ABSTRACT

Title of Thesis: PARENTAL DEPRESSION, PARENTING, AND CORTISOL REACTIVITY IN PRESCHOOLERS

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This study systematically examined individual differences in stress sensitivity as a vulnerability marker for depression in young children. We collected five salivary cortisol samples from 142 preschool-age children who were exposed to a laboratory stressor paradigm. Parents (*N* = 88 with family history of depression) completed clinical interviews and an observational parent-child interaction task. We found that hostile parenting behavior moderated the relation between maternal depression and offspring cortisol. Specifically, the offspring of mothers who had a history of depression during the child's life and whose mothers exhibited hostility evidenced increasing cortisol levels in response to the stressor paradigm. Conversely, the offspring of mothers who had no history of depression and whose mothers exhibited hostility evidenced decreasing cortisol levels in response to the stressor. The data highlight the critical role of the early caregiving environment on offspring's developing stress system and add to our understanding of transmission of depression risk.

PARENTAL DEPRESSION, PARENTING, AND CORTISOL REACTIVITY IN PRESCHOOLERS

By

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Chapter 1: Introduction

Clinical Significance of Depression

Depression is a chronic and recurrent mental illness that incurs grave negative outcomes on affected individuals, as well as the larger society. Major depressive disorder (MDD) is ranked as a leading cause of disability worldwide and the second largest cause of disability in women in the United States (Michaud, Murray, & Bloom, 2001). Depression is associated with significant impairment in physical, social, and occupational functioning, and higher rates of mortality and morbidity (Cuijpers, 2001; Wells et al., 1989). There is growing evidence for the association between depression and physical health conditions, including coronary heart disease (Evans et al., 2005; Ormel et al., 2007; Wulsin & Singal, 2003), obesity (Freedland & Carney, 2009), and diabetes (Knol et al., 2006). Furthermore, depression bears a considerable economic burden, contributing to the rising societal costs of primary and mental healthcare (Greenberg & Birnbaum, 2005).

Depression affects a significant number of individuals. The lifetime prevalence of major depression is approximately 20.8% in adults with its roots often emerging in childhood and adolescence (Kessler et al., 2005; Kessler & Wang, 2009). The rates of depression in childhood are relatively low (0-3%) (Costello, Foley, & Angold, 2006; Egger & Angold, 2006) and increase substantially in adolescence to rates comparable to those observed in adults (Lewinsohn, Hops, Roberts, Seeley, & Andrews, 1993; Rudolph, 2009). Depression is highly comorbid with other psychiatric disorders, including anxiety disorders (59%), impulse control disorders (32%), and substance-use disorders (24%) (Kessler & Wang, 2009). Clearly, a significant portion of the population is affected

adversely by depression and thus research examining its etiology and mechanisms of risk is warranted.

Offspring of Depressed Parents

The offspring of parents with a lifetime history of depression are three times more likely to develop depression and are at greater risk for anxiety and substance-use disorders (Hammen, 2009; Lieb, Isensee, Höfler, Pfister, & Wittchen, 2002; Weissman et al., 2006). Children of depressed parents are also at greater risk for medical health problems, earlier mortality and evidence greater social impairment than children of non-depressed parents (Weissman, Warner, Wickramaratne, Moreau, & Olfson, 1997; Weissman et al., 2006). Furthermore, the offspring of depressed parents who develop depression evidence a more severe and chronic course than depressed offspring of parents with no history of depression (Hammen, 2009). Further identification of those at risk for depression and, in particular, the mechanisms of risk are of utmost importance for prevention and/or early intervention efforts (Cuijpers, van Straten, Smit, Mihalopoulos, & Beekman, 2008). In order to develop preventive interventions and early interventions for children of depressed parents, it is imperative to understand the underlying mechanisms for their increased risk of mental and physical disorders. The aim of this investigation is to focus on one of the body's major stress-response systems, the hypothalamic-pituitary-adrenal (HPA) axis, as one potential mechanism in the intergenerational transmission of risk for depression.

A Hypothesized Mechanism of Risk: Dysregulation of the HPA Axis

One hypothesized mechanism for the intergenerational transmission of risk is abnormalities in the HPA axis (Holsboer, 2000). The relation between depression and

HPA axis dysregulation is one of the most consistent and robust biological correlates in depressed adults (Gunnar & Vazquez, 2006; Thase, 2009). Furthermore, a recent meta-analytic review reported abnormalities of the HPA axis in depressed children and adolescents (Lopez-Duran, Kovacs, & George, 2009) and has even been observed in depressed preschoolers (Luby et al., 2003). It has been hypothesized that dysregulation of the HPA axis is a mechanism for the increased vulnerability to life stress observed in depression, which suggests a possible explanation as to why stress and depression are consistently related (Hammen, 2009). Thus, individual differences in HPA axis function and reactivity to stress are important factors in understanding vulnerability for depression (Gunnar & Vazquez, 2006; Monroe, Slavich, & Georgiades, 2009).

Overview of the HPA axis

For decades, researchers have investigated the HPA axis, as it is one of two primary stress response systems. The HPA axis regulates several key systems in the body, including metabolism, immune system functioning, and the cardiovascular system (Dickerson & Kemeny, 2004). When faced with a threat, the HPA axis functions as an adaptive system that maintains homeostasis and promotes short-term survival in the organism (Gunnar &Vazquez, 2006).

Activation of the HPA axis involves a cascade of neurobiological events (Gunnar & Quevedo, 2007). First, the paraventricular nucleus of the hypothalamus releases corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) into the anterior pituitary. The CRH and AVP then stimulate the release of adrenocorticotropic hormone (ACTH). The ACTH then causes an increase in the synthesis and release of glucocorticoids from the adrenal cortex, including cortisol, the primary stress hormone in

humans (Gunnar & Vazquez 2006; Meaney, 2001; Thase, 2009). Cortisol binds to two types of receptors: mineralocorticoid and glucocorticoid (Gunnar & Quevedo, 2007). The effects of cortisol depend on receptor type. One important function of glucocorticoid receptors (GRs) is to regulate the negative feedback loop of the HPA axis. Activation of the GRs inhibits the further synthesis and release of CRH, which then terminates the HPA response. When faced with a psychological threat, the limbic system (including the amygdala, hippocampus, and prefrontal cortex) perceives the event as stressful and then activates the paraventricular nucleus of the hypothalamus (Gunnar & Quevedo, 2007).

The HPA axis exhibits a 24 hour circadian rhythm in which cortisol levels peak 30 minutes after awakening, fall over the course of the day, and reach the nadir in the evening before bedtime (Gunnar & Vazquez, 2006; Thase, 2009). This diurnal rhythm influences cortisol reactivity throughout the course of the day. Specifically, the HPA axis is more reactive when basal cortisol levels are lower in the afternoon and evening hours (Gunnar & Vazquez, 2006). During the preschool years, when young children acquire a sleep routine that excludes daytime naps, cortisol levels begin to regulate and follow the aforementioned pattern of HPA axis activity observed in adults (Gunnar & Vazquez, 2006).

Although the HPA axis serves an adaptive function during periods of acute stress, prolonged periods of HPA axis hyperactivity are associated with several negative outcomes, including damage of hippocampal neurons resulting in memory problems, inhibition of the immune system, and development of adverse physical health conditions (Dickerson & Kemeny 2004; McEwen, 1998). Furthermore, dysregulation of the HPA axis seems to be related to the etiology and development of several stress-related

psychiatric disorders, including depression (Meaney, 2001). It is important to investigate further the development and functioning of the HPA axis and the role it plays in risk for depression.

Depression and HPA Axis

Studies have demonstrated a relatively consistent relation between depression and HPA axis dysregulation (Burke, Davis, Otte, & Mohr, 2005; Lopez-Duran et al., 2009; Thase, 2009). Depression has been linked with various abnormalities in HPA axis functioning, including differences in basal levels, diurnal rhythm, and reactivity to psychological stressors and pharmacological challenge tests (Burke et al., 2005; Thase, 2009). In studies of depressed adults, there have been reports of HPA axis hyperactivity (Bhagwagar, Hafizi, & Cowen, 2005; Christensen & Kessing, 2001; Gillespie & Nemeroff, 2005; Holsboer et al., 1986), as well as hypoactivity (Stetler & Miller, 2005). In a recent meta-analysis, Burke and colleagues (2005) observed abnormalities in stress reactivity in depressed adults. Seven laboratory studies that implemented psychological stressor tasks (cognitive and public speaking) were included in the analysis. Depressed adults exhibited higher cortisol levels during the recovery period (at least 25 minutes after the presentation of the stressor) than non-depressed adults. Thus, across the seven studies, the depressed adults demonstrated a dysregulated pattern of HPA axis reactivity.

Research examining HPA axis dysfunction in depressed youth is much more limited, but findings generally support such an association. Specifically, a recent meta-analytic review reported higher basal cortisol levels and higher levels of cortisol following the Dexamethasone Suppression Test (DST) in depressed youth compared to non-depressed youth (Lopez-Duran et al., 2009). Failure to observe decreased cortisol

levels following the administration of the DST suggests a dysregulation of the negative feedback mechanism of the HPA axis (Burke et al., 2005; Gillespie & Nemeroff, 2005; Lopez-Duran et al., 2009).

To our knowledge, only two investigations examined the relation between pediatric depression and cortisol reactivity to a laboratory psychological stressor (for a review, see Lopez-Duran et al., 2009). Rao, Hammen, Ortiz, Chen, and Poland (2008) found that depressed adolescents exhibited higher peak cortisol levels in response to a social stressor compared to non-depressed adolescents. In addition, Luby and colleagues (2003, 2004) found increasing cortisol reactivity to both a parental separation and frustrating laboratory task in depressed preschoolers compared to psychiatric and no-disorder comparison groups. Overall, these studies support HPA axis abnormalities, including increased cortisol reactivity, in both depressed adults and depressed youth.

Parental Depression and Offspring HPA Axis Functioning

The offspring of depressed parents have been widely studied in an effort to identify mechanisms of the intergenerational transmission of risk for depression. One hypothesized mechanism is the transmission of a sensitive stress-response system to the child that confers risk for later depression. From as early as 24 hours after birth, newborns of depressed mothers display outcomes that mirror those of adults with depression, including a biochemical profile with elevated basal cortisol levels (Diego et al., 2004; Field, Diego, Hernandez-Reif, Vera, & Gil, 2004; Field, 1998; Lundy et al., 1999). Thus, from a young age, the offspring of depressed parents exhibit dysregulated HPA axis activity. Further evidence of elevated basal cortisol levels (baseline samples in laboratory studies or morning samples from home assessments) in offspring of depressed

parents have been reported in infants (Brennan et al., 2008; Bugental, Martorell, & Barraza, 2003; Feldman et al., 2009), preschoolers (Essex, Klein, Eunsuk, & Kalin, 2002), prepubertal children (Young, Vazquez, Jian, & Pfeffer, 2006), adolescents (Halligan, Herbert, Goodyer, & Murray, 2004), and young adults (Mannie, Harmer, & Cowen, 2007). Furthermore, Halligan, Herbert, Goodyer, and Murray (2007a) found that elevated morning basal cortisol levels at age 13 predicted depressive symptoms at age 16 in adolescents of mothers with depression. These prospective findings provide further support for the connection between HPA axis dysregulation and vulnerability for depression in at-risk offspring.

Significance of physiological reactivity to stress. Most of the research in the offspring of depressed parents has focused on basal cortisol levels; however, it has been hypothesized that individuals' *responsivity* or *reactivity* to stressors, in particular, may underlie vulnerability to developing depression. This hypothesis is consistent with the large literature supporting an association between exposure to life stress and depression onset and recurrence (Brown & Harris, 1978; Hammen, 2009; Monroe et al., 2009), which also appears to be at least partially causal (Kendler, Karkowski, & Prescott, 1999). Moreover, given the significant variability in individuals' physiological reactivity to stress (Gunnar & Quevedo, 2007), it is hypothesized that certain individuals are more susceptible to the depressogenic effects of stress at both the biological and behavioral levels (Dougherty, Klein, & Davila, 2004; Dougherty, Klein, Congdon, Canli, & Hayden, 2010; Gotlib, Joormann, Minor, & Hallmayer, 2008; Gunnar & Quevedo, 2007; Monroe et al., 2009). Therefore, it is important to assess individual differences in stress sensitivity in at-risk offspring in order to elucidate intergenerational transmission of risk.

Offspring of depressed parents and cortisol reactivity. Research examining stress reactivity in the offspring of depressed parents is quite sparse, and the little existing research leaves many unanswered questions due to conflicting findings, varying sample characteristics (i.e., age), and potential moderating factors, including parental characteristics, parenting, and exposure to life stress. Infants of mothers with a lifetime history of depression (Azar, Paquette, Zoccolillo, Baltzer, & Tremblay, 2007) and peripartum depression (Brennan et al., 2008) have been found to exhibit increased cortisol reactivity to an arm restraint stressor task. In addition, two studies have observed higher cortisol reactivity in infants of mothers who were both depressed and anxious postpartum (Brennan et al., 2008; Feldman et al., 2009).

Furthermore, a few studies reported that parental depressive *symptoms* were related to a hypoactive (i.e., blunted) pattern of activity in the offspring (Badanes, Watamura, & Hankin, 2011; Bouma, Ormel, Verhulst, & Oldehinkel, 2011; Fernald, Burke, & Gunnar, 2008; Gump et al., 2009). Fernald and colleagues (2008) found that higher maternal depressive symptoms were related to reduced basal cortisol levels and lower cortisol reactivity in young children (between 2.5-6 years). These findings were based on a sample of low-income families and the male children exhibited greater evidence of hypocortisolism than the female children (Fernald et al., 2008). Furthermore, Gump and colleagues (2009) found that mothers who reported chronically elevated depressive symptoms for 10 years since childbirth had children with significantly lower basal cortisol levels. In a recent study, Badanes and colleagues (2011) found that clinical levels of maternal depressive symptoms were related to a blunted pattern of cortisol reactivity in a sample of preschool-age children. Additionally, Bouma and colleagues

(2011) found that parental depressive symptoms were related to blunted cortisol reactivity in female adolescent offspring.

It is important to note that all of these studies focused on parental depressive symptoms during the child's life, rather than parental diagnostic history, which may be related to different neuroendocrine profiles in the offspring. Although these findings appear inconsistent with the evidence supporting hypercortisolaemia in depression, they are consistent with the large body of research demonstrating an association between chronic stress, which is inherent in chronic exposure to parental depression, and blunted cortisol levels (e.g., Ronsaville et al., 2006). Despite these findings demonstrating a blunted pattern of cortisol in the offspring, we hypothesize increased cortisol reactivity in the offspring of depressed parents, given the larger support for hyperactivity than hypoactivity in depression (Burke et al., 2005; Lopez-Duran et al., 2009; Thase, 2009).

In summary, there are currently only a very limited number of studies examining cortisol reactivity to stress in the offspring of depressed parents, and findings are somewhat mixed. It is likely that multiple factors are involved in the origins of cortisol reactivity, and an investigation into the relation between parental depression history and offspring cortisol reactivity requires a multifactorial approach. Next, we will briefly review the potential developmental origins of stress responsivity, including familial, possibly genetic, and/or environmental factors.

Potential Origins of Cortisol Reactivity

Familial and genetic origins. As reviewed above, a number of studies have found unique effects of parental depression history on offspring HPA axis function, independent of parenting (Azar et al., 2007; Mannie et al., 2007), which provides support for the

heritability of HPA axis function. Furthermore, twin studies have found a moderate degree of heritability in basal cortisol levels (Bartels, de Geus, Kirschbaum, Sluyter, & Boomsma, 2003; Young, Aggen, Prescott, and Kendler, 2000) and cortisol reactivity (Steptoe, van Jaarsveld, Semmler, Plomin, & Wardle, 2009). Recent studies have also revealed relations between specific genes and HPA axis reactivity (e.g., Dougherty et al., 2010; Gotlib et al., 2008).

Environmental origins. A significant contribution to the *causal* role of environmental influences on the regulation of the HPA axis has emerged from animal research (e.g., Francis, Diorio, Liu, & Meaney, 1999; Meaney, 2001). This research has provided considerable support for both the detrimental and ameliorative effects of early care on offspring neuroendocrine function (Gunnar & Donzella, 2002; Gunnar & Vazquez, 2006; Higley, Suomi, & Linnoila, 1992).

Consistent with the animal research, studies have also observed a relation between early adverse experiences, including sexual abuse and maltreatment, and HPA axis dysregulation in humans, which appear to have lasting effects on the developing HPA axis system (Heim, Newport, Mletzko, Miller, & Nemeroff, 2008; Goodman & Brand, 2009). In addition to severe stressors, several specific parental caregiving behaviors as well as parenting styles and attachment quality have been found to contribute to offspring HPA axis functioning (Azar et al., 2007; Bugental et al., 2003; Dougherty, Klein, Rose, & Laptook, 2011; Ellenbogen & Hodgins, 2009; Gunnar & Donzella, 2002; Gunnar, Larson, Herstgaard, Harris, & Brodersen, 1992; Gunnar & Quevedo, 2007; Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996). For instance, Ellenbogen and Hodgins (2009) found that low levels of parental structure (i.e. consistency) during middle

childhood predicted increased cortisol reactivity in adolescents. Furthermore, several studies have observed a significant relation between low parental sensitivity, characterized by low parental support/engagement and high parental hostility, and elevated offspring basal cortisol levels (Bugental et al., 2003; Murray, Halligan, Goodyer, & Herbert, 2010), as well as greater reactivity (Feldman et al., 2009). Other studies have examined the relations between harsh and intrusive parenting styles and offspring's stress reactivity. Infants of overcontrolling mothers (Azar et al., 2007) and mothers who frequently used corporal punishment (Bugental et al., 2003) exhibited increased cortisol reactivity to laboratory stressors. It is important to note that harsh parenting styles have also been found to be related to risk for depression in children. Specifically, in a recent meta-analysis, McLeod, Weisz, and Wood (2007) found that parental hostility was the strongest predictor of childhood depression. In sum, the research supports the environmental effects of low parental support and high parental hostility on offspring's neuroendocrine development (Gunnar & Quevedo, 2007).

Given the significant environmental contributors on offspring neuroendocrine function, it is difficult to determine whether findings on the deleterious effects of maternal depression on offspring neuroendocrine function are due to a familial, possibly genetic, liability for the disorder or to subsequent disruptions in parenting as a result of the parent's depression (e.g., Ashman, Dawson, Panagiotindes, Yamada, & Wilkinson, 2002; Essex et al., 2002; Halligan et al., 2004). For instance, depressed mothers have been found to display higher levels of negative affect and hostile behaviors (Lovejoy, Graczyk, O'Hare, & Neuman, 2000) and to be less responsive to their infants' behavioral

cues and more negative during interactions (Murray, Fiori-Cowley, & Hooper, 1996) compared to non-depressed mothers.

A limited number of studies has examined the influence of both parental depression diagnostic status and parenting quality on offspring's HPA axis functioning (Azar et al., 2007; Dougherty et al., 2011; Feldman et al., 2009; Kaplan, Evans, & Monk, 2008; Murray et al., 2010). Three studies have observed main effects of caregiving behaviors above and beyond parental diagnostic status on offspring's HPA axis functioning (Azar et al., 2007; Feldman et al., 2009; Murray et al., 2010), and one study found that maternal sensitivity moderated the relation between parental psychiatric illness (depression or anxiety) and infants' basal cortisol levels, such that only the infants of mothers with a psychiatric diagnosis *and* who were less sensitive exhibited significantly higher basal cortisol levels (Kaplan et al., 2008).

Clearly, more work is needed in investigating the unique and joint, interactive, effects of parental depression history and maladaptive parenting behaviors on offspring's neuroendocrine functioning, particularly beyond infancy. Recently, Dougherty and colleagues (2011) conducted the first study of the interactive effects of parental depression history and parenting on offspring cortisol reactivity in preschoolers. They found that the combination of parental depression history and high parental hostility was associated with increased offspring cortisol reactivity in preschool-age children. Furthermore, the moderating effect of parental hostility was found to be specific to children who were exposed to maternal depression during the first few years of life.

Dougherty and colleagues (2011) made a significant contribution to the literature as their study was the first to examine the interactive effects of parental depression

history and parenting on stress reactivity in children beyond infancy as well as highlighting the importance of exposure to maternal depression during the child's life. In light of these important findings, there are methodological limitations to consider. First, the study did not utilize a standardized stressor task that could be used as a reference for understanding cortisol reactivity. Additionally, the sample was a relatively homogeneous sample consisting of three-year old children from predominantly white, middle class, two-parent families. Lastly, a limited number of fathers with depression were included in the sample, which limited power in the analyses examining the specificity of findings related to paternal depression.

Chapter 2: Purpose of Current Study

It has been hypothesized that individuals' *responsivity* or *reactivity* to stressors, in particular, may underlie vulnerability to developing depression. However, few studies have assessed cortisol reactivity in young children beyond infancy (Dougherty et al., 2011; Hankin, Badanes, Abela, & Watamura, 2010; Kryski, Smith, Sheikh, Singh, & Hayden, 2011), and to date only one study has assessed cortisol reactivity to a laboratory paradigm in preschool-age children at risk for depression. Assessing cortisol reactivity during early childhood is particularly important for several reasons: (1) given that depression is rare in preschool-age children, we can better isolate risk or vulnerability factors during this developmental period (Egger & Angold, 2006); (2) early perturbations in HPA axis functioning have been shown to have lasting effects on the developing child (Heim et al., 2008; Meaney, 2001); and (3) early identification of those at risk for depression and a greater understanding of the mechanisms of risk may lead to the development of more effective prevention and early intervention programs.

Given the complexity of the development and regulation of the HPA axis, familial (possibly genetic) and environmental factors likely both contribute to individual differences in stress reactivity. However, only a few studies have examined the influence of both parental depression diagnostic status and parenting quality on offspring's HPA axis functioning, with only one study assessing preschool-age offspring. The present study aimed to build upon and replicate Dougherty and colleagues' (2011) findings by addressing the aforementioned limitations in a larger, more diverse high-risk sample of preschool-age offspring of depressed parents. Specifically, we assessed young children's cortisol reactivity in response to a developmentally appropriate stressor paradigm, using

the only standardized task that has been found to be effective in eliciting a mean cortisol increase in preschool-age children (Kryski, Smith, Sheikh, Singh, & Hayden, 2011)[1]. In addition, the current study included a comprehensive assessment of multiple cortisol samples following the presentation of a psychological stressor, which included four post-stressor samples to better capture children's peak and recovery cortisol levels. This is critical as salivary cortisol levels reach their peak sometime between 20-40 minutes following the presentation of the laboratory stressor (Dickerson & Kemeny, 2004). Nevertheless, previous studies with young children have generally assessed cortisol reactivity only through differences in one pre-stressor and one post-stressor saliva samples, which limits the ability to assess cortisol reactivity to the stressor. We also conducted our cortisol reactivity assessment during the child's second visit to the laboratory with the same experimenter in an effort to minimize the novelty of the laboratory visit. Furthermore, we explored the influence of multiple parenting dimensions on children's stress response, examining the interactive effects of parental depression history with parental hostility and parental support. We also recruited more fathers with a history of depression to allow for further examination of the specificity of parental depression findings. To examine potential developmental differences in HPA axis functioning, we examined cortisol reactivity in a wider age range of preschool-age offspring (3-5 years old). Lastly, we attempted to replicate Dougherty and colleagues' (2011) findings in a more diverse sample of children and their biological parents.

[1] To date, two studies have observed increasing cortisol reactivity in preschool-age children (Hankin et al., 2010; Kryski et al. 2011). Although Hankin and colleagues (2010) observed mean increases in children's cortisol, their stress reactivity assessment involved a number of positive and negative tasks. Therefore, their study did not use a standardized stressor paradigm that could be used as a reference for understanding cortisol reactivity to a specific stressor task.

Current Study

The current study aimed to examine salivary cortisol reactivity in the preschool-age offspring of parents with a history of depression and parents with no history of depression and to examine the potential moderating role of current parenting behaviors on the relation between parental depression history and offspring cortisol function. Any child with a current depressive disorder was excluded from the study in order to investigate cortisol reactivity as a potential early emerging vulnerability marker or trait marker, rather than a correlate of the disorder.

In order to examine the effects of both parental psychopathology and current parenting on preschoolers' cortisol reactivity, parental clinical interviews of psychopathology and observations of parenting behaviors were conducted. Assessing these two factors afforded an examination of the unique and joint, interactive effects of parental depression history and current maladaptive parenting on children's cortisol reactivity.

In sum, the proposed study tested **two specific aims**:

Aim 1: Examine the association between parental depression history and offspring's cortisol reactivity in preschool-age children. We hypothesized that offspring of parents with a history of depression would evidence increased cortisol reactivity compared to the offspring of parents with no history of depression.

Aim 2: Examine whether current maladaptive parenting moderated the relation between parental depression history and preschoolers' cortisol reactivity. We hypothesized that offspring of parents with a history of depression and whose parents

exhibited maladaptive parenting behaviors would evidence the greatest increase in cortisol reactivity.

Exploratory Aim: Examine whether the moderating effect of maladaptive parenting was specific to children whose parents had been depressed during the child's lifetime. Consistent with Dougherty et al. (2011), we hypothesized that the offspring of parents who were depressed during the child's first few years of life and whose parents exhibited maladaptive parenting behaviors would evidence the greatest increase in cortisol reactivity.

Chapter 3: Methodology

Participants

Participants (*N* = 175) consisted of a sample of preschool-age children and their biological parents. Potential participants were identified through several methods. Some participants were recruited using a purchased commercial mailing list (http://www.surveysampling.com) (27.0%). The mailing list included a list of phone numbers of families with children aged three to five years who lived within 20 contiguous miles from the University of Maryland, College Park campus. Undergraduate and graduate research assistants called families from this list to recruit for participation in the study. Through print advertisements, participants were also recruited from the Washington, DC metropolitan area (i.e., Maryland, DC, Virginia) (63.8%). Flyers were distributed to local schools, daycares, community centers, and health care providers (medical and specialty clinics, pediatricians). Within the sample, we made an attempt to recruit a group of parents with a lifetime history of depression through the use of flyers specifically focused on this population. Additionally, some participants were referred to the study by a friend or family member (9.2%). Children who: (1) were between the ages of three and five years (36-60 months); (2) never had been diagnosed with mental retardation or a pervasive developmental disorder (PDD); (3) did not have a current physical health condition (including diabetes), (4) were not taking corticosteroids; (5) did not have a biological parent who met criteria for psychosis or bipolar disorder as indicated by clinical interviews; and (6) had a biological parent with at least 50% physical custody who consented to participate were eligible. The study recruited both mothers and/or fathers with a history of depression. Mental health history was assessed

in both parents using structured clinical diagnostic interviews or using a family history approach. Control families could not have either a mother or father with a history of depression.

Of the 175 children participating in the study, one child did not speak English well enough to understand the laboratory tasks and therefore was excluded from the study and 17 (9.8%) children did not return for the second laboratory visit that included the cortisol reactivity assessment. Of the 157 preschool-age children who participated in the second laboratory visit, 15 children were excluded from the analyses due to the following reasons: 1) child was sick with a fever or currently taking antibiotic medication on the day of the cortisol reactivity assessment (n = 3); 2) child had a parent with a lifetime history of bipolar disorder-Not Otherwise Specified (NOS) (n = 2); 3) child met diagnostic criteria for a current depressive disorder (n = 4); 4) diagnostic information was not obtained for either of the child's parents (n = 2); and 5) child did not provide complete cortisol reactivity samples (n = 4). Thus, the total sample for this study resulted in 142 preschool-age children (71 boys and 71 girls).

Compared to the children who were not included in this subsample, participating children had significantly lower internalizing ($t(33.30) = -2.68$, $p = .011$) and externalizing ($t(168) = -2.56$, $p = .011$) behavior problems, older mothers ($t(33.39) = 2.34$, $p = .025$), and parents who exhibited significantly more support ($t(34.17) = 2.52$, $p = .017$) and less hostility ($t(32.10) = -2.33$, $p = .026$) during the parent-child interaction task. In addition, compared to families who were not included in this subsample, participating families had marginally significantly higher yearly income ($t(163) = 1.94$, $p = .055$). Children included in this subsample did not differ significantly from those not

included in terms of rates of parental history of depression, anxiety, or substance-use disorders.

Children's mean age was 50.7 months (*SD* = 9.63). Participating families identified themselves as Caucasian (*N* = 70; 50%), African-American (*N* = 47; 33.6%), Asian (*N* = 3; 2.1%), or other race (*N* = 20; 14.3%). Twenty-two (15.8%) children were of Hispanic/Latino descent. Over a third of the participating families (37.0%) reported a family income greater than $100,001; 28.3% of families reported a family income ranging from $70,001 to $100,000; 19.6% of families reported a family income ranging from $40,001 to $70,000; 8.7% of families reported a family income ranging from $20,001 to $40,000; and 6.5% of families reported a family income less than $20,000. The majority of the children had at least one parent with a four year-college degree (*N* = 102; 71.8%). Children were of average cognitive ability as measured by the Peabody Picture Vocabulary Test (*M* = 110.98, *SD* = 15.47) (PPVT; Dunn & Dunn, 1997).

Overall Design

This study consisted of two laboratory visits. During the first visit, the child participated in a standardized temperament assessment battery and parent-child interaction tasks. In between laboratory visits, psychiatric clinical interviews were conducted with mothers and fathers on the telephone. If the biological co-parent did not participate in the telephone interview, mothers provided a history of the fathers' mental health. The second visit to the laboratory assessed children's cortisol reactivity to a developmentally appropriate laboratory paradigm. During the cortisol reactivity assessment, primary caregivers completed a psychiatric interview about their child's current emotional and behavioral problems.

Study Implementation

Phone Screening. Trained research assistants conducted a preliminary phone screening. The research assistant verified that the participant met all of the inclusion criteria for the study. The phone screening served as a gross initial screen for parental bipolar disorder and psychosis; however, this was re-visited in the parental psychiatric interview. Parents who met criteria for these disorders were excluded from the study.

Session One. Upon arrival at the laboratory, a graduate research assistant provided the parent with an overview of the study's purpose and procedures and obtained informed consent. During the initial visit, the child participated in a series of observational tasks designed to assess child temperament and parent-child dyadic characteristics. For the purposes of this study, we will describe the parent-child interaction task only. At the end of the first laboratory visit, primary caregivers were asked to participate in the second phase of the study. The parent and co-parent were asked to participate in a telephone interview about her/his own mental health history. This interview was used to classify parents into one of two groups: (1) parents with no lifetime history of depression and (2) parents with a lifetime history of depression.

Session two. During the second laboratory visit, children's cortisol reactivity to a laboratory stressor was assessed. Children participated in a developmentally appropriate, stress-inducing laboratory task. One baseline salivary cortisol sample was collected prior to the onset of the task followed by four post-stressor samples. While children were completing this task, parents were interviewed about their child's current emotional and behavioral problems.

Measures

Demographic Information. During the initial visit, parents completed a demographic questionnaire that included information about race, age, socioeconomic status, marital status, parental education, and child-birth complications/premature status. For the full questionnaire, refer to Appendix C.

Parental depression history. The Structured Clinical Interview for Diagnostic Statistical Manual of Mental Disorders (DSM-IV-TR) (American Psychiatric Association, 2000), Axis I Disorders – non-patient version (SCID-NP; First, Spitzer, Gibbon, & Williams, 1996) was used to assess a lifetime history of depression in parents. The SCID is a widely used diagnostic assessment tool that has been documented to have excellent reliability and validity (Williams et al., 1992). Information about co-parents who do not complete the SCID was collected from parents through the Family History Research Diagnostic Criteria interview guide (FH-RDC; Andreasen, Endicott, Spitzer, & Winokur, 1977). The FH-RDC also has been documented as a reliable and valid method of assessing family history of affective disorders (Andreason et al., 1977).

All diagnostic interviews were conducted by a masters-level clinician who has extensive experience in the administration of these clinical assessment tools. Interviews were conducted on the telephone as several studies have demonstrated that face-to-face and telephone interviews yield similar results with non-patient samples (Rhode, Lewinsohn, & Seeley, 1997; Sobin, Weissman, Goldstein, & Adams, 1993). Interviews took approximately 30-90 minutes, depending on parents' psychiatric history. In addition, primary caregivers completed the Diagnostic Inventory for Depression (M = 6.83, SD = 6.91, α = .88) (DID; Zimmerman, Sheeran, & Young, 2004) to assess the

influence of current parental depressive symptoms on children's cortisol.

We had diagnostic information on 142 mothers and 132 fathers. Direct SCID interviews were obtained from all mothers and 72 (54.5%) fathers. Diagnostic information was obtained for 60 (45.5%) fathers using the FH-RDC. MDD and dysthymic disorder (DD) were collapsed into a single category reflecting depressive disorder. Of the parents, 70 (49.3%) mothers and 34 (25.8%) fathers had a history of MDD or DD. Children were considered to have a family history of depression if either parent had a diagnosis (*n* = 88; 62.0%). If a parent had lifetime MDD or DD based on the SCID, the onset and offset dates of all episodes were recorded to determine whether the parent was depressed during the child's life. Of the parents, 54 (38.0%; 43 mothers, 12 fathers) had MDD or DD during the child's life. Seventeen parents (12.0%) had a current depressive disorder.

Parent-child interaction task. All 142 children participated with a parent (94.4% mothers) in a series of structured teaching tasks adapted from Egeland et al.'s (1995) Teaching Task Battery. Five tasks were administered that were developmentally age-appropriate but moderately challenging. These tasks included book reading, a guessing game, a maze, a story sequencing task, and a set of puzzles. Each task was designed to elicit parents' involvement. Parents were instructed to provide any type of assistance or support in order for their child to complete the task successfully. During the first task, parents were instructed to tell their child a story using a picture book and to discuss the book with their child. The second task involved a guessing game, during which parents were instructed to help their child name as many things with *wheels* as he/she could. Next, parents had to help their child complete a maze on an Etch-A-Sketch toy. The

fourth task involved parents and children working together to sequence a series of picture cards. Finally, during the fifth task, parents taught their child to use plastic shape pieces to match designs shown on cards. All of the tasks were video-recorded for observational coding by trained research assistants.

For the purposes of this study, we used the parental hostility and parental supportive presence subscales to capture maladaptive parenting behaviors. Gunnar and Quevedo (2007) have argued that harsh and insensitive parenting behaviors significantly influence the development and regulation of offspring's HPA axis functioning. A recent meta-analytic review found that depressed mothers display higher levels of negative affect and hostile behaviors (Lovejoy et al., 2000). Furthermore, McLeod and colleagues (2007) found that parental hostility and absence of warmth were the strongest predictors of childhood depression. Coders rated the parent's hostility, based on expression of anger, frustration, and annoyance directed towards the child. Additionally, the parent's supportive presence was coded based on expression of positive regard and emotional support to the child. Both of these subscales were rated on a 5-point scale (1 is lowest possible score and 5 is the highest possible score). An aggregate score of parental hostility was created from the average of the 5 hostility scores across each episode (M = 1.12, SD = 0.25, Range: 1.0-2.2). The same procedure was done to create an aggregate score for parental supportive presence (M = 4.15, SD = 0.82, Range: 1.8-5.0). The parental hostility and supportive presence subscales evidenced satisfactory levels of internal consistency (hostility: $\alpha = .76$; supportive presence: $\alpha = .88$). The inter-rater reliability for the hostility and supportive presence scales was excellent (intraclass

correlation coefficient [ICC] = .91 and .96, respectively; *n* = 38). For a description of the parent-child interaction tasks, refer to Appendix D.

Child Depression. Children's current depression diagnoses were assessed using a structured psychiatric diagnostic interview with parents, the Preschool Age Psychiatric Assessment (PAPA; Version 1.4; Egger, Ascher, & Angold, 1999). The PAPA is a parent-reported interview that assesses emotional and behavioral problems in young children (between the ages of 2 and 5 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. We had diagnostic information on all 142 children. The PAPA was used to exclude any child (*n* = 4, 2.8%) with a current depressive disorder (major depressive disorder and/or dysthymic disorder).

Pervasive developmental disorder screener. During the first visit, parents were administered the Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003) to screen for pervasive developmental disorders. The SCQ is a parent-report measure of typical autistic behavior in preschool-age children. Recent reports have supported the validity and reliability of the SCQ (Chandler et al., 2007; Charman et al.,

2007). No participating children were excluded based on total SCQ score.

Cortisol reactivity assessment. During the second laboratory visit, children engaged in an acute psychological stressor paradigm that was developed by Kryski and colleagues (2011) and based on a modified version of Lewis and Ramsay's (2002) matching task. Kryski et al. (2011) demonstrated that this standardized stressor task was effective in eliciting a mean cortisol increase during a home visit with a sample of preschool-age children. As highlighted by Kryski and colleagues (2011), the stressor task incorporates the essential characteristics (uncontrollability, motivated task performance, and social evaluative threat) of laboratory stressor paradigms that have been found to be successful at eliciting a cortisol response in adults (Dickerson & Kemeny, 2004; Gunnar, Talge, & Herrera, 2009).

The stress assessment first consisted of a 30-minute period of quiet play (e.g., coloring, watching emotionally neutral videos, reading picture books), after which the experimenter collected the first saliva sample (T0 – baseline). After the baseline sample had been obtained, children participated in the structured stressor task. First, children were presented with a desirable and undesirable toy and were told that they could win their preferred prize if they successfully completed a matching game. During the task, children were asked to match colored chips with animal pictures based on a key they were given. Children were told that they had three minutes to complete each trial, and were shown a timer that the experimenter used to track the time. Children were also told that most children can finish the trials before the timer goes off. During the explanation of the task, children completed practice turns to ensure understanding of the rules of the game. Following the actual task trials, the experimenter manipulated the timer such that

children failed the following three trials. During each of the trials, the experimenter sat with a clipboard and pretended to take notes on the child's performance. Following each of the failed trials, the experimenter said, "Uh oh. You didn't finish in time." At the end of the third failed trial, the experimenter acted confused and said, "Wait a minute! My timer isn't working right! It's been going off after only 2 minutes, not 3 minutes, so you didn't have enough time to finish." After the child was informed that the timer was broken, the experimenter presented the child with the desired prize and worked together to successfully complete the matching game.

Cortisol samples were obtained prior to the start of the task (T0), and then at 20 (T1), 30 (T2), 40 (T3), and 50 (T4) minutes following the completion of the task. Saliva samples were obtained by having children dip a cotton dental roll into a small amount of Kool-Aid® mix. The children then placed the cotton roll in their mouths until saturated. The wet cotton was expressed into a vial by the experimenter. After each visit, the vials were kept frozen at -20° Celsius until assayed in duplicate using a time-resolved fluorescence immunoassay with flourometric end-point detection (DELFIA). Salivary cortisol samples were assayed at the Biochemical Laboratory at the University of Trier, Germany. The use of the oral stimulant was carefully monitored across all samples. The procedures employed here have been shown to yield little-to-no effect on cortisol concentrations (Talge, Donzella, Kryzer, Gierens, & Gunnar, 2005). Inter- and intra-assay coefficients of variation were 7.1%-9.0% and 4.0%-6.7%, respectively. For a full description of the Cortisol Reactivity Task protocol refer to Appendix E.

The study's dependent variable was children's cortisol levels collected during the assessment. We collected a total of 5 cortisol samples during the reactivity assessment

(baseline, 20, 30, 40 and 50 minutes post-stressor). In an effort to limit the number of comparisons and conserve statistical power, we calculated the area under the curve (AUC) with respect to ground (AUC_g) and with respect to increase (AUC_i) derived from the trapezoid formula (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). The AUC_g is a measure of the magnitude of total cortisol secretion across the 5 samples whereas the AUC_i is a measure of the change in cortisol levels across the 5 time points. Prior to analyses, we examined the descriptive statistics and frequency distributions of the variables, as cortisol values are often found to be positively skewed (Gunnar & Talge, 2008), and performed the appropriate transformations as necessary (i.e., $\log_{10}$ transformations).

Potential Confounds

Several factors that have been found to influence cortisol levels were assessed, including food and caffeine intake, time of laboratory visit, time of waking, body mass index (BMI), child health and medications, and stressful events occurring on the assessment day (Gunnar & Talge, 2008; Hibel, Granger, Kivlighan, & Blair, 2006). These factors were reported by the parent during the second laboratory visit and examined as potential confounds on children's cortisol reactivity. We also assessed parental lifetime anxiety (n = 87; 61.7%) and substance-use (n = 54; 38.3%) disorders, observer-rated child activity level during the second laboratory visit, and parent-reported child internalizing (α = .84) and externalizing (α = .90) behavior problems as assessed using the Child Behavior Checklist/1½-5 (Achenbach & Rescorla, 2000).

Chapter 4: Results

Preliminary Analyses

Table 1 shows the means, standard deviations, and N's for demographic variables, potential covariates, and cortisol levels in nanomoles per liter (nmol/L) by parental depression history. Cortisol values in Table 1 reflect raw values for greater ease of interpretation (in nmol/L), but a logarithmic transformation of these cortisol values was used in all analyses. Compared to the offspring of parents with no lifetime history of depression, offspring of parents with a history of depression were significantly more likely to also have a parent with a lifetime anxiety disorder ($\chi^2(1,141) = 11.03$, $p = .001$), had significantly more externalizing problems ($t(140) = 2.11$, $p = .037$), were more likely to have taken medication on the day of cortisol reactivity assessment ($\chi^2(1,142) = 4.24$, $p = .040$), and had a trend for completing fewer trials of the stressor during the reactivity assessment ($t(138.30) = -1.76$, $p = .081$). Parental depression history was not significantly associated with current parental support ($r = -.08$, p = .373) or current parental hostility ($r = .07$, $p = .432$). Parents who experienced depression in the past month ($n = 17$) were rated significantly less supportive ($r = -.17$, $p = .049$) than parents who did not experience depression in the past month. Current depression in the past month was not significantly associated with parental hostility ($r = .10$, $p = .242$). Parents' self-report of current depressive symptoms was not significantly associated with parental support ($r = -.03$, $p = .769$) or hostility ($r = -.004$, $p = .963$). We observed a significant negative relation between current parental support and parental hostility ($r = -.53$, $p < .001$).

Cortisol level comparisons across the sample. In order to understand children's cortisol responses to the laboratory stressor, paired samples *t*-tests were used to test differences between mean cortisol levels across time for the whole sample. As seen in Figure 1, mean cortisol levels decreased significantly from baseline to 20 minutes post-stress ($t(141)$, = -2.60, p = .010). There were no significant changes in mean cortisol levels from 20 to 30 minutes post-stress ($t(141)$, = -.80, p = .425), 30 to 40 minutes post-stress ($t(141)$, = -.32, p = .749), or 40 to 50 minutes post-stress ($t(141)$, = 1.32, p = .189). The decreasing mean salivary cortisol response observed in this investigation is similar to those observed in other studies of preschoolers using stress inducing laboratory tasks (Dougherty et al., 2010; Gunnar et al., 2009; Luby et al., 2003). Nevertheless, as seen in Table 1, a subgroup of children in the current sample (36.6%, N = 52) exhibited a positive $AUC_{i.}$ There were no significant differences for mean cortisol values at any of the 5 salivary cortisol samplings between offspring of parents with a history of depression and offspring of parents with no history of depression (see Table 1).

Potential confounds on child cortisol AUC. We first examined potential confounds on child AUC_g and AUC_i using t-tests and Pearson product moment correlation coefficients. The number of trials the child completed during the stressor paradigm was positively related to AUC_g (r = .18, p = .030) and whether the child ate a meal one hour prior to the cortisol reactivity assessment was negatively related to AUC_i (r = -.24, p = .004). Thus, children who completed more trials of the stressor paradigm exhibited greater total cortisol secretion across the cortisol reactivity assessment, and children who ate a meal prior to the visit exhibited less of an increase in cortisol across the 4 post-stress samples. Child sex, child age, time of visit, parental lifetime anxiety and

substance-use, socioeconomic status, child activity, and child internalizing and externalizing symptoms were not significantly associated with child AUC_g or AUC_i. In all remaining analyses, number of trials failed and whether the child ate a meal were included as covariates in analyses of AUC_g and AUC_i, respectively. Lastly, we did not observe a significant relation between child AUC_g and AUC_i (r = .12, p = .161). Thus, it appears that AUC_g and AUC_i are two distinct measures of HPA axis functioning that capture different aspects of cortisol responses.

Stress reactivity, parental depression, and parenting

We examined differences in child AUC_g and AUC_i between the offspring of depressed and non-depressed parents while controlling for significant covariates. No significant differences were observed for child AUC_g between offspring of parents with lifetime depression (M = 14.56, SD = 14.82, N = 88) and without lifetime depression (M = 15.87, SD = 12.71, N = 54), $F(1,139)$ = .06, p = .801. Similarly, no significant differences were observed for child AUC_i between the offspring of parents with lifetime depression (M = -2.22, SD = 10.70, N = 88) and without lifetime depression, (M = -2.21, SD = 9.26, N = 54), $F(1,139)$ = .06, p = .807. We also examined differences in child AUC_g and AUC_i between the offspring of currently depressed parents (depression in the past month) and non-depressed parents. No significant differences were observed for child AUC_g between offspring of currently depressed parents (M = 13.71, SD =12.52, N = 17) and non-depressed parents (M = 14.71, SD = 13.72, N = 116), F (1,130) = .05, p = .820. Similarly, no significant differences were observed for child AUC_i between the offspring of parents with current depression (M = -5.68, SD =12.25, N = *17*) and without current depression (M = -1.97, SD = 9.42, N = 116), F (1,130) = 1.46, p = .229. Finally,

parents' self-report of current depressive symptoms was not significantly associated with child AUC_g (B = .01, SE= 1.20, p = .948) or child AUC_i (B = -.11, SE= .85, p = .192).

We also examined group differences in child AUC_g and AUC_i between offspring of parents with lifetime history of anxiety and substance-use disorders. No significant differences were observed for child AUC_g between offspring of parents with lifetime anxiety (M = 13.84, SD = 13.90, N = 87) and without lifetime anxiety (M = 16.36, SD = 13.44, N = 54), $F(1,138)$ = 1.29, p = .258 or for child AUC_i between offspring of parents with lifetime anxiety (M = -1.79, SD = 10.49, N = 87) and without lifetime anxiety (M = -3.52, SD = 8.55, N = 54), $F(1,138)$ = .34, p = .562. Finally, no significant differences were observed for child AUC_g between offspring of parents with lifetime substance-use disorders (M = 13.86, SD = 13.53, N = 54) and without lifetime substance-use disorders (M = 15.39, SD = 13.90, N = 87), $F(1,138)$ = .17, p = .680 or for child AUC_i between offspring of parents with lifetime substance-use disorders (M = -2.14, SD = 8.78, N = 54) and without lifetime substance-use disorders (M = -2.64, SD = 10.43, N = 87), $F(1,138)$ = .12, p = .732.

Next, we examined the associations between the parenting dimensions and children's cortisol while controlling for significant covariates. Parental support was not significantly associated with either child AUC_g (β = -.10, SE = 1.16, p = .238) or child AUC_i (β = .08, SE = .83, p = .362). Similarly, parental hostility was not significantly associated with child AUC_g (β = .08, SE = 1.15, p = .318) or child AUC_i (β = .01, SE = .83, p = .886).

We then examined the interaction between parental history of depression and each parenting dimension separately, to conserve statistical power, using multiple linear

regression analyses. Parental lifetime depression was dummy coded (0, 1) for absence or presence of a lifetime depressive disorder. Prior to creating interaction terms, continuous variables (parenting and covariates) were standardized using a z-transformation to reduce problems associated with multicollinearity (Aiken & West, 1991). Interaction terms were created by multiplying parental depression history and parenting variables. After entering parental depression history, support and the covariates in Step 1, we examined whether the interaction term entered at Step 2 was associated with child AUC_g or AUC_i. The interaction between parental depression history and parental support was not significantly associated with child AUC_g (β = .18, *SE* = 2.50, *p* = .223) or child AUC_i (β = -.02, *SE* = 1.78, *p* = .915). Next, we conducted regression models entering parental depression history, hostility and the covariates in Step 1 and the interaction term entered at Step 2 for child AUC_g and AUC_i. The interaction between parental depression history and hostility was not significantly associated with child AUC_g (β = -.04, *SE* = 2.65, *p* = .810) but was significantly associated with child AUC_i (β = .35, *SE* = 1.86, *p* = .031).

To understand the significant interaction, we plotted the relations between estimated levels of offspring's AUC_i across estimated levels of high and low parental hostility for offspring of parents with and without lifetime depression. As seen in Figure 2, for the offspring of parents with no lifetime history of depression, there was a trend for high hostility predicting lower child AUC_i (β = -.29, *SE* = 1.60, *p* = .075). In contrast, for children of parents with lifetime history of depression, there was no significant association between parental hostility and child AUC_i (β = .12, *SE* = .96, *p* = .212). Thus, only the combination of no parental depression history and high parental hostility was associated with decreasing offspring AUC_i. When controlling for parental anxiety and

substance-use disorders, the interaction between parental depression history and parental hostility was reduced to a trend level of significance (β = .28, SE = 1.84, p = .077). We did not observe significant interactions between parenting behaviors and parental anxiety or parental substance-use disorders when predicting child cortisol.

Child exposure to parental depression. Given previous findings (Dougherty et al., 2011), we examined whether the findings varied as a function of the timing of parents' depression. First, we examined differences in child AUC_g and AUC_i among the offspring of parents who had no lifetime depression, who experienced depression prior to the birth of the child, and who experienced depression during the child's life. No significant differences were observed for child AUC_g among offspring of parents with no lifetime depression (M = 16.21, SD = 13.05, N = 58), depression prior to the birth of the child (M = 12.96, SD = 9.94, N = 30), and depression during the child's life (M = 15.00, SD = 16.80, N = 54), $F(2,138)$ = .54, p = .583. Similarly, there were no significant differences for child AUC_i among offspring of parents with no lifetime depression (M = -2.45, SD = 9.07, N = 58), depression prior to the birth of the child (M = -1.23, SD = 7.85, N = 30), and depression during the child's life (M = -2.51, SD = 12.29, N = 54), F (2,138) = .39, p = .676.

We next examined whether the interaction between parental depression history and parental hostility varied as a function of the timing of parents' depression. We created dummy coded variables indicating the timing of parents' depression: parental depression occurring only prior to the birth of the child and parental depression occurring during the child's life. Next, we conducted regression models entering parental depression history (dummy coded depression occurring prior to child's birth and dummy

coded depression occurring during child's life), hostility and the covariates in Step 1 and their respective interaction terms with hostility entered at Step 2 for child AUC_g and AUC_i. When predicting child AUC_g, we did not observe a significant interaction between parental depression occurring during the child's life and hostility (β = -.01, *SE* = 2.73, *p* = .972) or between parental depression occurring prior to the child's life and hostility (β = .02, *SE* = 4.17, *p* = .870). When predicting child AUC_i, we found a significant interaction between parental depression occurring during the child's life and parental hostility (β = .40, *SE* = 1.89, *p* = .007). The interaction between parental depression prior to the child's life and parental hostility was not significant for child AUC_i (β = .01, *SE* = 2.90, *p* = .896).

As seen in Figure 3, for children who were exposed to parental depression during their first few years of life, parental hostility was significantly associated with higher child AUC_i (β = .22, *SE* = 1.06, *p* = .036). In contrast, for children whose parents had no lifetime history of depression, parental hostility was marginally significantly related to lower child AUC_i (β = -.29, *SE* = 1.57, *p* = .064). For children whose parents had a lifetime history of depression prior to the child's life, there was no significant association between hostility and child AUC_i (β = -.25, *SE* = 2.43, *p* = .297).

Specificity of findings to mothers and fathers. We next examined whether findings differed with respect to whether the depressed parent was the mother or father. Parallel analyses were conducted examining the effects of lifetime history of maternal (*n* = 70) and paternal (*n* = 34) depression separately. No significant differences were observed for child AUC_g between offspring of mothers with lifetime depression (*M* = 15.27, *SD* = 15.14, *N* = 70) and without lifetime depression (*M* = 14.86, *SD* = 12.95, *N* = 72),

$F(1,139) = .24$, $p = .626$. Similarly, no significant differences were observed for child AUC_i between offspring of mothers with lifetime depression ($M = -1.94$, $SD = 10.68$, $N = 70$) and without lifetime depression ($M = -2.49$, $SD = 9.67$, $N = 72$), $F(1,139) = .01$, $p = .914$. No significant differences were observed for child AUC_g between offspring of mothers with lifetime anxiety ($M = 13.74$, $SD = 14.39$, $N = 66$) and without lifetime anxiety ($M = 15.74$, $SD = 13.16$, $N = 75$), $F(1,138) = .68$, $p = .411$. However, children of mothers with lifetime anxiety ($M = -0.43$, $SD = 10.11$, $N = 66$) exhibited marginally significantly greater AUC_i compared to children of mothers with no lifetime anxiety ($M = -4.22$, $SD = 9.22$, $N = 75$), $F(1,138) = 3.84$, $p = .052$. No significant differences were observed for child AUC_g between offspring of mothers with lifetime substance-use disorders ($M = 14.92$, $SD = 15.30$, $N = 25$) and without lifetime substance-used disorders ($M = 14.78$, $SD = 13.44$, $N = 116$), $F(1,138) = .05$, $p = .819$. Similarly, no significant differences were observed for child AUC_i between offspring of mothers with lifetime substance-use disorders ($M = -0.70$, $SD = 9.38$, $N = 25$) and without lifetime substance-use disorders, ($M = -2.82$, $SD = 9.89$, $N = 116$), $F(1,138) = .82$, $p = .367$.

No significant differences were observed for child AUC_g between offspring of fathers with lifetime depression ($M = 12.97$, $SD = 12.53$, $N = 34$) and without lifetime depression ($M = 15.01$, $SD = 13.90$, $N = 98$), $F(1,128) = .46$, $p = .500$. Similarly, no significant differences were observed for child AUC_i between offspring of fathers with lifetime depression ($M = -3.47$, $SD = 9.76$, $N = 34$) and without lifetime depression ($M = -1.95$, $SD = 9.86$, $N = 98$), $F(1,128) = .31$, $p = .580$. No significant differences were observed for child AUC_g between offspring of fathers with lifetime anxiety ($M = 13.34$, $SD = 11.38$, $N = 46$) and without lifetime anxiety ($M = 15.09$, $SD = 14.60$, $N = 86$),

$F(1,128) = .71$, $p = .400$. Similarly, no significant differences were observed for child AUC_i between offspring of fathers with lifetime anxiety ($M = -3.00$, $SD = 10.37$, $N = 46$) and without lifetime anxiety ($M = -1.99$, $SD = 9.55$, $N = 86$), $F(1,128) = .28$, $p = .598$. No significant differences were observed for child AUC_g between offspring of fathers with lifetime substance-use disorders ($M = 11.43$, $SD = 12.10$, $N = 38$) and without lifetime substance-use disorders ($M = 15.66$, $SD = 14.02$, $N = 93$), $F(1,128) = 1.56$, $p = .214$. Finally, no significant differences were observed for child AUC_i between offspring of fathers with lifetime substance-use disorders ($M = -3.37$, $SD = 7.44$, $N = 38$) and without lifetime substance-use disorders ($M = -2.11$, $SD = 10.54$, $N = 93$), $F(1,128) = .39$, $p = .532$.

We then examined the interactions between maternal and paternal history of depression with the parenting dimensions on children's AUC_g and AUC_i. Consistent with the parental model, the interaction between maternal lifetime depression and parental hostility was not significantly associated with child AUC_g ($\beta = -.04$, $SE = 2.60$, $p = .815$) but was significantly associated with child AUC_i ($\beta = .34$, $SE = 1.83$, $p = .029$). To understand the significant interaction, we plotted the relations between estimated levels of offspring's AUC_i across estimated levels of high and low parental hostility for offspring of mothers with and without lifetime depression. As seen in Figure 4, for the offspring of mothers with no lifetime history of depression, there was a trend for high hostility predicting lower child AUC_i ($\beta = -.28$, $SE = 1.55$, $p = .076$). For children of mothers with a lifetime history of depression, there was no significant association between parental hostility and child AUC_i ($\beta = .13$, $SE = .98$, $p = .201$). When controlling for maternal anxiety and substance-use disorders, the interaction between

maternal depression and parental hostility was reduced to marginal significance (β = .28, *SE* = 1.78, *p* = .063).

We did not observe a significant interaction between paternal lifetime depression and parental hostility with child AUC_g (β = .133, SE = 3.73, *p* = .184) or child AUC_i (β = .06, *SE* = 2.67, *p* = .503). Thus, as seen in Figure 5, the interaction between history of depression and hostility appeared to be specific to mothers as we did not observe a significant interaction between lifetime history of depression and parental hostility for fathers.

Similarly, the interaction between exposure to parental depression and parental hostility on child AUC_i was specific to exposure to maternal depression only. We found a significant interaction between maternal depression occurring during the child's life and parental hostility (β = .35, *SE* = 1.91, *p* = .016), whereas the interaction between maternal depression occurring prior to the child's life and parental hostility was not significant (β = .03, *SE* = 2.92, *p* = .753).

As seen in Figure 6, for children who were exposed to maternal depression during their first few years of life, there was a trend for parental hostility predicting higher child AUC_i (β = .19, *SE* = 1.12, *p* = .092). For children whose mothers had no lifetime history of depression, there was a trend for parental hostility predicting lower child AUC_i (β = -.28, *SE* = 1.55, *p* = .076). For children whose mothers had a lifetime history of depression prior to the child's life, there was no significant association between parental hostility and child AUC_i (β = -.18, *SE* = 2.47, *p* = .457).

No significant interactions between paternal depression occurring during the child's life and parental hostility (β = .06, *SE* = 9.83, *p* = .737) or between paternal

depression occurring prior to the child's life and parental hostility (β = .11, *SE* = 9.95, *p* = .483) on child AUC_i were observed.

To determine whether these effects are specific to parental depression history or other forms of parental psychopathology, we examined interactions between lifetime maternal and paternal anxiety with parenting on child AUC_g and AUC_i. We found a significant interaction between lifetime maternal anxiety and parental hostility for child AUC_g (β = .21, *SE* = 2.41, *p* = .048). As seen in Figure 7, we plotted the relations between estimated levels of offspring's AUC_g across estimated levels of high and low parental hostility for offspring of mothers with and without lifetime anxiety. For the offspring of mothers with a lifetime history of anxiety, there was a trend for high hostility predicting higher child AUC_g (β = .25, *SE* = 1.93, *p* = .070). For children of mothers without a lifetime history of anxiety, there was no significant association between parental hostility and child AUC_g (β = -.09, *SE* = 1.44, *p* = .371). Results were similar when controlling for maternal depressive and substance-use disorders. We did not observe any other significant interactions between maternal and paternal anxiety with the parenting variables when predicting child AUC_g or AUC_i. No significant interactions between maternal or paternal substance-use disorders with parenting were observed for either child AUC_g or AUC_i.

Developmental Differences.

Finally, we examined whether the findings varied as a function of child age using multiple linear regressions analyses. The sample consisted of three (*N* = 60, 42.3%), four (*N* = 51, 35.9%), and five (*N* = 31, 21.8%) year-old children. As mentioned above, child age was not significantly associated with offspring AUC_g (β = -.12, *SE* = 1.17, *p* = .159)

or AUC_i (β = -.06, *SE* = .83, *p* = .494). Furthermore, the relation between parental depression history and offspring cortisol reactivity did not vary as a function of child age when predicting child AUC_g (β = .05, *SE* = 2.40, *p* = .700) or child AUC_i (β = -.10, *SE* = 1.71, *p* = .415). Lastly, the interaction between parental depression history and parental hostility did not vary as a function of child age when predicting child AUC_g (β = -.08, *SE* = 2.65, *p* = .545) or child AUC_i (β = -.06, *SE* = 1.87, *p* = .633).

Chapter 5: Discussion and Conclusions

This study examined whether the non-depressed, preschool-age offspring of parents with lifetime depression demonstrated increased cortisol reactivity to a standardized developmentally-appropriate laboratory stressor paradigm and whether current observed parenting behavior moderated this relation. In support of our hypothesis and consistent with Dougherty et al. (2011), we found that the offspring who were exposed to parental depression during the first few years of life and whose parents demonstrated high levels of hostility during the parent-child interaction task evidenced increasing cortisol levels in response to the stressor paradigm. The relation between high parental hostility and increasing offspring cortisol reactivity was not observed for the offspring of parents who had a history of depression *prior to the birth* of the child, underscoring the importance of child exposure to parental depression. In addition, we found that the offspring of parents with *no lifetime depression* and whose parents displayed high hostility evidenced decreasing cortisol levels in response to the laboratory stressor paradigm.

When we examined the specificity of the parental depression findings to mothers and fathers with depression, we found that the moderating effect of parental hostility was specific to children whose mothers had been depressed during the child's lifetime, highlighting the significant role of early maternal caregiving experiences on the regulation of young children's HPA axis. Furthermore, parental hostility moderated the relation between maternal history of anxiety and offspring's total cortisol secretion in response to the stressor paradigm, illustrating the importance of examining the influence of other forms of maternal psychopathology on offspring's neuroendocrine functioning.

Our work replicates and extends the literature examining the unique and interactive influences of parental depression history and parenting behaviors on high-risk offspring's cortisol reactivity. Notably, we replicated the findings observed by Dougherty et al. (2011) using a standardized stressor paradigm with an independent, more diverse, larger high-risk young sample, providing further evidence that early exposure to parental depression *and* parental hostility is specifically related to young children's increased stress reactivity. This observed pattern of increasing cortisol levels in our high-risk sample of preschool-age children is consistent with the literature documenting elevated cortisol responses in depressed adults (Burke et al., 2005) and youth (Lopez-Duran et al., 2009). It is possible that increased stress sensitivity may render high-risk offspring more vulnerable to the depressogenic effects of stress later in life, suggesting that early dysregulation of the HPA axis is one mechanism involved in the intergenerational transmission of depression.

Our findings highlight the critical influence of early environmental experiences, particularly parenting, on the development and functioning of young children's neuroendocrine system. Currently, few studies have examined the relations between both parental depression history and parenting behavior on high-risk offspring's stress reactivity, with only one study assessing preschool-age children (Dougherty et al., 2011). Our findings are consistent with recent studies that found that parenting behavior moderated the relation between maternal psychopathology and basal cortisol levels in infants (Kaplan et al., 2008) and cortisol reactivity in preschoolers (Dougherty et al., 2011). Thus, our findings add to this emerging body of work, which suggests that

parental hostility exacerbates the relation between exposure to parental depression and offspring HPA axis functioning.

As described above, the moderating effect of parental hostility was found to be specific to *maternal* depression occurring during the first few years of the child's life, illustrating the importance of the quality of the mother-child relationship on the development and functioning of young children's HPA axis. Our findings are consistent with the animal literature that has observed deleterious and ameliorative effects of early maternal caregiving behavior on offspring stress reactivity (Francis et al., 1999; Gunnar & Vazquez, 2006; Meaney, 2001). For instance, naturally occurring variations in maternal licking and grooming and arched back nursing (LG-ABN) have been found to be directly related to the regulation of the stress system in rodents (Gunnar & Vazquez, 2006; Meaney, 2001). The rat pups of mothers who engage in low levels of LG-ABN exhibit greater neuroendocrine responses to stress in comparison to the offspring of mothers who engage in high levels of LG-ABN (Meaney, 2001). In addition, Francis and colleagues (1999) conducted a cross-fostering study with rodents that provided evidence for the intergenerational transmission of individual differences in stress reactivity through variations in maternal caregiving behavior. Our findings are also consistent with the large body of literature in humans examining the influence of insensitive parenting behaviors on offspring cortisol (e.g. Azar et al., 2007; Bugental et al., 2003; Dougherty et al., 2011; Gunnar & Donzella, 2002; Gunnar et al., 1992; Murray et al., 2010; Nachiamas et al., 1996).

Given the significant neuroplasticity during a child's first few years of life, stressful environmental experiences, including exposure to maternal depression and harsh

parenting, may have lasting influences on children's developing neuroendocrine system (Gunnar & Vazquez, 2006). Recent work has suggested that exposure to maternal depression during particular sensitive periods (e.g. prenatal or postnatal period) may render offspring more vulnerable to later emotional and/or behavioral problems (Ashman et al., 2002; Davis et al., 2007; Essex et al., 2002; Field, 2011; Halligan et al., 2007a). For instance, Davis and colleagues (2007) found that infants of mothers who were depressed during pregnancy exhibited more negative temperament compared to infants of mothers who were not depressed during pregnancy. Additionally, Ashman and colleagues (2002) found that maternal depression during the postnatal period, specifically the first two years of life, was the strongest predictor of elevated basal cortisol levels in seven year-old children. Furthermore, they found that higher offspring basal cortisol levels were related to more internalizing problems in the children of mothers with a history depression. Similarly, Halligan and colleagues (2007a) found that exposure to maternal postnatal depression predicted adolescent offspring's depressive symptoms at age 16, and this relation was mediated by offspring's elevated morning basal cortisol levels assessed at age 13.

More work in this area is needed to examine whether there are specific critical time periods of exposure to maternal depression that render offspring more vulnerable to dysregulated HPA axis functioning and later psychiatric outcomes. Specifically, it will be important to examine the differential impact of exposure to maternal depression across various proposed sensitive periods (e.g. pregnancy, first two years of life). Alternatively, it is possible that the cumulative effects of early exposure to maternal depression may be more related to young children's neuroendocrine functioning and related psychiatric

outcomes. In support of this explanation, Ashman and colleagues (2002) found that the depressed mothers in their sample tended to experience more months of depression throughout the offspring's early years of life. Thus, it is possible that the duration of early exposure to maternal depression may be more influential than the timing of the depression exposure. Similarly, Halligan, Murray, Martins, and Cooper (2007b) found that exposure to maternal postnatal depression was associated with increased rates of depression diagnoses in adolescent offspring *only* if mothers had subsequent episodes of depression later in the offspring's life. It will be important for further work to tease apart the influence of the timing and chronicity of maternal depression on young children's HPA axis functioning, as well as the role that parenting plays in these relations.

In addition to replicating the findings observed in Dougherty et al. (2011) regarding the cortisol reactivity of the offspring of parents who experienced depression during the child's life, we also found that higher levels of parental hostility were associated with a pattern of decreasing cortisol levels in the offspring of parents *without* a history of depression. This finding may reflect a dampening pattern of neuroendocrine activity that is consistent with what has been observed in prior studies assessing the impact of chronic stress on the HPA axis (Badanes et al., 2011; Ronsaville et al., 2006). Given that elevated and blunted patterns of HPA axis functioning have been associated with negative physical and mental health outcomes (McEwen, 1998), it will be important for future studies to examine why certain factors or environmental circumstances lead to divergent cortisol profiles and whether these profiles are associated with distinct risk pathways for the onset of subsequent psychopathology.

This study is also the first to report that parental hostility moderated the relation between maternal lifetime history of anxiety and offspring's total cortisol secretion, even after controlling for lifetime maternal depression and substance-use disorders. In addition, existing research on the relation between maternal anxiety diagnosis and offspring cortisol reactivity has been limited to work in infants. Thus, our study was the first to examine the main and interactive effects of maternal anxiety diagnosis and parenting behaviors on offspring's cortisol reactivity beyond infancy. Specifically, we found that the offspring of mothers with a lifetime history of anxiety and whose mothers demonstrated high levels of hostility evidenced a higher total output of cortisol following the stressor task. This finding underscores the importance of examining maternal anxiety, as well as maternal depression history.

Prior studies have observed relations between maternal anxiety and offspring cortisol (e.g. Brennan et al., 2008; Feldman et al., 2009; Grant et al., 2009; O'Connor et al., 2005; Van den Bergh, Van Calster, Smits, & Van Huffel, 2008; Warren et al., 2003). For instance, maternal prenatal anxiety symptoms have been found to predict elevated basal cortisol in pre-adolescent (O'Connor et al., 2005) and adolescent (Van den Bergh et al., 2008) offspring. Additionally, three studies observed increased cortisol reactivity in the infants of mothers with prenatal anxiety disorder (Grant et al., 2009), comorbid anxiety and depressive disorders (Brennan et al., 2008), and lifetime panic disorder (Warren et al., 2003). To date, only two studies have examined the relations between maternal anxiety and parenting behavior on offspring cortisol reactivity in infants (Feldman et al., 2009; Grant et al., 2009). In contrast to our findings, both Feldman et al. (2009) and Grant et al. (2009) found independent main effects, and not an interactive

effect, of maternal anxiety disorder and parenting on infant cortisol reactivity. Importantly, both of these studies included samples of women with current anxiety disorders. Given that current maternal anxiety has been found to be related to less sensitive parenting behavior (Nicol-Harper, Harvey, & Stein, 2007), it is possible that the main effects reported in the aforementioned studies may have masked an interaction between maternal anxiety history and parenting that we observed in our study. It is also important to note that in the current study we did not assess the timing of maternal anxiety disorders. Given our findings about the significance of early exposure to maternal depression, further work is necessary that examines the impact of exposure to maternal anxiety across the child's life on offspring's neuroendocrine functioning.

Our results suggest that the combination of hostile parenting and maternal history of depression and anxiety is related to elevated patterns of HPA axis responses in high-risk offspring. These findings suggest that the HPA axis may serve as a mechanism of risk for both the offspring of depressed and anxious mothers. This shared mechanism of risk is consistent with findings that depression and anxiety share common, as well as distinct, etiological mechanisms (Kendler, Neale, Kessler, Heath, & Eaves, 1992). It is important to highlight that the interactions between maternal anxiety and depression and parental hostility were related to different indices of HPA axis functioning, AUC_g and AUC_i, respectively. While AUC_g assesses *total cortisol secretion* following stress, AUC_i assesses *total change in cortisol* following stress. Thus, the two indices are likely capturing different components of the reactivity of the neuroendocrine system (Pruessner et al., 2003), which is further suggested by the low correlation we observed between the AUC indices (r = .12). Given our findings, it is possible that parental hostility incurs

distinct abnormalities in neuroendocrine functioning based on whether the child is at familial risk for depression or anxiety. However, this interpretation is speculative and further studies are warranted that assess multiple indices of HPA axis functioning in children at risk for depression and anxiety disorders.

Consistent with Dougherty et al. (2011), the preschool-age offspring of parents with a history of depression did not exhibit increased cortisol reactivity. This finding is in contrast to those observed in infants of depressed mothers who exhibited increased cortisol reactivity (Azar et al., 2007; Brennan et al., 2008; Feldman et al., 2009). Notably, many of these prior studies included women who experienced depression during the prenatal and/or postnatal periods. As mentioned above, it is possible that exposure to maternal depression during these proposed sensitive periods may be associated with dysregulation of the HPA axis. Additionally, many of these prior studies focused on women who were currently depressed. Given that current maternal depression is related to maladaptive parenting (Lovejoy et al. 2000), the main effects reported in prior studies may have masked an interaction between maternal depression history and parenting that was observed in our study.

Surprisingly, we did not observe a relation between maladaptive parenting and cortisol reactivity, which has previously been reported in studies examining the effects of parenting on offspring's cortisol function (e.g. Azar et al., 2007; Dougherty et al., 2011; Feldman et al., 2009; Murray et al., 2010). One possible explanation for this inconsistent finding is that our assessment of parenting did not capture sufficiently high levels of hostility. For instance, we recorded the parent-child interaction through a one-way mirror. It is possible that the parents in our study may have been more aware of being

filmed than in other studies and minimized their hostility throughout the interaction. Nevertheless, we replicated previous findings demonstrating that parental hostility moderated the relation between parental depression history and offspring cortisol, which suggests that the level of observed hostility, even low levels in this context, captures meaningful parenting characteristics.

The aim of the current study was to examine the impact of parental depression and parenting behavior on offspring cortisol reactivity. However, only a subgroup of the children in our sample (36.6%) exhibited increases in cortisol following the laboratory stressor paradigm, which makes it difficult to interpret the results in terms of HPA axis *reactivity* (Dougherty et al., 2011; Gunnar et al., 2009). Nevertheless, some children did exhibit increasing cortisol in response to the stressor paradigm and the independent variables in the study did correlate with unique patterns of cortisol responses in the offspring. It is important to note that in the adult literature as well, which has more reliably observed mean elevations in cortisol to laboratory stressor paradigms, a subgroup of individuals do not respond or demonstrate a decline in cortisol levels after presentation of the laboratory stressor (Dickerson & Kemeny, 2004). This phenomenon is also observed, and considerably more frequently, in studies examining youths' cortisol reactivity to a laboratory stressor paradigm (Gunnar et al., 2009).

In fact, no study has observed a mean increase in cortisol following a standardized psychosocial stressor paradigm conducted in the laboratory in preschool-age children (Gunnar et al., 2009). The two studies that have observed increases in cortisol following a stressor were both conducted in the child's home (Hankin et al., 2010; Kryski et al., 2011). Taken together, it appears that young children can respond to psychosocial

paradigms but that there are important methodological challenges in assessing cortisol reactivity during laboratory visits. One potential challenge is that children's baseline samples may be more reflective of a response to coming to the laboratory than their true basal level.

We attempted to minimize a cortisol response to the novelty of the research setting by conducting the cortisol reactivity assessment during the child's second visit to the laboratory with the same experimenter from the child's initial visit. Nevertheless, many parents reported that their child was excited to return to the laboratory in anticipation of their second visit. Additionally, the experimenters noted that a few of the children in the sample appeared fearful and reluctant to separate from their parents prior to participating in the cortisol reactivity assessment. Given these narrative accounts and observations, the baseline sample that we collected 30 minutes following the child's arrival may be an index of the child's anticipatory response to returning to the laboratory and/or related to the child's distress over separating from his/her parent, rather than a measure of the child's true baseline cortisol levels. Therefore, the baseline samples we obtained at the laboratory, even following a period of 30 minutes of quiet play with a familiar individual, may not match baseline samples obtained at home (Gunnar & Talge, 2008). Thus, future research examining cortisol reactivity in samples of young children may want to consider implementing a longer period of acclimation to the laboratory (e.g. an hour; Badanes et al., 2011) or conducting home visits to obtain a more accurate index of baseline cortisol and overall cortisol reactivity.

This study had several strengths. First, we assessed parental psychopathology in both parents using clinical interviews and used an observational parent-child interaction

task. Second, we studied the impact of exposure to parental depression across the child's life on offspring's cortisol responses. Third, although depression is rare in young children, we excluded preschoolers with current depression to study cortisol reactivity as mechanism of risk or vulnerability marker, rather than a consequence of the disorder. Fourth, the study included a larger sample of fathers with a lifetime history of depressive disorder, than previously reported in the literature. In fact, fathers have been excluded from most prior work assessing cortisol reactivity in high-risk offspring with the exception of two recent studies (Bouma et al., 2011; Dougherty et al., 2011). Fifth, we assessed other forms of psychopathology in both parents, which is important given that depression is highly comorbid with other disorders and that cortisol dysfunction is linked with other psychiatric disorders besides depression. Sixth, we collected five cortisol samples, including four post-stressor samples to better capture individual differences in children's reactivity and recovery responses to the stressor paradigm. Finally, we recruited a more ethnically diverse sample of high-risk children than obtained in many previous studies.

The present study also has a number of limitations. First, only a subgroup of the participating children exhibited increasing cortisol levels to the stressor paradigm. This finding is not surprising given prior work assessing cortisol reactivity in this age range (Gunnar et al., 2009). Given that most of the children exhibited decreasing cortisol levels following the stressor, the findings are difficult to interpret in terms of cortisol *reactivity*. However, we observed variability in cortisol responses across children and were able to examine individual differences in cortisol responses to the stressor paradigm. Although we attempted to obtain an accurate index of the child's baseline cortisol levels by having

the child play quietly for 30 minutes prior to collecting the first sample, it is possible that this acclimation period should have been longer (e.g. 40-60 minutes). Future studies should consider assessing cortisol reactivity at home or waiting longer than 30 minutes to obtain the baseline sample in the laboratory.

Second, the participating parent in the parent-child interaction task was most commonly the child's biological mother (94.4%); therefore, the specificity of the findings regarding maternal psychopathology must be interpreted with caution. Future studies should attempt to obtain measures of parenting behavior from both parents. Third, we were not able to obtain diagnostic data from all fathers; however, we made considerable attempts to obtain diagnostic information on fathers by direct interview or the family history method (93.0%). Fourth, although we assessed whether parental depression occurred during the child's life, we did not examine possible sensitive periods of exposure or assess parenting behaviors across the child's life. Moreover, we did not assess the timing of other parental psychiatric disorders; therefore, we cannot assume specificity of our findings regarding exposure to maternal depression only.

Fifth, prior studies have found support for the role of genetics on offspring cortisol reactivity (e.g. Dougherty et al., 2010; Gotlib et al., 2008). However, the present study did not examine genetic influences on offspring HPA axis functioning. Finally, due to the cross-sectional nature of our design, we cannot assume causality or directionality from our findings. Although our data suggest that offspring's cortisol respsones are related to parenting and maternal psychiatric history; we do not know whether these factors lead to the onset of subsequent psychopathology without a

longitudinal design. Nevertheless, our findings provide a good avenue and basis for future longitudinal research to study a specific pathway for later risk.

Conclusion

Research has consistently reported that the offspring of parents with a history of depression are at increased risk for developing depression and other psychiatric problems (Weissman et al., 2006). It is of utmost importance to identify possible mechanisms of risk for the intergenerational transmission of depression. Several studies suggest that early dysregulation of the HPA axis may be a mechanism of risk for depression. Consistent with Dougherty et al. (2011), our findings suggest that the combination of early exposure to maternal depression *and* current parental hostility is related to a pattern of increasing cortisol responses in preschool-age children, highlighting the impact of environmental influences on the regulation of young children's developing neuroendocrine system. Additionally, our study was the first to observe the impact of parental hostility on the relation between maternal anxiety and offspring stress reactivity in high-risk preschool-age offspring. It is important to note that several possible explanations or mechanisms exist for the intergenerational transmission of depression, including genetics, insensitive maternal caregiving behaviors, timing and course of maternal depression, exposure to life stress, and dysregulated HPA axis functioning (Goodman & Gotlib, 1999). Therefore, the processes involved in these relations are likely complex and further multifactorial investigations into the transmission of risk are warranted.

It is possible that early life experiences, particularly insensitive parenting and exposure to maternal depression, may impose lasting changes on offspring HPA axis

functioning that render young children more vulnerable to the onset of subsequent depression and other stress-related psychiatric disorders. Thus, although our data are preliminary, they suggest implications for prevention and early intervention efforts. Recent encouraging findings have emerged from the animal literature, which suggest that changes in environmental experience (e.g. maternal caregiving behavior, enriched environments) can reverse the deleterious effect of early adverse rearing experiences on rats' neuroendocrine and behavioral responses to fear (Francis, Diorio, Plotsky, & Meaney, 2002). Therefore, early interventions may be particularly critical for the identification of high-risk young children, particularly during a period of neuroplasticity, prior to the onset of subsequent psychopathology. Our findings suggest that parenting interventions designed to strengthen the quality of mother-child relationships may be particularly important for reducing young children's vulnerability to later physical and psychiatric disorders associated with dysregulated HPA axis functioning.

Table 1: Subject characteristics and cortisol indicators for offspring of parents with and without a lifetime history of depression

	Offspring of parents with no lifetime depression (n = 54)	Offspring of parents with lifetime depression (n = 88)
Characteristic		
Child Sex, male (%)	26 (48.15)	45 (51.14)
Child Age, mean (SD), months	46.78 (9.64)	44.28 (8.78)
$\geq$ 1 parent college graduate (%)	39 (72.22)	63 (71.59)
Time of lab visit after noon (%)	35 (64.81)	68 (77.27)
Child attended school day of visit (%)	12 (22.22)	21 (23.86)
Child was sick day of visit (%)	4 (7.41)	5 (5.68)
Child took medication day of visit (%)	1 (1.85)	10 (11.36)
Child ate a meal within 1 hr of visit (%)	24 (44.44)	32 (36.36)
Child had caffeine within 2 hrs of visit (%)	3 (5.56)	4 (4.55)
Child had difficulty sleeping night prior (%)	3 (5.56)	9 (10.23)
Child activity level, mean (SD)	2.60 (0.58)	2.59 (0.76)
Number of trials failed during task (SD)	2.91 (0.40)	2.76 (0.59)
Child CBCL Internalizing, mean (SD)	6.91 (5.35)	8.43 (5.95)
Child CBCL Externalizing, mean (SD)	9.70 (7.21)	12.48 (7.88)
Parental lifetime anxiety disorder (%)	24 (44.44)	63 (71.59)
Parental lifetime substance use disorder (%)	20 (37.04)	34 (38.64)
Parenting behavior		
PCI Parental hostility, mean (SD)	1.10 (.21)	1.13 (.27)
PCI Parental support, mean (SD)	4.22 (0.66)	4.11 (0.74)
Salivary cortisol indicator, mean (SD)		
Cortisol level at time 1, nmol/L	2.55 (3.69)	2.35 (2.15)

Cortisol level at time 2, nmol/L	2.13 (1.95)	2.05 (1.54)
Cortisol level at time 3, nmol/L	2.11 (1.94)	2.05 (1.84)
Cortisol level at time 4, nmol/L	2.05 (1.46)	2.13 (2.26)
Cortisol level at time 5, nmol/L	2.23 (2.12)	2.11 (1.65)
AUC_g, mean (SD), nmol/L	136.35 (126.61)	132.95 (95.51)
AUC_i, mean (SD), nmol/L	-18.76 (106.53)	-13.72 (103.97)
AUC_i positive, N (%)	17 (31.48)	35 (39.77)

Note. AUC_g and AUC_i = area under the curve with respect to ground and increase, respectively.

Figure Captions

Figure 1. Offspring's mean cortisol values in response to the laboratory stressor by parental depression history. The graph shows mean cortisol values for each of the five times of cortisol data collection: 30 minutes after adaptation to the laboratory (Time 1), 20 minutes post-stressor (Time 2), 30 minutes post-stressor (Time 3), 40 minutes post-stressor (Time 4), and 50 minutes post-stressor (Time 5).

Figure 2. Offspring's total change in cortisol as a function of parental depression history and parental hostility. Cortisol change was calculated as area under the curve with respect to increase (AUC_i).

Figure 3. Offspring's total change in cortisol as function of the timing of parental depression history and parental hostility. Cortisol change was calculated as area under the curve with respect to increase (AUC_i).

Figure 4. Offspring's total change in cortisol as a function of maternal depression history and parental hostility. Cortisol change was calculated as area under the curve with respect to increase (AUC_i).

Figure 5. Offspring's total change in cortisol as a function of paternal depression history and parental hostility. Cortisol change was calculated as area under the curve with respect to increase (AUC_i).

Figure 6. Offspring's total change in cortisol as function of the timing of maternal depression history and parental hostility. Cortisol change was calculated as area under the curve with respect to increase (AUC_i).

Figure 7. Offspring's total cortisol secretion as a function of maternal anxiety history and parental hostility. Cortisol secretion was calculated as area under the curve with respect to ground (AUC_g).

Figure 1.

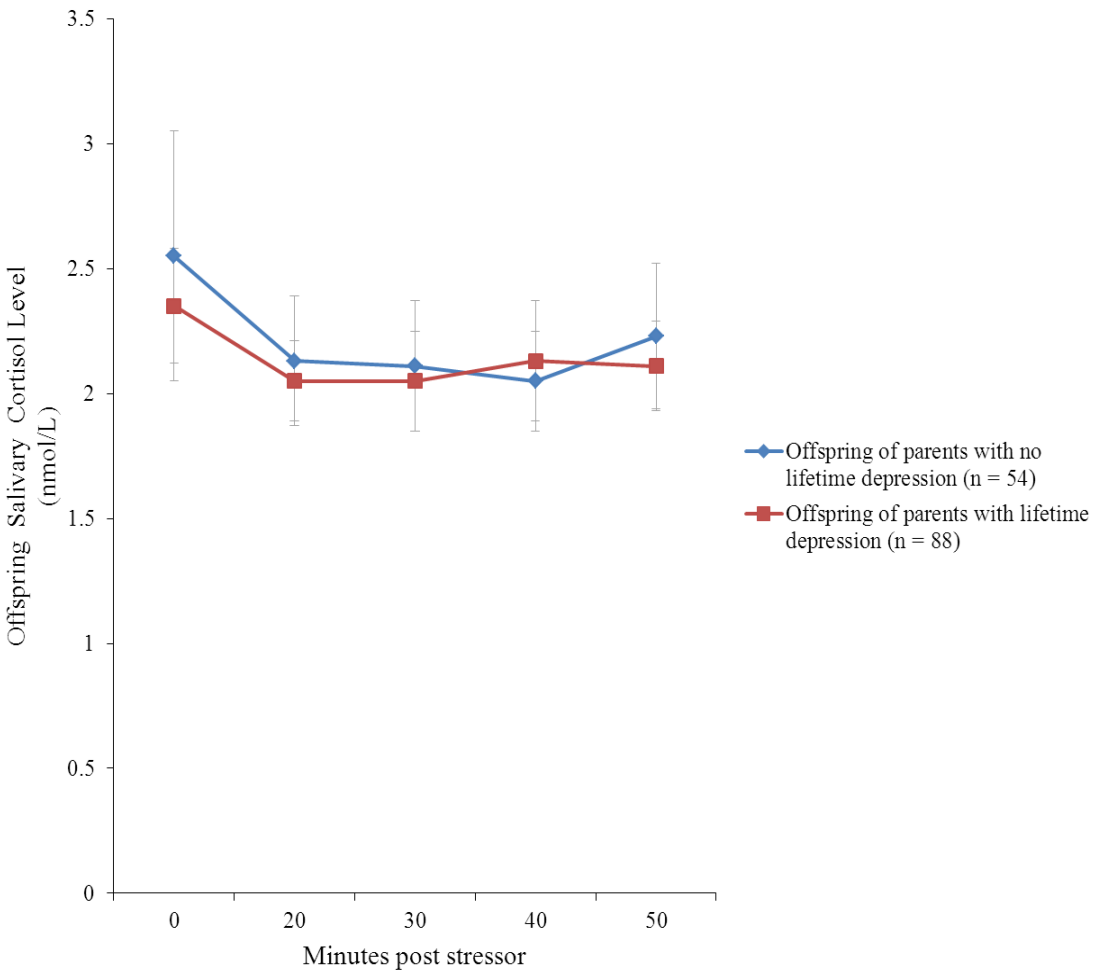

3.5
3
2.5
2
1.5
1
0.5
0
Offspring Salivary Cortisol Level (nmol/L)
0
20
30
40
50
Minutes post stressor
Offspring of parents with no lifetime depression (n = 54)
Offspring of parents with lifetime depression (n = 88)

Figure 2.

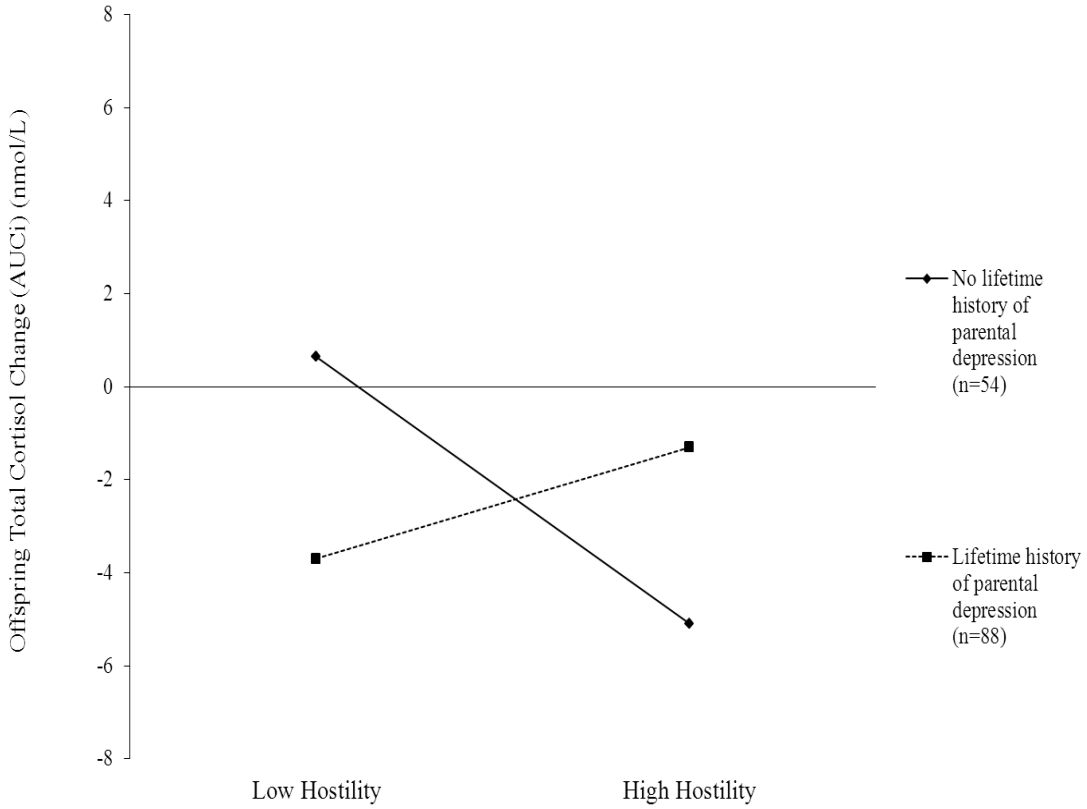

Offspring Total Cortisol Change (AUCi) (nmol/L)
8
6
4
2
0
-2
-4
-6
-8
Low Hostility
High Hostility
No lifetime history of parental depression (n=54)
Lifetime history of parental depression (n=88)

Figure 3.

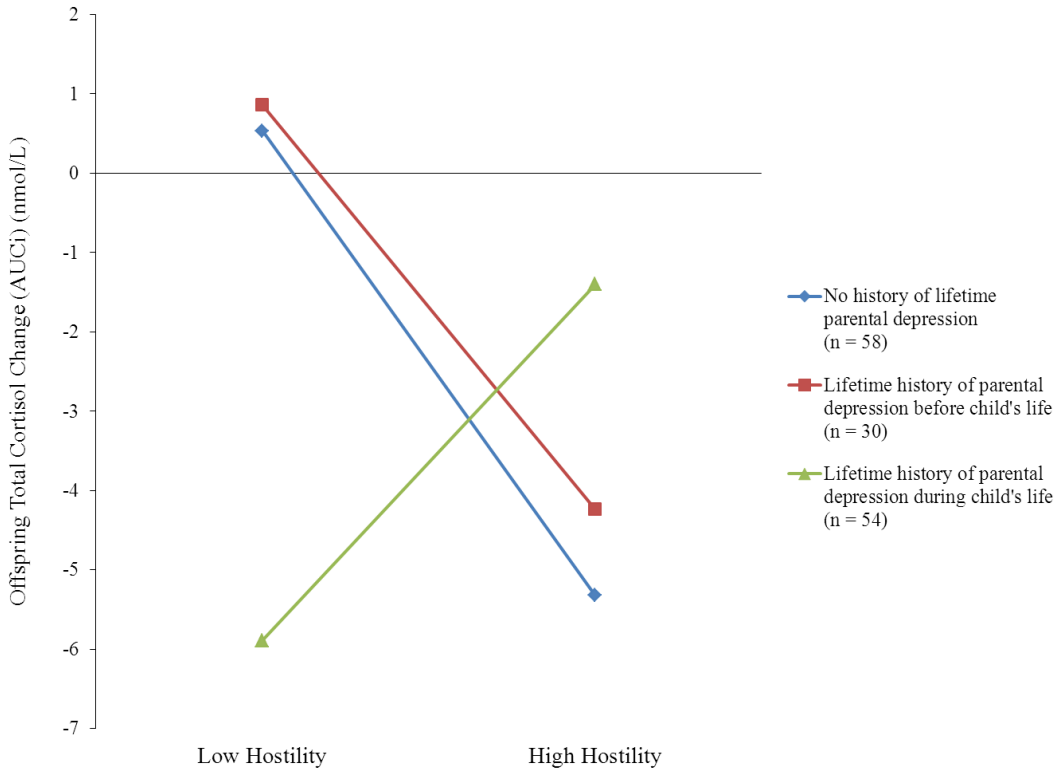

2
1
0
-1
-2
-3
-4
-5
-6
-7
Offspring Total Cortisol Change (AUCi) (nmol/L)
Low Hostility
High Hostility
No history of lifetime parental depression (n = 58)
Lifetime history of parental depression before child's life (n = 30)
Lifetime history of parental depression during child's life (n = 54)

Figure 4.

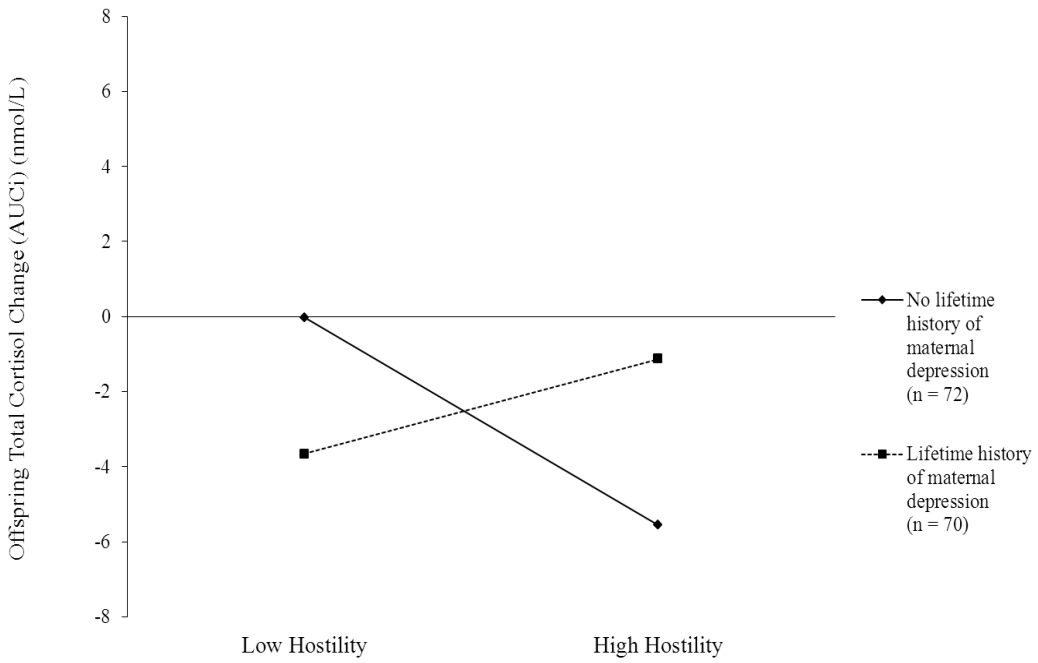

Offspring Total Cortisol Change (AUCi) (nmol/L)
8
6
4
2
0
-2
-4
-6
-8
Low Hostility
High Hostility
No lifetime history of maternal depression (n = 72)
Lifetime history of maternal depression (n = 70)

Figure 5.

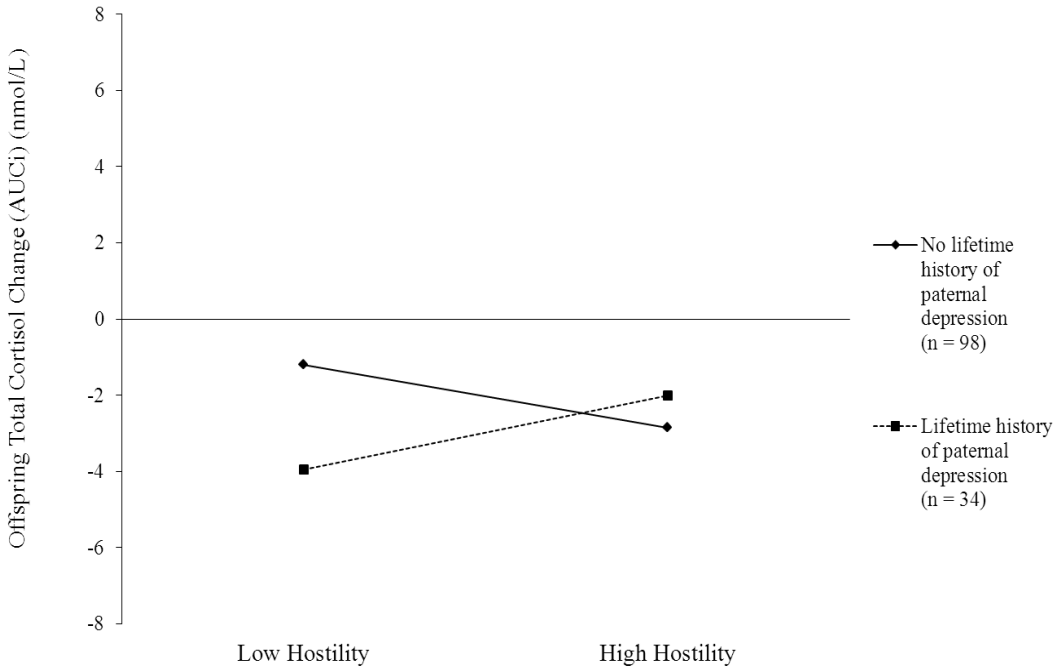

Offspring Total Cortisol Change (AUCi) (nmol/L)
8
6
4
2
0
-2
-4
-6
-8
Low Hostility
High Hostility
No lifetime history of paternal depression (n = 98)
Lifetime history of paternal depression (n = 34)

Figure 6.

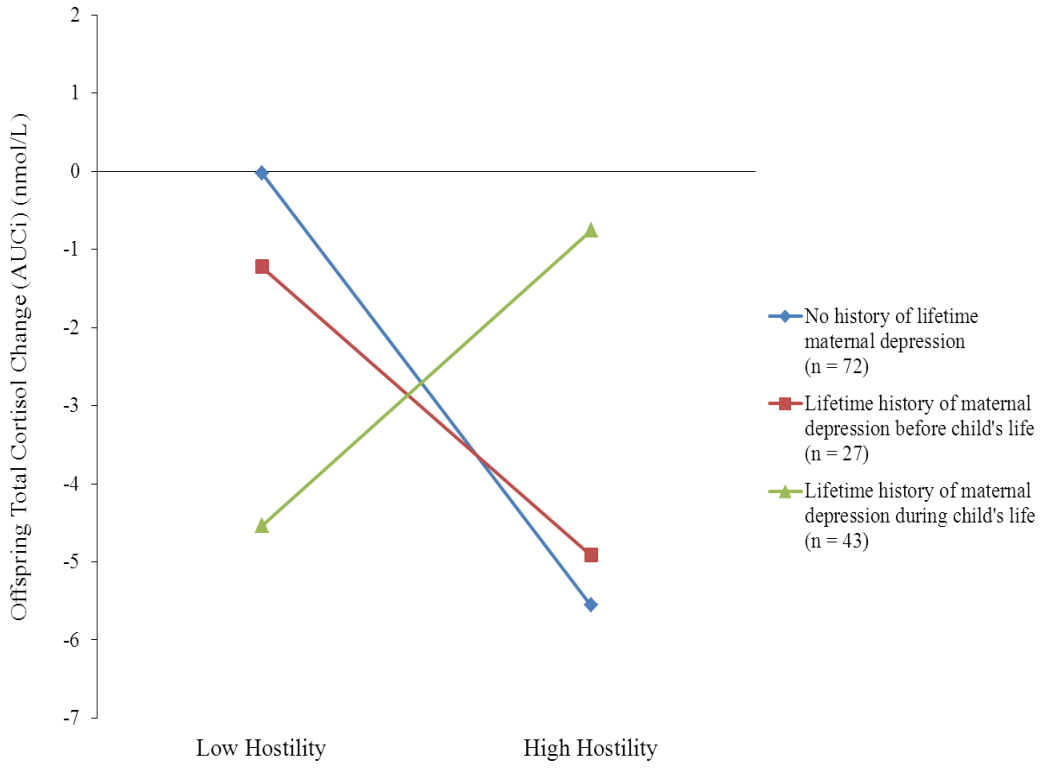
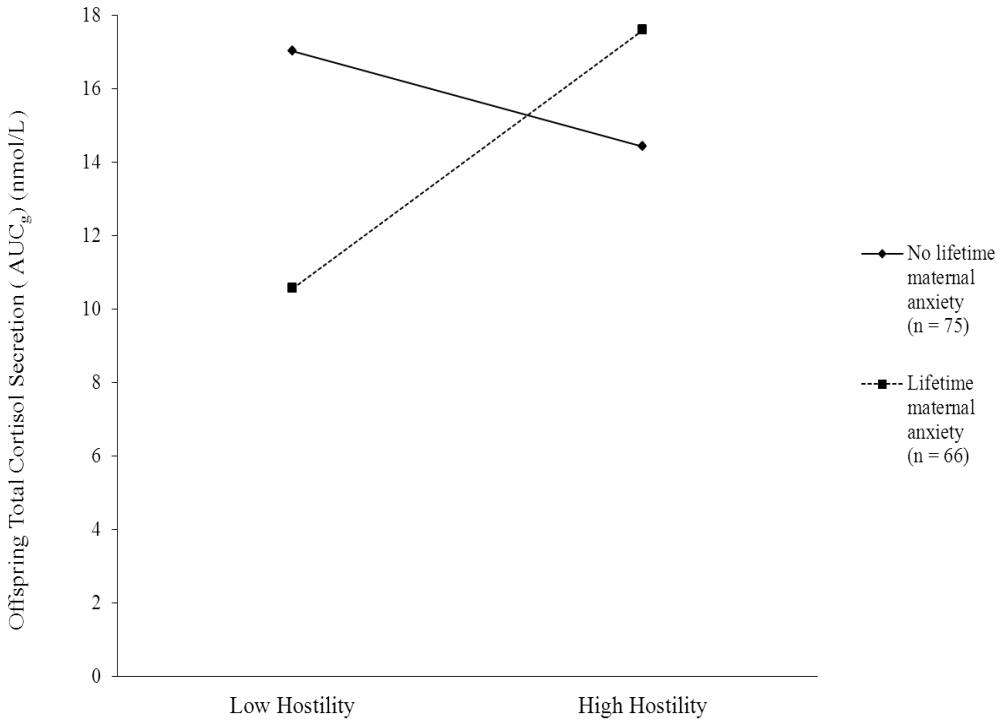

Offspring Total Cortisol Change (AUCi) (nmol/L)
2
1
0
-1
-2
-3
-4
-5
-6
-7
Low Hostility
High Hostility
No history of lifetime maternal depression (n = 72)
Lifetime history of maternal depression before child's life (n = 27)
Lifetime history of maternal depression during child's life (n = 43)

Figure 7.


18
16
14
12
10
8
6
4
2
0
Offspring Total Cortisol Secretion (AUCg) (nmol/L)
Low Hostility
High Hostility
No lifetime maternal anxiety (n = 75)
Lifetime maternal anxiety (n = 66)

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