will be your turn soon."

Episode 7: Snack Delay

5 min

Have a seat and I will show you what we have here. Look we have these yummy (crackers)? We're going to play a game with crackers. Let me show you how to play. I'll put a cracker under the cup like this and when I ring the bell you pick up the cup and eat the cracker. Let's do one for practice. When you're done chewing, we'll start again.

*Hold bell level with child's head. Can ask child questions (Are they yummy? Which color should we choose next? Etc.)

Pauses: 5sec, 10sec, No Pause, 20sec, No Pause, 30sec.

Do you want to ring the bell for me now and I will eat the cracker? How about you ring the bell and eat the cracker? All right, you did a great job! How about we go into the other room while the toy people put out new toys for us? We can bring these crackers with us for a snack.

Episode 9: Box Empty

Hey X, guess what? Since you have been such a good boy/girl today and helped me so much, I'm going to give you a present to take home with you. You sit right here and I will be right back with your present. 15-30 second pause

Here is your present! You've done such a good job that you can unwrap it and take the present home with you! It's a really great present! I wish I could keep it for myself, but I'm going to give it to you! You can go ahead and unwrap your present. I'll be right back. (Leave room 2.5min, set stopwatch on belt)

I'm sorry! I was in such a hurry when I wrapped your present that I forgot to put the present in the box. Wasn't that silly of me, I am really sorry. But I brought the present with me; do you want to see it? (Show each present) Do you want to show your mom what you got?

Appendix D. Salivary Cortisol Sampling Instructions

INSTRUCTIONS FOR TAKING SALIVA SAMPLES AT HOME (CHILD)

Parent should collect individual samples from their child at the following scheduled times on

TWO consecutive weekdays:

1. IN THE MORNING UPON CHILD AWAKENING
2. 30 MINUTES AFTER CHILD AWAKENING
3. 45 MINUTES AFTER CHILD AWAKENING
4. 30 MINUTES BEFORE CHILD BEDTIME

Use the timers to help keep track of the sampling times. Use the Sticker Sheet to help keep track of your progress.

RULES: As you collect saliva, we ask that:

- You select **2 consecutive weekdays** for sampling. Aim for two typical weekdays. AVOID especially troublesome or exciting days or a weekend.
- Do not collect saliva samples if you or your child is sick or taking antibiotics.
- Do not brush your teeth before sampling.
- Drinking a glass of water upon waking is acceptable.
- Do not eat or drink anything prior to sampling, other than water.
- Avoid caffeinated and dairy products prior to sampling.
- Both you and your child MUST use the Kool-Aid crystals to collect saliva samples.
- Complete the Daily Diary for your child at the end of each sampling day.

Step 1: **Set the Timer.** In the morning upon child awakening, set the timer for 30 minutes.

Step 2: **Open Vial.** Pop open the cap from the plastic vial.

Step 3: **Eat Kool-Aid.** Dip the cotton roll in just a few crystals, less than 1/16th of a teaspoon. Do not add water to the Kool-Aid.

Step 4: **Chew Cotton.** Chew the cotton in your mouth until it is very moist. Parents should tell the child that she or he is not to swallow the cotton, only to chew it. This usually takes about one minute of chewing. We recommend counting to 60.

Step 5: **Fill Vial.** Separate the plunger from the barrel of the syringe. Put wet cotton roll into barrel. Re-insert plunger, push down, and collect the saliva into vial. Hold the vial firmly, as it can slip. Try to fill at least one-third of the vial. (Discard cotton & syringe.)

- Step 6: **Label the Vial.** Record the date and time of day on the correct pre-printed label. Attach this label onto the vial so that it forms a “flag” around the vial. Use a permanent marker/pen to write on the label if you have one. If not, use a pencil.
- Step 7: **Refrigerate!** Place the sample into the plastic storage bag. Refrigerate the sample. Refrigeration is a Very Important Step.
- Step 8. **When the timer buzzes, repeat Steps 1-7** to collect the next sample. Set the timer for 15 minutes as a reminder to collect the saliva for the third time point.
- Step 9: **At night, set the timer for 30 minutes before bed.** Repeat Steps 1-7 when the timer buzzes.
- Step 10: **Fill out the Daily Diary.** After you have collected all the saliva samples, fill out and your child’s Daily Diary for Day 1.
- Step 11: **Tomorrow, repeat steps 1-10 for your child.**
- Step 12: **Return samples along with the Daily Diaries.** After two days of sampling, your child should have completed a total of 8 samples. Please return the samples, along with the Daily Diaries to our lab on your second lab visit.

If you have ANY questions, please give us a call. No question is strange regarding this process, and we would be happy to answer your questions. Please call the Child Stress and Emotions Lab at 301-405-9880.

Appendix E. Daily Diary

**Day 1 – Daily Diary (CHILD Form)
Home Saliva Collection**

REMINDER:

Across 2 consecutive weekdays, collect Samples: (1) upon waking, (2) 30 minutes after waking, (3) 45 minutes after waking, and (4) before bed. There should be a total of 4 samples collected from your child on each day. Complete the daily diary after all samples for that day have been collected.

1. Day 1: Date of saliva collection
_____MM/_____DD/_____YY
2. Day of week (circle one): SUN MON TUES WED THURS
FRI SAT
3. Time of child's waking:
_____AM
4. Was this the child's normal time of waking?
NO YES
5. If NO, when does the child normally awaken?
_____AM
6. Time child went to sleep this evening
_____PM
7. Time of Sample 1 (to be collected upon waking):
_____AM
8. Time of Sample 2 (30 minutes after waking):
_____AM
9. Time of Sample 3 (45 minutes after waking):
_____AM
10. Time of Sample 4 (30 minutes before bed):
_____PM
11. Did your child go to school or daycare today?
NO YES

12. Does your child have difficulty falling asleep? (Circle one)

Never

Sometimes

Frequently

13. Circle approximately how long it took your child to fall asleep the night before the morning sampling. (Circle one)

1-15 min
min

16-30 min

> 30

14. How many hours of sleep did your child get on the night prior to the morning sampling? _____hours

15. Circle the best description of your child's health today? (Circle one)

Healthy

Sick

If your child was not feeling well today, please comment briefly on his/her symptoms:

16. Does your child use an inhaler for asthma?

NO YES

a. If yes, when did your child last use the inhaler?

_____MM/_____DD/_____/YY

b. Did your child use the inhaler the day before or on the day of saliva sampling?

NO YES

c. What is the name of the inhaler? _____

17. Is your child currently using any medications?

NO YES

a. If yes, please list medication(s):

18. Please mark which activities your child did on the day of sampling:

- School
- Daycare
- Shopping
- Visiting friends
- Family outing
- Club meeting
- Sport participant
- School event
- Quiet activity at home (homework or TV)
- Playing at home
- Other, please specify: _____

a. Please mark any of the following that apply to your child on the day of the saliva sampling:

- Argument(s) with parent that lasted more than a few moments
- Argument(s) with sibling(s) that lasted more than a few moments
- Argument(s) with friend(s) that lasted more than a few moments
- Prolonged concerns or things that cause your child to worry
- Other events causing anxiety or distress for your child
- None of the above

19. Did your child eat a meal within the hour before any of the samplings?

- Before Sample 1?
 NO YES If yes, when? _____ AM/PM
- Before Sample 2?
 NO YES If yes, when? _____ AM/PM
- Before Sample 3?
 NO YES If yes, when? _____ AM/PM
- Before Sample 4?
 NO YES If yes, when? _____ AM/PM

- a. Did your child eat or drink any caffeinated products (e.g., soda, chocolate, iced tea) within two hours prior to any sampling?

Before Sample 1? _____NO _____YES

Before Sample 2? _____NO _____YES

Before Sample 3? _____NO _____YES

Before Sample 4? _____NO _____YES

- b. Did your child eat or drink any dairy products within 15 minutes prior to any sampling?

Before Sample 1? _____NO _____YES

Before Sample 2? _____NO _____YES

Before Sample 3? _____NO _____YES

Before Sample 4? _____NO _____YES

20. Has your child had a recent tooth loss? (Circle one)
NO YES

- a. If yes, when? _____MM/_____DD/_____YY

- b. Does your child have any cuts in his/her mouth? Or is there any reason that there would be blood in your child's mouth? (Circle one)

NO YES

- a. If yes, what is the reason?

Thanks! One more day to go...

Please label and refrigerate the samples.

Day 2 – Diary (CHILD)
Home Saliva Collection

REMINDER:

Across 2 consecutive weekdays, collect samples: (1) upon waking, (2) 30 minutes after waking, (3) 45 minutes after waking, and (4) before bed. There should be a total of 4 samples collected from your child on each day. Complete the daily diary after all samples for that day have been collected.

1. Day 2: Date of saliva collection
_____MM/_____DD/_____YY
2. Day of week (circle one): SUN MON TUES WED THURS FRI
SAT
3. Time of child's waking:
_____AM
4. Was this the child's normal time of waking?

 NO YES
5. If NO, when does the child normally awaken?
_____AM
6. Time child went to sleep this evening
_____PM
7. Time of Sample 1 (to be collected upon waking): _____AM
8. Time of Sample 2 (30 minutes after waking):
_____AM
9. Time of Sample 3 (45 minutes after waking):
_____AM
10. Time of Sample 4 (30 minutes before bed):
_____PM
11. Did your child go to school or daycare today?

 NO YES

12. Does your child have difficulty falling asleep? (Circle one)

Never

Sometimes

Frequently

13. Circle approximately how long it took your child to fall asleep the night before the morning sampling. (Circle one)

1-16 min

16-30 min

> 30 min

14. How many hours of sleep did your child get on the night prior to the morning sampling? _____ hours

15. Circle the best description of your child's health today?

Healthy

Sick

If your child was not feeling well today, please comment briefly on his/her symptoms:

16. Does your child use an inhaler for asthma? NO YES

a. If yes, when did your child last use the inhaler?

____MM/____DD/____/YY

b. Did your child use the inhaler the day before or on the day of saliva sampling?

NO

YES

c. What is the name of the inhaler? _____

17. Is your child currently using any medications? NO YES

a. If yes, please list medication(s):

18. Please mark which activities your child did on the day of sampling:

___School

___Daycare

___Shopping

- Visiting friends
- Family outing
- Club meeting
- Sport participant
- School event
- Quiet activity at home (homework or TV)
- Playing at home
- Other, please specify: _____

19. Please mark any of the following that apply to your child on the day of the saliva sampling:

- Argument(s) with parent that lasted more than a few moments
- Argument(s) with sibling(s) that lasted more than a few moments
- Argument(s) with friend(s) that lasted more than a few moments
- Prolonged concerns or things that cause your child to worry
- Other events causing anxiety or distress for your child
- None of the above

20. Did your child eat a meal within the hour before any of the samplings?

- Before Sample 1?
 NO YES If yes, when? _____ AM/PM
- Before Sample 2?
 NO YES If yes, when? _____ AM/PM
- Before Sample 3?
 NO YES If yes, when? _____ AM/PM
- Before Sample 4?
 NO YES If yes, when? _____ AM/PM

21. Did your child eat or drink any caffeinated products (e.g., soda, chocolate, iced tea) within two hours prior to any sampling?

- Before Sample 1? NO YES
- Before Sample 2? NO YES
- Before Sample 3? NO YES
- Before Sample 4? NO YES

22. Did your child eat or drink any dairy products within 15 minutes prior to any sampling?

- Before Sample 1? NO YES
- Before Sample 2? NO YES
- Before Sample 3? NO YES

Before Sample 4? _____NO _____YES

23. Has your child had a recent tooth loss? (Circle one)
NO YES

a. If yes, when? _____MM/_____DD/_____YY

24. Does your child have any cuts in his/her mouth? Or is there any reason that there would be blood in your child's mouth? (Circle one)
NO YES

a. If yes, what is the reason?

Thanks again! Please label and refrigerate all samples and bring them with you on your second visit to our lab . If you are not returning to the lab, please mail the envelope and questionnaires to us in your pre-paid envelope.

Appendix F. Design Considerations.

Eight design considerations are important to address:

First, we chose to study a sample of preschool-age children (3-5 years old). To date, the majority of studies examining the CAR and depression risk have used samples of adolescents and adults, many of whom have already developed significant psychopathology and impairment. As depression is rare in preschool-age children (Egger & Angold, 2006), examining the CAR in this age group would provide the opportunity to examine HPA axis abnormalities in at-risk individuals *prior* to the onset of a subsequent disorder, and provide fundamental insight into the origins of HPA axis dysregulation and its role in depression risk. Furthermore, given the high degree of neuroplasticity during early childhood (Nelson et al., 2006), and as early perturbations in HPA axis functioning have been found to demonstrate lasting impacts on the developing child (Heim et al., 2008; Meaney, 2001), there is a vital need to identify risk factors for depression in early childhood, as it may provide a stronger foundation for early and potentially more effective intervention efforts. Notably, the preschool period is when cortisol levels, seen initially in early infancy, stabilize to an adult-equivalent rhythm (Gunnar & Vasquez, 2006). Lastly, any child with a current major depressive disorder will be excluded from the study in order to investigate the CAR as a potential early emerging vulnerability marker or trait marker, rather than a correlate of the disorder.

Second, the proposed study will assess history of depression in both mothers and fathers. Children of at least one parent with a history of depression will be considered a member of our at-risk group. Previous research has found that a history of depression in either parent is associated with increased risk for depression (Weissman et al., 2006), and

elevated CAR (Vreeburg et al., 2010) in offspring. Nonetheless, consistent with prior research, we anticipate that the majority of participating primary caregivers will be mothers.

Third, the proposed study will examine both temperamental NE and PE. Previous research has linked high levels of NE and low levels of PE to depression. Specifically, high NE and low PE have been concurrently (Joiner et al., 1996) and prospectively (Dougherty et al., 2010) associated with depression in youth. In addition, both NE and PE have been linked to abnormalities in HPA axis functioning.

Fourth, we decided to assess child temperament using an observational laboratory measure, the Laboratory Temperament Assessment Battery (Lab-TAB; Goldsmith et al., 1995). To date, most studies have assessed child temperament through use of parent-reports. Parent reports have several strengths, including the ability to assess child behavioral quickly with minimal expense. However, parent-reports may be influenced by the parent's own psychopathology, mood, and expectations (Youngstrom et al., 1999). It is noteworthy that this issue would be particularly problematic in our study examining child temperament and parental depression, as parents would report on both variables. Thus, we decided to assess child temperament using an observational laboratory measure in order to provide a more objective and controlled approach to examining child temperament. Moreover, laboratory observations of temperament show moderate stability over time (Durbin, Hayden, Klein, & Olino, 2007), and have demonstrated links to both parental depression (Olino et al., 2010) and later depression (Dougherty et al., 2010), providing further support for the validity of laboratory observations as a measure of child temperament.

Fifth, existing studies in youth have collected morning samples at fixed-times. Given that cortisol rhythms depend on person-specific sleep-wake schedules (Kudielka & Kirschbaum, 2003; Wilhelm et al., 2008), fixed-time sampling is problematic as cortisol levels may differ as a function of time awake, rather than true individual differences in cortisol levels. To date, only four studies in youth have implemented multi-morning sample assessments of the CAR at person-specific wake times, and no study investigated the CAR in a sample of offspring at risk for depression. Thus, this study will assess children's cortisol at waking, 30 and 45 minutes post waking to better capture children's CAR.

Sixth, research suggests that cortisol samples should be assessed across days to reliably assess the CAR (Hellhammer et al., 2007). Thus, we instructed parents to collect cortisol samples across two days. In addition, as the type of day has been associated with cortisol levels (Kunz-Ebrecht et al., 2004), parents were instructed to collect cortisol samples on weekdays only. Lastly, children who took corticosteroid, stimulant, and analgesic medications and who were sick with a fever during the time of sample collection, will be excluded from analyses, as these factors have been shown to impact cortisol levels (Granger et al., 2009; Gunnar & Talge, 2007).

Seventh, as noncompliance to sampling times has been found to influence cortisol data (Broderick et al., 2004; Kudielka et al., 2003, 2007; Smith & Dougherty, 2014), we will control for parental compliance to sampling times in analyses. Compliance data will be based on a diary measure, as well as the MEMS TrackCap (Aardex Ltd., Zug, Switzerland). Although it would be ideal to have compliance data based on the MEMS for the full sample, inclusion of this information for 55.2% of the sample would help

provide some indication of the extent to which our results are robust to the effects of noncompliance (Adam et al., 2006).

Eighth, as depressive disorders are often comorbid with anxiety disorders (Kessler et al., 2003) and substance use disorders (SUD; Swendsen & Merikangas, 2000), these disorders will be included in models as covariates, in order to examine the specific relationship between parental depression and children's CAR.

References

- Adam, E. K., Doane, L. D., Zinbarg, R. E., Mineka, S., Craske, M. G., & Griffith, J. W. (2010). Prospective prediction of major depressive disorder from cortisol awakening responses in adolescence. *Psychoneuroendocrinology, 35*, 921-931.
- Adam, E. K., Hawkley, L. C., Kudielka, B. M., & Cacioppo, J. T. (2006). Day-to-day dynamics of experience–cortisol associations in a population-based sample of older adults. *Proceedings of the National Academy of Sciences, 103*, 17058-17063.
- Adam, E.K., & Kumari, M., (2009). Assessing salivary cortisol in largescale epidemiological research. *Psychoneuroendocrinology 34*, 1423-1436.
- Adam, E. K., Sutton, J. M., Doane, L. D., & Mineka, S. (2008). Incorporating hypothalamic–pituitary–adrenal axis measures into preventive interventions for adolescent depression: Are we there yet?. *Development and Psychopathology, 20*, 975-1001.
- Adam, E. K., Vrshek-Schallhorn, S., Kendall, A. D., Mineka, S., Zinbarg, R. E., & Craske, M. G. (2014). Prospective associations between the cortisol awakening response and first onsets of anxiety disorders over a six-year follow-up–2013 Curt Richter Award Winner. *Psychoneuroendocrinology, 44*, 47-59.
- Aiken, L.S., & West, S.G. (1991). *Multiple regression: Testing and interpreting interactions*. Newbury Park, CA: Sage.
- Alink, L.R., Ijzendoorn, M.H., Bakermans-Kranenburg, M.J., Mesman, J., Juffer, F., & Koot, H.M. (2008). Cortisol and externalizing behavior in children and adolescents: mixed metaanalytic evidence for the inverse relation of basal cortisol

- and cortisol reactivity with externalizing behavior. *Developmental Psychobiology*, 50, 427–450.
- Andreasen, N. C., Endicott, J., Spitzer, R. L., & Winokur, G. (1977). The family history method using diagnostic criteria: Reliability and validity. *Archives of General Psychiatry*, 34, 1229–1235.
- Anthony, J. L., Lonigan, C. J., Hooe, E. S., & Phillips, B. M. (2002). An affect-based, hierarchical model of temperament and its relations with internalizing symptomatology. *Journal of Clinical Child and Adolescent Psychology*, 31, 480-490.
- Ashman, S.B., Dawson, G., Panagiotides, H., Yamada, E., & Wilkinson, C.W. (2002). Stress hormone levels of children of depressed mothers. *Development and Psychopathology*, 14, 333 – 349.
- Backhaus, J., Junghanns, K., & Hohagen, F. (2004). Sleep disturbances are correlated with decreased morning awakening salivary cortisol. *Psychoneuroendocrinology*, 29, 1184-1191.
- Bartels, M., de Geus, E. J., Kirschbaum, C., Sluyter, F., & Boomsma, D. I. (2003). Heritability of daytime cortisol levels in children. *Behavior Genetics*, 33, 421-433.
- Bäumler, D., Kirschbaum, C., Kliegel, M., Alexander, N., & Stalder, T. (2013). The cortisol awakening response in toddlers and young children. *Psychoneuroendocrinology*, 38, 2485-2492.
- Beach, S. R., & Amir, N. (2003). Is depression taxonic, dimensional, or both?. *Journal of Abnormal Psychology*, 112(2), 228-236.

- Belli, R.F., Shay, W.L., & Stafford, F.P. (2001). Event history calendars and question list surveys: A direct comparison of interviewing methods. *Public Opinion Quarterly*, 65, 45–74.
- Bhagwagar, Z., Hafizi, S., & Cowen, P.J. (2003). Increase in concentration of waking salivary cortisol in recovered patients with depression. *The American Journal of Psychiatry*, 160, 1890-1891.
- Bhagwagar, Z., Hafizi, S., & Cowen, P.J. (2005). Increased salivary cortisol after waking in depression. *Psychopharmacology*, 182, 54-57.
- Bijttebier, P., & Roeyers, H. (2009). Temperament and vulnerability to psychopathology: Introduction to the special section. *Journal of Abnormal Child Psychology*, 37, 305-308.
- Block, J. H., Gjerde, P. F., & Block, J. H. (1991). Personality antecedents of depressive tendencies in 18-year-olds: A prospective study. *Journal of Personality and Social Psychology*, 60, 726–738.
- Bockting, C. L., Lok, A., Visser, I., Assies, J., Koeter, M. W., & Schene, A. H. (2012). Lower cortisol levels predict recurrence in remitted patients with recurrent depression: a 5.5 year prospective study. *Psychiatry research*, 200, 281-287.
- Bremmer, M. A., Deeg, D. J., Beekman, A. T., Penninx, B. W., Lips, P., & Hoogendijk, W. J. (2007). Major depression in late life is associated with both hypo-and hypercortisolemia. *Biological Psychiatry*, 62, 479-486.
- Broderick, J. E., Arnold, D., Kudielka, B. M., & Kirschbaum, C. (2004). Salivary cortisol sampling compliance: comparison of patients and healthy volunteers. *Psychoneuroendocrinology*, 29, 636–650.

- Brown, T. A., Chorpita, B. F., & Barlow, D. H. (1998). Structural relationships among dimensions of the DSM-IV anxiety and mood disorders and dimensions of negative affect, positive affect, and autonomic arousal. *Journal of Abnormal Psychology, 107*, 179.
- Burke, H.M., Davis, M.C., Otte, C., & Mohr, D.C. (2005). Depression and cortisol responses to psychological stress: A meta-analysis. *Psychoneuroendocrinology, 30*, 846–856.
- Carnegie, R., Araya, R., Ben-Shlomo, Y., Glover, V., O'Connor, T. G., O'Donnell, K. J., ... & Lewis, G. (2013). Cortisol awakening response and subsequent depression: prospective longitudinal study. *The British Journal of Psychiatry.*
- Caspi, A. (2000). The child is father of the man: Personality continuities from childhood to adulthood. *Journal of Personality and Social Psychology, 78*, 158–172.
- Caspi, A, Moffitt, T. E., Newman, D. L., & Silva, P. A. (1996). Behavioral observations at age 3 years predict adult psychiatric disorders. *Archives of General Psychiatry, 53*, 1033-1039.
- Chrousos, G. P., & Gold, P. W. (1998). A healthy body in a healthy mind—and vice versa—the damaging power of “uncontrollable” stress. *Journal of Clinical Endocrinology & Metabolism, 83*, 1842-1845.
- Cizza, G., Ronsaville, D. S., Kleitz, H., Eskandari, F., Mistry, S., Torvik, S., ... & Martinez, P. E. (2012). Clinical subtypes of depression are associated with specific metabolic parameters and circadian endocrine profiles in women: the power study. *PloS one, 7*, e28912.

- Clark, L. A., & Watson, D. (1999). Temperament: A new paradigm for trait psychology. In L. A. Pervin & O. P. John (Eds.), *Handbook of personality: Theory and research* (2nd ed) (pp. 399–423). New York: Guilford.
- Clark, L. A., Watson, D., & Mineka, S. (1994). Temperament, personality, and the mood and anxiety disorders. *Journal of Abnormal Psychology, 103*, 103-116.
- Clow, A., Thorn, L., Evans, P., & Hucklebridge, F. (2004). The awakening cortisol response: methodological issues and significance. *Stress, 7*, 29-37.
- Connell, A. M., & Goodman, S. H. (2002). The association between psychopathology in fathers versus mothers and children's internalizing and externalizing behavior problems: a meta-analysis. *Psychological Bulletin, 128*, 746 – 773.
- Contreras, F., Menchon, J. M., Urretavizcaya, M., Navarro, M. A., Vallejo, J., & Parker, G. (2007). Hormonal differences between psychotic and non-psychotic melancholic depression. *Journal of Affective Disorders, 100*, 65-73.
- Cuijpers, P. (2001). Mortality and depressive symptoms in inhabitants of residential homes. *International Journal of Geriatric Psychiatry, 16*, 131 – 138.
- Cuipers, P., & Smit, F. (2002). Excess mortality in depression: A meta-analysis of community studies. *Journal of Affective Disorders, 72*, 227-236.
- Dahl, R. E., Ryan, N. D., Puig-Antich, J., Nguyen, N. A., Al-Shabbout, M., Meyer, V. A., & Perel, J. (1991). 24-hour cortisol measures in adolescents with major depression: a controlled study. *Biological Psychiatry, 30*, 25-36.
- Davidson, R. J. (1998). Affective style and affective disorders: Perspectives from affective neuroscience. *Cognition & Emotion, 12*, 307-330.
- Degnan, K. A., Almas, A. N., & Fox, N. A. (2010). Temperament and the environment in

- the etiology of childhood anxiety. *Journal of Child Psychology and Psychiatry*, *51*, 497-517.
- Degnan K.A., Hane A.A., Henderson H.A., Moas O., Reeb-Sutherland B.C., & Fox N.A. (2011). Longitudinal stability of temperamental exuberance and social-emotional outcomes in early childhood. *Developmental Psychology*, *47*, 765–780.
- DeSantis, A. S., Adam, E. K., Doane, L. D., Mineka, S., Zinbarg, R. E., & Craske, M. G. (2007). Racial/ethnic differences in cortisol diurnal rhythms in a community sample of adolescents. *Journal of Adolescent Health*, *41*, 3-13.
- Detting, A. C., Gunnar, M. R., & Donzella, B. (1999). Cortisol levels of young children in full-day childcare centers: Relations with age and temperament. *Psychoneuroendocrinology*, *24*, 519-536.
- Doane, L. D., Franz, C. E., Prom-Wormley, E., Eaves, L. J., Mendoza, S. P., Hellhammer, D. H., ... & Jacobson, K. C. (2011). Negative emotionality, depressive symptoms and cortisol diurnal rhythms: Analysis of a community sample of middle-aged males. *Hormones and behavior*, *60*, 202-209.
- Dockray, S., Bhattacharyya, M. R., Molloy, G. J., & Steptoe, A. (2008). The cortisol awakening response in relation to objective and subjective measures of waking in the morning. *Psychoneuroendocrinology*, *33*, 77-82.
- Dockray, S., & Steptoe, A. (2010). Positive affect and psychobiological processes. *Neuroscience & Biobehavioral Reviews*, *35*, 69-75.
- Dougherty, L. R., Klein, D. N., Durbin, C. E., Hayden, E. P., & Olino, T. M. (2010).

- Temperamental positive and negative emotionality and children's depressive symptoms: A longitudinal prospective study from age three to age ten. *Journal of Social and Clinical Psychology, 29*, 462-488.
- Dougherty, L. R., Klein, D. N., Olino, T. M., Dyson, M., & Rose, S. (2009). Increased waking salivary cortisol and depression risk in preschoolers: The role of maternal history of melancholic depression and early child temperament. *Journal of Child Psychology and Psychiatry, 50*, 1495-1503.
- Dougherty, L. R., Smith, V. C., Olino, T. M., Dyson, M. W., Bufferd, S. J., Rose, S. A., & Klein, D. N. (2013). Maternal psychopathology and early child temperament predict young children's salivary cortisol 3 years later. *Journal of abnormal child psychology, 41*, 531-542.
- Dougherty, L. R., Tolep, M. R., Smith, V. C., & Rose, S. (2013). Early exposure to parental depression and parenting: associations with young offspring's stress physiology and oppositional behavior. *Journal of abnormal child psychology, 41*, 1299-1310.
- Dozier, M., Peloso, E., Lewis, E., Laurenceau, J. P., & Levine, S. (2008). Effects of an attachment-based intervention on the cortisol production of infants and toddlers in foster care. *Development and psychopathology, 20*, 845-859.
- Dunn, L.M., & Dunn, L.M. (1997). *Peabody Picture Vocabulary Test (3rd edition)*. Circle Pines, Minnesota: American Guidance Service.
- Durbin, C. E., Hayden, E. P., Klein, D. N., & Olino, T. M. (2007). Stability of laboratory-assessed temperamental emotionality traits from ages 3 to 7. *Emotion, 7*, 388-399.

- Durbin, C. E., Klein, D. N., Hayden, E. P., Buckley, M. E., & Moerk, K. C. (2005). Temperamental emotionality in preschoolers and parental mood disorders. *Journal of Abnormal Psychology, 114*, 28 – 37.
- Eaves, L. C., Wingert, H. D., Ho, H. H., & Mickelson, E. C. R. (2006). Screening for autism spectrum disorders with the social communication questionnaire. *Journal of Developmental and Behavioral Pediatrics, 27*, S95-S103.
- Egger, H.L., & Angold, A. (2006). Common emotional and behavioral disorders in preschool children: Presentation, nosology, and epidemiology. *Journal of Child Psychology and Psychiatry, 47*, 313–337.
- Egger, H.L., Ascher, B.H., & Angold, A. (1999). The Preschool Age Psychiatric Assessment: Version 1.1. Durham, NC: Center for Developmental Epidemiology, Department of Psychiatry and Behavioral Sciences, Duke University Medical Center.
- Eisenberg, N., Valiente, C., Spinrad, T. L., Cumberland, A., Liew, J., Reiser, M., ... & Losoya, S. H. (2009). Longitudinal relations of children's effortful control, impulsivity, and negative emotionality to their externalizing, internalizing, and co-occurring behavior problems. *Developmental Psychology, 45*, 988 –1008.
- Essex, M.J., Klein, M.H., Cho, E., & Kalin, N.D. (2002). Maternal stress beginning in infancy may sensitize children to later stress exposure: effects on cortisol and behavior. *Biological Psychiatry, 52*, 776–784.
- Feldman, R., Granat, A., Pariente, C., Kanety, H., Kuint, J., & Gilboa-Schechtman, E. (2009). Maternal depression and anxiety across the postpartum year and infant

- social engagement, fear regulation, and stress reactivity. *Journal of the American Academy of Child & Adolescent Psychiatry*, 48, 919-927.
- First, M. B., Spitzer, R.L., Gibbon, M., & Williams, J.B.W. (1996). *Structured Clinical Interview for DSM-IV Axis I Disorders: Non-patient edition (Version 2.0)*. New York, NY: New York State Psychiatric Institute, Biometrics Research.
- Fisher, P. A., Gunnar, M. R., Dozier, M., Bruce, J., & Pears, K. C. (2006). Effects of therapeutic interventions for foster children on behavioral problems, caregiver attachment, and stress regulatory neural systems. *Annals of the New York Academy of Sciences*, 1094, 215-225.
- Forbes E.E., Cohn J.F., Allen N.B., & Lewinsohn P.M. (2004). Infant affect during parent– infant interaction at 3 and 6 months: Differences between mothers and fathers and influence of parent history of depression. *Infancy*, 5, 61–84.
- Franken, I. H., Rassin, E., & Muris, P. (2007). The assessment of anhedonia in clinical and non-clinical populations: further validation of the Snaith–Hamilton Pleasure Scale (SHAPS). *Journal of affective disorders*, 99, 83-89.
- Fredrickson, B.L., & Levenson, R. W. (1998). Positive emotions speed recovery from the cardiovascular sequelae of negative emotions. *Cognition & Emotion*, 12, 191-220.
- Fries, E., Dettenborn, L., & Kirschbaum, C. (2009). The cortisol awakening response (CAR): Facts and future directions. *International Journal of Psychophysiology*, 72, 67-73.

- Geiss, A., Varadi, E., Steinbach, K., Bauer, H. W., & Anton, F. (1997). Psychoneuroimmunological correlates of persisting sciatic pain in patients who underwent discectomy. *Neuroscience Letters*, *237*, 65-68.
- Gerritsen, L., Geerlings, M. I., Bremmer, M. A., Beekman, A. T., Deeg, D. J., Penninx, B. W., & Comijs, H. C. (2009). Personality characteristics and hypothalamic-pituitary-adrenal axis regulation in older persons. *The American Journal of Geriatric Psychiatry*, *17*, 1077-1084.
- Gold, P. W., & Chrousos, G. P. (2002). Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. *Molecular psychiatry*, *7*, 254-275.
- Gold, P. W., & Chrousos, G. P. (2013). Melancholic and atypical subtypes of depression represent distinct pathophysiological entities: CRH, neural circuits, and the diathesis for anxiety and depression. *Molecular Psychiatry*, *18*, 632-634.
- Goldsmith, H.H., Reilly, J., Lemery, K.S., Longley, S., & Prescott, A. (1995). The Laboratory Temperament Assessment Battery: Preschool version. *Unpublished manuscript*.
- Goldstein, B. L., & Klein, D. N. (2014). A review of selected candidate endophenotypes for depression. *Clinical Psychology Review*, *34*, 417-427.
- Goodman, S. H., & Gotlib, I. H. (1999). Risk for psychopathology in the children of depressed mothers: a developmental model for understanding mechanisms of transmission. *Psychological Review*, *106*, 458 – 490.

- Goodman, S. H., Rouse, M. H., Connell, A. M., Broth, M. R., Hall, C. M., & Heyward, D. (2011). Maternal depression and child psychopathology: a meta-analytic review. *Clinical Child and Family Psychology Review, 14*, 1-27.
- Goodyer, I. M., Herbert, J., Altham, P. M. E., Pearson, J., Secher, S. M., & Shiers, H. M. (1996). Adrenal secretion during major depression in 8-to 16-year-olds, I. Altered diurnal rhythms in salivary cortisol and dehydroepiandrosterone (DHEA) at presentation. *Psychological medicine, 26*, 245-256.
- Goodyer, I. M., Herbert, J., Tamplin, A., & Altham, P. M. (2000). First-episode major depression in adolescents: Affective, cognitive and endocrine characteristics of risk status and predictors of onset. *British Journal of Psychiatry, 176*, 142-149.
- Goodyer, I. M., Park, R. J., & Herbert, J. (2001). Psychosocial and endocrine features of chronic first-episode major depression in 8–16 year olds. *Biological Psychiatry, 50*, 351-357.
- Gottesman, I. I., & Gould, T. D. (2003). The endophenotype concept in psychiatry: etymology and strategic intentions. *American Journal of Psychiatry, 160*, 636-645.
- Gould, M. S., Greenberg, T. E. D., Velting, D. M., & Shaffer, D. (2003). Youth suicide risk and preventive interventions: a review of the past 10 years. *Journal of the American Academy of Child & Adolescent Psychiatry, 42*, 386-405.
- Granger, D. A., Hibel, L. C., Fortunato, C. K., & Kapelewski, C. H. (2009). Medication effects on salivary cortisol: Tactics and strategy to minimize impact in behavioral and developmental science. *Psychoneuroendocrinology, 34*, 1437-1448.

- Greenberg, P. E., Kessler, R. C., Birnbaum, H. G., Leong, S. A., Lowe, S. W., Berglund, P. A., & Corey-Lisle, P. K. (2003). The economic burden of depression in the United States: how did it change between 1990 and 2000?. *Journal of Clinical Psychiatry, 64*, 1465-1475.
- Gribbin, C. E., Watamura, S. E., Cairns, A., Harsh, J. R., & LeBourgeois, M. K. (2012). The cortisol awakening response (CAR) in 2-to 4-year-old children: Effects of acute nighttime sleep restriction, wake time, and daytime napping. *Developmental Psychobiology, 54*, 412-422.
- Gruber, J., Culver, J. L., Johnson, S. L., Nam, J. Y., Keller, K. L., & Ketter, T. A. (2009). Do positive emotions predict symptomatic change in bipolar disorder?. *Bipolar Disorders, 11*, 330-336.
- Gruber, J., Johnson, S. L., Oveis, C., & Keltner, D. (2008). Risk for mania and positive emotional responding: too much of a good thing?. *Emotion, 8*, 23-33.
- Gunnar, M. R. & Donzella, B. (2002). Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology, 27*, 199–220.
- Gunnar, M. R., & Talge, N. M. (2007). Neuroendocrine measures in developmental research. In L. A. Schmidt & S. J. Segalowitz (Eds.), *Developmental Psychophysiology: Theory, Systems, and Methods*. (pp. 343–366). Cambridge: University Press.
- Gunnar, M. R., & Vazquez, D. M. (2001). Low cortisol and a flattening of expected daytime rhythm: Potential indices of risk in human development. *Development and Psychopathology, 13*, 515-538.

- Gunnar M.R. & White B. (2001). Salivary cortisol measures in infant and child assessment. In L.T Singer & P.S. Zeskind (Eds.), *Biobehavioral Assessment of the Newborn*. (p. 167-189). New York: Guilford Press.
- Halligan, S. L., Herbert, J., Goodyer, I. M., & Murray, L. (2004). Exposure to postnatal depression predicts elevated cortisol in adolescent offspring. *Biological Psychiatry*, *55*, 376–381.
- Halligan, S. L., Herbert, J., Goodyer, I., & Murray, L. (2007). Disturbances in morning cortisol secretion in association with maternal postnatal depression predict subsequent depressive symptomatology in adolescents. *Biological Psychiatry*, *62*, 40–46.
- Hammen, C. (2005). Stress and depression. *Annual Review of Clinical Psychology*, *1*, 293-319.
- Hammen, C.L. (2009). Children of depressed parents. In: I.H. Gotlib & C.L. Hammen (Eds.), *Handbook of depression* (pp.275-297). New York, NY: Guilford Press.
- Hardy, M.A. (1993). *Regression with Dummy Variables*. Newbury Park: Sage.
- Harris, T. O., Borsanyi, S., Messari, S., Stanford, K., Brown, G. W., Cleary, S. E., ... & Herbert, J. (2000). Morning cortisol as a risk factor for subsequent major depressive disorder in adult women. *The British Journal of Psychiatry*, *177*, 505-510.
- Hasler, G., Drevets, W. C., Manji, H. K., & Charney, D. S. (2004). Discovering endophenotypes for major depression. *Neuropsychopharmacology*, *29*, 1765-1781.

- Hauner, K. K., Adam, E. K., Mineka, S., Doane, L. D., DeSantis, A. S., Zinbarg, R., ... & Griffith, J. W. (2008). Neuroticism and introversion are associated with salivary cortisol patterns in adolescents. *Psychoneuroendocrinology*, *33*, 1344-1356.
- Hayden, E. P., Klein, D. N., Durbin, C. E., & Olino, T. M. (2006). Positive emotionality at age 3 predicts cognitive styles in 7-year-old children. *Development and Psychopathology*, *18*, 409-423.
- Heim, C., Ehlert, U., & Hellhammer, D. H. (2000). The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychoneuroendocrinology*, *25*, 1-35.
- Heim, C., Newport, D. J., Heit, S., Graham, Y. P., Wilcox, M., Bonsall, R., ... & Nemeroff, C. B. (2000). Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *Journal of the American Medical Association*, *284*, 592-597.
- Heim, C., Newport, D. J., Mletzko, T., Miller, A. H., & Nemeroff, C. B. (2008). The link between childhood trauma and depression: insights from HPA axis studies in humans. *Psychoneuroendocrinology*, *33*, 693-710.
- Heller, W., & Nitschke, J. B. (1998). The puzzle of regional brain activity in depression and anxiety: The importance of subtypes and comorbidity. *Cognition and Emotion*, *12*, 421-447.
- Hellhammer, J., Fries, E., Schweisthal, O. W., Schlotz, W., Stone, A. A., & Hagemann, D. (2007). Several daily measurements are necessary to reliably assess the cortisol rise after awakening: State-and trait components. *Psychoneuroendocrinology*, *32*, 80-86.

- Hill, E. M., Billington, R., & Krägeloh, C. (2013). The cortisol awakening response and the big five personality dimensions. *Personality and Individual Differences, 55*, 600-605.
- Holsboer, F. (2000). The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology, 23*, 477–501.
- Jacobs, N., Nicolson, N. A., Derom, C., Delespaul, P., Van Os, J., & Myin-Germeys, I. (2005). Electronic monitoring of salivary cortisol sampling compliance in daily life. *Life Sciences, 76*, 2431–2443.
- Jacobson, L. (2005). Hypothalamic-pituitary-adrenocortical axis regulation. *Endocrinology and Metabolism Clinics of North America, 34*, 271-292.
- Jessop, D. S., & Turner-Cobb, J. M. (2008). Measurement and meaning of salivary cortisol: a focus on health and disease in children. *Stress: The International Journal on the Biology of Stress, 11*, 114.
- Joiner Jr, T. E., Catanzaro, S. J., & Laurent, J. (1996). Tripartite structure of positive and negative affect, depression, and anxiety in child and adolescent psychiatric inpatients. *Journal of Abnormal Psychology, 105*, 401-409.
- Kagan, J. (1989). Temperamental contributions to social behavior. *American Psychologist, 44*, 668-674.
- Kagan, J. (1997). Temperament and the reactions to unfamiliarity. *Child Development, 68*, 139-143.
- Kagan, J., Reznick, J. S., & Snidman, N. (1987). The physiology and psychology of behavioral inhibition in children. *Child Development, 58*, 1459–1473.

- Kendler, K. S., Gardner, C. O., Gatz, M., & Pedersen, N. L. (2007). The sources of comorbidity between major depression and generalized anxiety disorder in a Swedish national twin sample. *Psychological Medicine, 37*, 453-462.
- Kendler, K. S., Gatz, M., Gardner, C. O., & Pedersen, N. L. (2006). Personality and major depression: a Swedish longitudinal, population-based twin study. *Archives of General Psychiatry, 63*, 1113-1120.
- Kessler, R.C., Berglund, P., Demler, O., Jin, R., Koretz, D., Merikangas, K.R.,... & Wang P.S. (2003). The epidemiology of major depressive disorder: Results from the National Comorbidity Study Replication (NCS-R). *Journal of the American Medical Association, 289*, 3095–3105.
- Kessler, R.C., Berglund, P., Demler, O., Jin, R., Merikangas, K.R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry, 62*, 593-602.
- Kessler, R. C., & Wang, P. S. (2002). Epidemiology of depression. In I. A. Gotlib & C. L. Hammen (Eds.), *Handbook of depression*. (pp. 23–42). New York, NY: Guilford Press.
- Klein, D. N., Kotov, R., & Bufferd, S. J. (2011). Personality and depression: explanatory models and review of the evidence. *Annual Review of Clinical Psychology, 7*, 269.
- Klein, D. N., Shankman, S. A., & McFarland, B. (2006). Classification of mood disorders. In D. J. Stein, D. J. Kupfer, & A. F. Schatzberg (Eds.), *The American*

Psychiatric Publishing textbook of mood disorders. Washington, DC: American Psychiatric Publishing.

Knorr, U., Vinberg, M., Kessing, L. V., & Wetterslev, J. (2010). Salivary cortisol in depressed patients versus control persons: a systematic review and meta-analysis. *Psychoneuroendocrinology*, *35*, 1275-1286.

Kudielka, B. M., Broderick, J. E., & Kirschbaum, C. (2003). Compliance with saliva sampling protocols: Electronic monitoring reveals invalid cortisol profiles in noncompliant subjects. *Psychosomatic Medicine*, *65*, 313–319.

Kudielka, Brigitte M, Hawkley, L. C., Adam, E. K., & Cacioppo, J. T. (2007). Compliance with ambulatory saliva sampling in the chicago health, aging, and social relations study and associations with social support. *Annals of Behavioral Medicine*, *34*, 209-216.

Kudielka, B. M., & Kirschbaum, C. (2003). Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. *Psychoneuroendocrinology*, *28*, 35-47.

Kumari, M., Shipley, M., Stafford, M., & Kivimaki, M. (2011). Association of diurnal patterns in salivary cortisol with all-cause and cardiovascular mortality: findings from the Whitehall II study. *The Journal of Clinical Endocrinology & Metabolism*, *96*, 1478-1485.

Kunz-Ebrecht, S. R., Kirschbaum, C., Marmot, M., & Steptoe, A. (2004). Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. *Psychoneuroendocrinology*, *29*, 516-528.

Laptook, R. S., Klein, D. N., Durbin, C. E., Hayden, E. P., Olino, T. M., & Carlson, G.

- (2008). Differentiation between low positive affectivity and behavioral inhibition in preschool-age children: A comparison of behavioral approach in novel and non-novel contexts. *Personality and Individual Differences, 44*, 758-767.
- Laptook, R. S., Klein, D. N., Olino, T. M., Dyson, M. W., & Carlson, G. (2010). Low positive affectivity and behavioral inhibition in preschool-age children: A replication and extension of previous findings. *Personality and Individual Differences, 48*, 547-551.
- Lamers, F., Vogelzangs, N., Merikangas, K. R., De Jonge, P., Beekman, A. T. F., & Penninx, B. W. J. H. (2013). Evidence for a differential role of HPA-axis function, inflammation and metabolic syndrome in melancholic versus atypical depression. *Molecular psychiatry, 18*, 692-699.
- Liang, K. Y., & Zeger, S. L. (1986). Longitudinal data analysis using generalized linear models. *Biometrika, 73*, 13–22.
- Lonigan, C. J., Carey, M. P., & Finch, A. J. (1994). Anxiety and depression in children and adolescents: negative affectivity and the utility of self-reports. *Journal of Consulting and Clinical Psychology, 62*, 1000 - 1008.
- Lonigan, C. J., Phillips, B. M., & Hooe, E. S. (2003). Relations of positive and negative affectivity to anxiety and depression in children: evidence from a latent variable longitudinal study. *Journal of Consulting and Clinical Psychology, 71*, 465-481.
- Lopez-Duran, N.L., Kovacs, M., & George, C.J. (2009). Hypothalamic–pituitary–adrenal axis dysregulation in depressed children and adolescents: A meta-analysis. *Psychoneuroendocrinology, 34*, 1272–1283.

- Lovejoy, M. C., Graczyk, P. A., O'Hare, E., & Neuman, G. (2000). Maternal depression and parenting behavior: A meta-analytic review. *Clinical Psychology Review, 20*, 561-592.
- Lupien, S. J., Ouellet-Morin, I., Trépanier, L., Juster, R. P., Marin, M. F., Francois, N., ... & Plusquellec, P. (2013). The DeStress for success program: effects of a stress education program on cortisol levels and depressive symptomatology in adolescents making the transition to high school. *Neuroscience, 249*, 74-87.
- Mackrell, S. V., Sheikh, H. I., Kotelnikova, Y., Kryski, K. R., Jordan, P. L., Singh, S. M., & Hayden, E. P. (2014). Child temperament and parental depression predict cortisol reactivity to stress in middle childhood. *Journal of Abnormal Psychology, 123*, 106-116.
- Mannie, Z. N., Harmer, C. J., & Cowen, P. J. (2007). Increased waking salivary cortisol levels in young people at familial risk of depression. *American Journal of Psychiatry, 164*, 617-621.
- McEwen, B. S., & Seeman, T. (1999). Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load. *Annals of the New York Academy of Sciences, 896*, 30-47.
- McEwen, B. S. (1998). Stress, adaptation, and disease: allostasis and allostatic load. *Annals of the New York Academy of Sciences, 840*, 33-44.
- Meaney, M. J. (2001). Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annual Review of Neuroscience, 24*, 1161-1192.

- Michaud, C.M., Murray, C.J.L., & Bloom, B.R. (2001). Burden of Disease—Implications for Future Research. *Journal of American Medical Association*, 285, 535–539.
- Michels, N., Sioen, I., De Vriendt, T., Huybrechts, I., Vanaelst, B., & De Henauw, S. (2012). Children's morning and evening salivary cortisol: pattern, instruction compliance and sampling confounders. *Hormone Research in Paediatrics*, 77, 27.
- Miller, R., Plessow, F., Kirschbaum, C., & Stalder, T. (2013). Classification criteria for distinguishing cortisol responders from nonresponders to psychosocial stress: Evaluation of salivary cortisol pulse detection in panel designs. *Psychosomatic Medicine*, 75, 832–840.
- Miller, G. A., & Rockstroh, B. (2013). Endophenotypes in psychopathology research: where do we stand?. *Annual Review of Clinical Psychology*, 9, 177-213.
- Mineka, S., Watson, D., & Clark, L. A. (1998). Comorbidity of anxiety and unipolar mood disorders. *Annual Review of Psychology*, 49, 377-412.
- Muris, P., & Dietvorst, R. (2006). Underlying personality characteristics of behavioral inhibition in children. *Child psychiatry and human development*, 36, 437-445.
- Munafò, M. R., Lee, L., Ayres, R., Flint, J., Goodwin, G., & Harmer, C. J. (2006). Early morning salivary cortisol is not associated with extraversion. *Personality and individual differences*, 40, 395-400.
- Nanni, V., Uher, R., & Danese, A. (2012). Childhood maltreatment predicts unfavorable course of illness and treatment outcome in depression: a meta-analysis. *American Journal of Psychiatry*, 169, 141-151.

- Nater, U. M., Hoppmann, C., & Klumb, P. L. (2010). Neuroticism and conscientiousness are associated with cortisol diurnal profiles in adults-role of positive and negative affect. *Psychoneuroendocrinology*, *35*, 1573-1577.
- Nelemans, S. A., Hale III, W. W., Branje, S. J., van Lier, P. A., Jansen, L. M., Platje, E., ... & Meeus, W. H. (2013). Persistent Heightened Cortisol Awakening Response and Adolescent Internalizing Symptoms: A 3-year Longitudinal Community Study. *Journal of Abnormal Child Psychology*.
- Nelson, C.A., de Haan, M., & Thomas, K.M. (2006). *Neuroscience of cognitive development*. Hoboken, NJ: John Wiley & Sons.
- O'Connor, T. G., Ben-Shlomo, Y., Heron, J., Golding, J., Adams, D., & Glover, V. (2005). Prenatal anxiety predicts individual differences in cortisol in pre-adolescent children. *Biological Psychiatry*, *58*, 211-217.
- O'Donnell, K. J., Glover, V., Jenkins, J., Browne, D., Ben-Shlomo, Y., Golding, J., & O'Connor, T. G. (2013). Prenatal maternal mood is associated with altered diurnal cortisol in adolescence. *Psychoneuroendocrinology*, *38*, 1630-1638.
- Olino, T. M., Klein, D. N., Dyson, M. W., Rose, S. A., & Durbin, C. E. (2010). Temperamental emotionality in preschool-aged children and depressive disorders in parents: associations in a large community sample. *Journal of Abnormal Psychology*, *119*, 468 - 478.
- Olino, T. M., Lopez-Duran, N. L., Kovacs, M., George, C. J., Gentzler, A. L., & Shaw, D. S. (2011). Developmental trajectories of positive and negative affect in children at high and low familial risk for depressive disorder. *Journal of Child Psychology and Psychiatry*, *52*, 792-799.

- Oswald, L. M., Zandi, P., Nestadt, G., Potash, J. B., Kalaydjian, A. E., & Wand, G. S. (2006). Relationship between cortisol responses to stress and personality. *Neuropsychopharmacology, 31*, 1583-1591.
- Peeters, F., Nicolson, N. A., & Berkhof, J. (2004). Levels and variability of daily life cortisol secretion in major depression. *Psychiatry Research, 126*, 1-13.
- Phillips, B. M., Lonigan, C. J., Driscoll, K., & Hooe, E. S. (2002). Positive and negative affectivity in children: A multitrait-multimethod investigation. *Journal of Clinical Child and Adolescent Psychology, 31*, 465-479.
- Penninx, B. W., Beekman, A. T., Corsi, A. M., Bremmer, M., Hoogendijk, W. J., Guralnik, J. M., ... & Bandinelli, S. (2007). Late-life depressive symptoms are associated with both hyperactivity and hypoactivity of the hypothalamo-pituitary-adrenal axis. *The American Journal of Geriatric Psychiatry, 15*, 522-529.
- Pizzagalli, D. A. (2014). Depression, stress, and anhedonia: toward a synthesis and integrated model. *Annual Review of Clinical Psychology, 10*, 393-423.
- Polk, D. E., Cohen, S., Doyle, W. J., Skoner, D. P., & Kirschbaum, C. (2005). State and trait affect as predictors of salivary cortisol in healthy adults. *Psychoneuroendocrinology, 30*, 261-272.
- Portella, M. J., Harmer, C. J., Flint, J., Cowen, P., & Goodwin, G. M. (2005). Enhanced early morning salivary cortisol in neuroticism. *American Journal of Psychiatry, 162*, 807-809.
- Pruessner, J. C., Hellhammer, D. H., & Kirschbaum, C. (1999). Burnout, perceived

stress, and cortisol responses to awakening. *Psychosomatic Medicine*, 61, 197-204.

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology*, 28, 916–931.

Pruessner, J. C., Wolf, O. T., Hellhammer, D. H., Buske-Kirschbaum, A., von Auer, K., Jobst, S., Kaspers, F., et al. (1997). Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. *Life Sciences*, 61, 2539-2549.

Rende, R. D. (1993). Longitudinal relations between temperament traits and behavioral syndromes in middle childhood. *Journal of the American Academy of Child & Adolescent Psychiatry*, 32, 287-290.

Roberts, A. D., Wessely, S., Chalder, T., Papadopoulos, A., & Cleare, A. J. (2004). Salivary cortisol response to awakening in chronic fatigue syndrome. *The British Journal of Psychiatry*, 184, 136-141.

Rohde, P., Lewinsohn, P.M., & Seeley, J.R. (1997). Comparability of Telephone and Face-to-Face Interviews in Assessing Axis I and II Disorders. *American Journal of Psychiatry*, 154, 1593–1598.

Rosmalen, J. G. M., Oldehinkel, A. J., Ormel, J., De Winter, A. F., Buitelaar, J. K., & Verhulst, F. C. (2005). Determinants of salivary cortisol levels in 10–12 year old children; a population-based study of individual differences. *Psychoneuroendocrinology*, 30, 483-495.

- Rosmond, R., & Björntorp, P. (2000). The hypothalamic–pituitary–adrenal axis activity as a predictor of cardiovascular disease, type 2 diabetes and stroke. *Journal of Internal Medicine*, 247, 188-197.
- Rothbart, M. K., & Bates, J. E. (2006). Temperament. In N. Eisenberg, W. Damon, & R. M. Lerner (Eds.), *Handbook of child psychology Vol 3: Social, emotional, and personality development* (6th ed., pp. 99-166). Hoboken, NJ: John Wiley & Sons Inc.
- Rutter, M., Bailey, A. & Lord, C. (2003). *Social Communication Questionnaire (SCQ)*. Western Psychological Services.
- Seifer, R., Sameroff, A. J., Barrett, L. C., & Krafchuk, E. (1994). Infant temperament measured by multiple observations and mother report. *Child Development*, 65, 1478-1490.
- Shankman, S. A., Katz, A. C., DeLizza, A. A., Sarapas, C., Gorka, S. M., & Campbell, M. L. (2014). The Different Facets of Anhedonia and Their Associations with Different Psychopathologies. In M.S. Ritsner (Ed.), *Anhedonia: A Comprehensive Handbook Volume I* (pp. 3-22). Netherlands: Springer Netherlands.
- Shankman, S.A., & Klein, D.N. (2003). The comorbidity between depression and anxiety: An evaluation of the tripartite, approach-withdrawal, and valence-arousal models. *Clinical Psychology Review*, 23, 605-637.
- Shankman, S. A., Tenke, C. E., Bruder, G. E., Durbin, C. E., Hayden, E. P., & Klein, D. N. (2005). Low positive emotionality in young children: Association with EEG asymmetry. *Development and Psychopathology*, 17, 85-98.

- Shannon, K.E., Beauchaine, T.P., Brenner, S.L., Neuhaus, E., & Gatzke-Kopp, L. (2007). Familial and temperamental predictors of resilience in children at risk for conduct disorder and depression. *Developmental Psychopathology, 19*, 701–727.
- Shiner, R., & Caspi, A. (2003). Personality differences in childhood and adolescence: Measurement, developments, and consequences. *Journal of Child Psychology and Psychiatry, 44*, 2-32.
- Silberg, J., & Rutter, M. (2002). Nature-nurture interplay in the risks associated with parental depression. In C. L. Hammen & I. A. Gotlib (Eds.), *Children of depressed parents: Mechanisms of risk and implications for treatment*. (pp. 13–36). Washington, DC: American Psychological Association.
- Smith, V. C., & Dougherty, L. R. (2014). Noisy spit: Parental noncompliance with child salivary cortisol sampling. *Developmental psychobiology, 56*, 647-656.
- Snaith, R. P., Hamilton, M., Morley, S., Humayan, A., Hargreaves, D., & Trigwell, P. (1995). A scale for the assessment of hedonic tone: the Snaith-Hamilton Pleasure Scale. *The British Journal of Psychiatry, 167*, 99-103.
- Spence, S. H., Najman, J. M., Bor, W., O'Callaghan, M. J., & Williams, G. M. (2002). Maternal anxiety and depression, poverty and marital relationship factors during early childhood as predictors of anxiety and depressive symptoms in adolescence. *Journal of Child Psychology and Psychiatry, 43*, 457-469.
- Stalder, T., Bäumlér, D., Miller, R., Alexander, N., Kliegel, M., & Kirschbaum, C. (2013). The cortisol awakening response in infants: Ontogeny and associations with development-related variables. *Psychoneuroendocrinology, 38*, 552-559.

- Step toe, A., Leigh Gibson, E., Hamer, M., & Wardle, J. (2007). Neuroendocrine and cardiovascular correlates of positive affect measured by ecological momentary assessment and by questionnaire. *Psychoneuroendocrinology*, *32*, 56-64.
- Step toe, A., & Wardle, J. (2005). Positive affect and biological function in everyday life. *Neurobiology of Aging*, *26*, 108-112.
- Step toe, A., Wardle, J., & Marmot, M. (2005). Positive affect and health-related neuroendocrine, cardiovascular, and inflammatory processes. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 6508-6512.
- Stetler, C., & Miller, G. E. (2005). Blunted cortisol response to awakening in mild to moderate depression: regulatory influences of sleep patterns and social contacts. *Journal of Abnormal Psychology*, *114*, 697.
- Stetler, C., & Miller, G. E. (2011). Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. *Psychosomatic Medicine*, *73*, 114-126.
- Stifter C.A., Putnam S., & Jahromi L. (2008). Exuberant and inhibited toddlers: stability of temperament and prediction to behavior problems. *Development and Psychopathology*, *20*, 401-421.
- Stone, A. A., Schwartz, J. E., Smyth, J., Kirschbaum, C., Cohen, S., Hellhammer, D., & Grossman, S. (2001). Individual differences in the diurnal cycle of salivary free cortisol: a replication of flattened cycles for some individuals. *Psychoneuroendocrinology*, *26*, 295-306.
- Swendsen, J. D., & Merikangas, K. R. (2000). The comorbidity of depression and substance use disorders. *Clinical Psychology Review*, *20*, 173-189.

- Talge, N. M., Donzella, B., Kryzer, E. M., Gierens, A., & Gunnar, M. R. (2005). It's not that bad: Error introduced by oral stimulants in salivary cortisol research. *Developmental Psychobiology, 47*, 369-376.
- Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *Journal of Psychosomatic Research, 53*, 865-871.
- Tugade, M. M., & Fredrickson, B. L. (2004). Resilient individuals use positive emotions to bounce back from negative emotional experiences. *Journal of Personality and Social Psychology, 86*, 320-333.
- Van den Bergh, B. R. H., Van Calster, B., Smits, T., Van Huffel, S., & Lagae, L. (2008). Antenatal maternal anxiety is related to HPA-axis dysregulation and self-reported depressive symptoms in adolescence: a prospective study on the fetal origins of depressed mood. *Neuropsychopharmacology, 33*, 536-545.
- van Os, J., Jones, P., Lewis, G., Wadsworth, M., & Murray, R. (1997). Developmental precursors of affective illness in a general population birth cohort. *Archives of General Psychiatry, 54*, 625-631.
- van Santen, A., Vreeburg, S. A., Van der Does, A. J., Spinhoven, P., Zitman, F. G., & Penninx, B. W. (2011). Psychological traits and the cortisol awakening response: results from the Netherlands Study of Depression and Anxiety. *Psychoneuroendocrinology, 36*, 240-248.
- Veen, G., van Vliet, I. M., DeRijk, R. H., Giltay, E. J., van Pelt, J., & Zitman, F. G. (2011). Basal cortisol levels in relation to dimensions and DSM-IV categories of depression and anxiety. *Psychiatry Research, 185*, 121-128.

- Vreeburg, S. A., Hartman, C. A., Hoogendijk, W. J., van Dyck, R., Zitman, F. G., Ormel, J., & Penninx, B. W. (2010). Parental history of depression or anxiety and the cortisol awakening response. *The British Journal of Psychiatry, 197*, 180-185.
- Vreeburg, S. A., Hoogendijk, W. J., DeRijk, R. H., van Dyck, R., Smit, J. H., Zitman, F. G., & Penninx, B. W. (2013). Salivary cortisol levels and the 2-year course of depressive and anxiety disorders. *Psychoneuroendocrinology, 38*, 1494-1502.
- Vreeburg, S. A., Hoogendijk, W. J., van Pelt, J., DeRijk, R. H., Verhagen, J. C., van Dyck, R., ... & Penninx, B. W. (2009). Major depressive disorder and hypothalamic-pituitary-adrenal axis activity: results from a large cohort study. *Archives of General Psychiatry, 66*, 617-626.
- Vreeburg, S. A., Zitman, F. G., van Pelt, J., DeRijk, R. H., Verhagen, J. C., van Dyck, R., ... & Penninx, B. W. (2010). Salivary cortisol levels in persons with and without different anxiety disorders. *Psychosomatic Medicine, 72*, 340-347.
- Vrshek-Schallhorn, S., Doane, L. D., Mineka, S., Zinbarg, R. E., Craske, M. G., & Adam, E. K. (2013). The cortisol awakening response predicts major depression: predictive stability over a 4-year follow-up and effect of depression history. *Psychological Medicine, 43*, 483-493.
- Wardenaar, K. J., Vreeburg, S. A., van Veen, T., Giltay, E. J., Veen, G., Penninx, B. W., & Zitman, F. G. (2011). Dimensions of depression and anxiety and the hypothalamo-pituitary-adrenal axis. *Biological Psychiatry, 69*, 366-373.
- Watamura, S. E., Donzella, B., Alwin, J., & Gunnar, M. R. (2003). Morning-to-afternoon increases in cortisol concentrations for infants and toddlers at child care: Age differences and behavioral correlates. *Child Development, 74*, 1006-1020.

- Weissman, M.M., Wickramaratne, P., Nomura, Y., Warner, V., Pilowsky, D., & Verdeli, H. (2006). Offspring of depressed parents: 20 years later. *American Journal of Psychiatry, 163*, 1001-1008.
- Wilhelm, I., Born, J., Kudielka, B.M., Schlotz, W., & Wüst, S. (2007). Is the cortisol awakening rise a response to awakening? *Psychoneuroendocrinology, 32*, 358–366.
- Williams, J. B., Gibbon, M., First, M. B., Spitzer, R. L., Davies, M., Borus, J., Howes, M. J., et al. (1992). The Structured Clinical Interview for DSM-III-R (SCID). II. Multisite test-retest reliability. *Archives of General Psychiatry, 49*, 630–636.
- Wüst, S., Federenko, I., Hellhammer, D. H., & Kirschbaum, C. (2000a). Genetic factors, perceived chronic stress, and the free cortisol response to awakening. *Psychoneuroendocrinology, 25*, 707-720.
- Wüst, S., Wolf, J., Hellhammer, D.H., Federenko, I., Schommer, N., Kirschbaum, C., (2000b). The cortisol awakening response—normal values and confounds. *Noise Health 2*, 79–88.
- Young, E. A., Vazquez, D., Jiang, H., & Pfeffer, C. R. (2006). Saliva cortisol and response to dexamethasone in children of depressed parents. *Biological Psychiatry, 60*, 831–836.
- Youngstrom, E., Izard, C., & Ackerman, B. (1999). Dysphoria-related bias in maternal ratings of children. *Journal of Consulting and Clinical Psychology, 67*, 905-91.