Stony Brook University

SALIVARY CORTISOL AND DEPRESSION RISK: RELATIONS WITH CHILD TEMPERAMENT, MATERNAL HISTORY OF DEPRESSION, PARENTING, AND LIFE STRESS

A Dissertation Presented

by

Lea Rose Dougherty

The Graduate School

In Partial fulfillment of the

Requirements

for the Degree of

Doctor of Philosophy

in

Clinical Psychology

Stony Brook University

August 2008

STONY BROOK UNIVERSITY

The Graduate School

Lea Rose Dougherty

We, the dissertation committee for the above candidate for the

Doctor of Philosophy degree, hereby recommend

acceptance of this dissertation.

Daniel N. Klein, Ph.D. - Dissertation Advisor
Professor, Department of Psychology

Joanne Davila, Ph.D. - Chairperson of Defense
Associate Professor, Department of Psychology

Brenda J. Anderson, Ph.D.
Associate Professor, Department of Psychology

Gabrielle Carlson, M.D.
Professor, Department of Psychiatry, Stony Brook University

This dissertation is accepted by the Graduate School

Lawrence Martin
Dean of the Graduate School

Abstract of the Dissertation

Salivary Cortisol and Depression Risk: Relations with Child Temperament, Maternal History of Depression, Parenting, and Life Stress

by

Lea Rose Dougherty

Doctor of Philosophy

in

Clinical Psychology

Stony Brook University

2008

Dysregulation of the HPA system has been consistently associated with the affective disorders. The current study aims to test the hypothesis that low positive emotionality (PE), a possible temperamental precursor to depression, is associated with HPA axis functioning in children prior to the onset of a depressive disorder. This study also aims to examine the unique and joint effects of some of the complex factors related to children's early HPA axis functioning and depression risk, including maternal history of depression, parenting and life stress, alongside early temperament. These factors were examined in relation to children's salivary cortisol levels in response to a laboratory stressor and to children's home basal cortisol levels. One hundred and sixty-six preschool-aged children and their biological parents were recruited from a larger study examining temperamental risk for mood disorders: 166 children completed all 4 salivary cortisol samples during the lab visit; 94 children provided a home morning salivary cortisol sample; and 93 children provided an evening salivary cortisol sample. Child temperament and parenting were

assessed using laboratory observational measures, and maternal depression and child life stressors were assessed with semi-structured clinical interviews. First, mixed effects modeling was used to examine predictors of children's laboratory cortisol reactivity. We found that temperamental negative emotionality (NE), behavioral inhibition (BI), parental hostility and life stress were significantly associated with components of the trajectory of cortisol during the lab visit. Furthermore, several significant interactions emerged that predicted greater laboratory cortisol reactivity, including child low PE X maternal melancholic depression, child BI X maternal melancholic depression, child low PE X parental hostility, and maternal depression X parental hostility. In addition, child BI interacted with parental support to predict lower laboratory baseline cortisol levels. Next, multiple regression was used to examine predictors of children's home basal cortisol levels. We found that both child low PE and maternal melancholic depression were significantly associated with higher morning cortisol levels, suggesting potential depressive endophenotypes. The study's findings are summarized in terms of risk, resilience, and potentiation as they relate to the development of neuroendocrine dysfunction in young children, and suggest several potential avenues for future research.

Table of Contents

Tables

Figures

INTRODUCTION

Dysregulation of the HPA system has been consistently associated with the affective disorders (Bradbury, Akana, & Dallman, 1994; de Kloet, 1994; Johnson, Kamilaris, Chrousos, & Gold, 1992), and prospective studies provide evidence that abnormalities of the HPA axis contribute to their etiology (Goodyer, Herbert, Tamplin, & Altman, 2000; Harris et al., 2000). Research suggests that the HPA axis plays a central role in mediating the effects of stress on psychopathology (Arborelius, Owens, Plotsky, & Nemeroff, 1999). During acute stress, there is a rapid increase in the secretion of cortisol, an adrenocortical steroid hormone, and in secretions from the corticotropin-releasing factor (CRF). However, adaptive advantages conferred by cortisol secretion during stress are limited to its acute effects, rather than chronic release, as chronic elevation in cortisol is almost always deleterious and may have long-term detrimental consequences (Gold & Chrousos, 1999; Meyer, Chrousos, & Gold, 2001).

The present study aims to examine the factors associated with neuroendocrine dysfunction that may be involved in the subsequent development of depression via HPA axis functioning. Similar to theoretical conceptualizations of the development and course of the depressive disorders, we plan to examine temperamental, familial and environmental factors as likely factors associated with neuroendocrine dysfunction, with a particular emphasis on the relation between temperamental vulnerability to depression and HPA axis functioning. Recently, researchers hypothesize that an underactivation of positive affect, approach behavior, and responsiveness to reward (which we will refer to as “positive emotionality” or PE) may constitute a temperamental vulnerability to the depressive disorders (Clark, Watson, Mineka, 1994; Davidson, 1998; Meehl, 1975). The

current study will examine the relation between low PE, as a potential temperamental precursor to depression, and HPA functioning in preschoolers. While several researchers have examined the relation between other temperament dimensions, such as behavioral inhibition (BI) and HPA functioning (e.g., Kagan, Reznick, & Snidman, 1987), very little research has examined the relation between low PE and HPA functioning in young children. We are particularly interested in the unique effects of child temperament on HPA axis functioning in a young sample prior to the onset of depression. It is important to establish this relation prior to the onset of a depressive disorder in order to eliminate any confound between temperament and psychopathology. Moreover, the current study aims to tease apart some of the complex factors related to children's early HPA axis functioning and depression risk, including familial risk for depression, parenting, and life stress, alongside early temperament. This study should provide a foundation for future longitudinal studies to prospectively test a more comprehensive causal model positing that the relation between temperament and subsequent depression is mediated in part by the body's major physiological stress response system.

Below, we will review the literature on the association between depression and abnormalities of the HPA axis, followed by a section on the relation between temperament and depression. Then, we will review the developmental literature examining the possible temperamental, familial, and environmental contributions to neuroendocrine dysfunction, including early child temperament, maternal depression, parenting, and life stress.

Depression and HPA axis functioning. Studies over several decades have reported abnormalities in cortisol secretion in a significant proportion of adults with major

depressive disorder (MDD). The most prominent neuroendocrine abnormality in MDD is hyperactivity of the HPA axis, occurring in approximately 50% of patients (Thase & Howland, 1995). These elevated levels of cortisol are thought to be a function of hypersecretion of CRF by the HPA system and an impairment of negative feedback regulation, preventing elevated cortisol levels from returning to basal levels (Chrousos & Gold, 1992). Studies have reported associations between HPA axis abnormalities and depression severity (Nelson & Davis, 1997), melancholia/endogenicity (Rush, Giles, Schlesser, Orsulak, Parker, Weissenburger et al., 1996), psychotic features (Nelson & Davis, 1997), length of current depressive episode (Posener, DeBattista, Williams, Kraemer, Kalehzan, & Schatzberg, 2000), episode onset (Harris et al., 2000), chronicity (Watson, Gallagher, Del-Estal, Hearn, Ferrier, & Young, 2002), relapse (Aubry, Gervasoni, Osiek, Perret, Rossier, Bertschy et al., 2007), and treatment response (Vythilngam et al., 2004).

The link between depression and hypercortisolemia appears to be most pronounced and prevalent in patients with melancholic depression (Kupfer, 1991). In order to meet criteria for melancholic depression, individuals must meet criteria for anhedonic mood, which is defined as a failure to derive pleasure from all or nearly all activities and/or a lack of appetitive responding to usually pleasurable stimuli, in addition to at least three of the following: (a) depressive symptoms that are usually worse in the morning, (b) early morning awakening, (c) psychomotor retardation, (d) weight loss, and/or (e) inappropriate guilt. The subtype of melancholic depression has been shown to identify a subset of affected individuals with distinct clinical features (e.g., greater number of episodes, more impairment) and a particularly high familial liability to

depressive illness (Kendler, 1997). Specifically, in a sample of female twins with major depression, the presence of melancholic subtype in one twin substantially increased the risk for major depression in her co-twin, and the increased risk was due to the effect of melancholic features, rather than a general effect of clinical severity (Kendler, 1997). Recent evidence suggests that melancholia arises from different etiologic mechanisms than other forms of depression (Beauchaine, 2003; Beauchaine & Marsh, 2006). Patients with melancholic depression display elevated basal levels of cortisol, despite normal ACTH levels, in comparison with nondepressed individuals (Gold, Calabrese, et al., 1986; Holboer, Vonbardeleben, Girken, Stalla, & Muller, 1984; Wong et al., 2000; Young, Carlson, & Brown, 2002); evidence an attenuated ACTH response, but similar cortisol response, after administration of exogenous corticotrophin-releasing hormone (CRH) (Gold, Calabrese et al., 1986); evidence elevated cerebrospinal fluid norepinephrine levels around the clock (Wong et al., 2000); and evidence alterations in 5-HT2a receptor density compared with individuals with non-melancholic depression (e.g., Akin, Manier, Sanders-Bush, & Shelton, 2004).

HPA axis functioning has also been examined in other subtypes and populations of depressed individuals. In contrast to melancholic depression, patients with atypical depression (a subtype of depression characterized by mood reactivity, increased appetite, and hypersomnia) are hypothesized to have a downregulated HPA axis (Gold, Licinio, Wong, & Chrousos, 1995); however, this hypocortisolemia appears to be present only in those individuals with atypical depression characterized by an early onset (i.e., before age 20 years) and a chronic course (i.e., no spontaneous well-being greater than 2 months) rather than in individuals with atypical depression with either a later onset or a less

chronic course of illness (Levitan, Vaccarino, Brown, & Kennedy, 2002; Stewart, Quitkin, McGrath, & D. F. Klein, 2005). In addition, erratic cortisol, rather than hyper- or hypocortisolism, has been linked to HPA axis dysregulation in outpatients with MDD (Peeters, Nicolson, & Berkhof, 2003). Thus, the fundamental neuroendocrine deficit in depression is complex and the type of dysfunction seems to vary according to specific features of the depressive illness.

Research linking HPA axis dysfunction and depression in children and adolescents has yielded an even more ambiguous array of findings than for adults. In contrast to findings in depressed adults, many investigations of 24-hour cortisol secretion have demonstrated no differences between depressed child and adolescent outpatients and control groups (Birmaher et al., 1992; Feder et al., 2004; Kutcher et al., 1991; Puig-Antich et al., 1989), whereas a few studies have found such differences (Debellis et al., 1996; Goodyer, Herbert, Altham, Pearson, Secher, & Shiers, 1996). Nevertheless, alterations in the HPA system have been associated with onset of MDD in at-risk adolescents (Goodyer et al., 2001; Goodyer, Herbert, Tamplin, & Altham, 2000), chronic depression in children and adolescents (Goodyer, Park, & Herbert, 2001), and internalizing problems in clinic-referred children (Granger, Weisz, McCracken, Ideda, & Douglas, 1996).

Interestingly, the specific relation between melancholic depression and hypercortisolism appears to be present in depressed children as well as depressed adults. Birmaher et al. (1996) found significantly higher baseline cortisol levels in children with melancholic depression than children with nonmelancholic depression, and melancholic depressed children evidenced lower ACTH secretion after CRH infusion (similar to

findings in adults with melancholic depression). In addition, Luby et al. (2003) reported elevations in salivary cortisol in depressed preschoolers, particularly those with anhedonia (a characteristic feature of melancholia), during a laboratory separation paradigm, compared to nondepressed children.

Origins of HPA axis dysfunction in depression. Research has provided support for the hypothesis that dysregulation of stress hormones plays a major role in the pathophysiology of depression, especially as one pathway to depression (Greden, Gardner, King, Grunhaus, Carroll, & Kronfol, 1983; Holsboer, 2000; Holsboer, Liebl, & Hofschuster, 1982). However, the origins of the neuroendocrine dysfunction are not well understood. On the one hand, some argue that neuroendocrine dysregulation is due to cumulative physiological changes produced by excessive challenge (i.e., allostatic load; McEwen, 1998), such that repeated or chronic psychosocial stressors alter brain structure and function in key regions thought to underlie depression (Nemeroff, 1996). Indeed, developmental research has shown that early neuroendocrine dysfunction is associated with poor attachment relationships, neglect, maltreatment, and trauma (Gunnar & Donzella, 2002). Moreover, both animal and human research suggests that the heightened HPA axis reactivity associated with early adversity persists into adulthood (Heim & Nemeroff, 2001; Heim et al., 2000). On the other hand, geneticists contend that MDD is transmitted through an intermediate phenotype, such as a temperament trait, rather than by the direct transmission of the depressive syndrome (Silberg & Rutter, 2002). This trait (or set of traits) may be associated with a tendency for HPA axis dysregulation that renders individuals with the trait more vulnerable to stress, thereby increasing the risk of a depressive episode. It may also be likely that a complex interplay between genetic

susceptibility and environmental exposures is involved in the neuroendocrine dysfunction in depression. This notion has been supported by findings that individuals with high genetic risk for depression are more susceptible to the depressive episode-triggering effect of an adverse life event (Kendler, Gardner, & Prescott, 2002).

As we aim to take a multifactorial and comprehensive approach in our understanding of the origins of neuroendocrine dysfunction, we will first orient the reader to the literature supporting early temperament as a developmental precursor to depression, particularly as early temperament has often been overlooked in models of psychopathology. Then, we will review temperamental, familial and environmental contributors as possible determinants of neuroendocrine functioning.

Temperamental precursors to depression. Theorists have long posited that the predisposition for the depressive disorders may be rooted in individual differences in temperament (e.g., Akiskal, 1989; Kraepelin, 1921). Temperament refers to characteristic patterns of emotional reactivity and regulation that are relatively stable and at least partially influenced by early-developing biological systems (Rothbart & Bates, 2006). Two traits that are central to most models of child temperament and adult personality are positive emotionality (PE) and negative emotionality (NE). PE is a higher order construct that includes a number of facets, including: (a) positive affect and enthusiasm; (b) approach, reward seeking, and appetitive behavior; (c) energy, activity, and liveliness; (d) affiliation, warmth, and gregariousness; and (e) ascendance, surgency, and dominance (Shiner & Caspi, 2003). It is generally viewed as orthogonal to the higher-order construct of negative emotionality (NE), which includes such facets as anxiety, sadness, irritability, and negative mood reactivity [although see Russell and Carroll (1999) for an opposing

perspective]. Both PE and NE are partially heritable and fairly stable over time in both adults and children (Durbin, Hayden, Klein, & Olino, 2007; Goldsmith, Buss, & Lemery, 1997; Roberts & DelVecchio, 2000).

Several theorists have developed temperament models that link both PE and NE to depression. Most notably, Clark, Watson, and Mineka (1994) developed the tripartite model, which hypothesizes that low PE is a specific temperamental predisposition to depression while NE is non-specific to a variety of forms of psychopathology, including depressive and anxiety disorders. Other theorists have also posited that facets of PE, such as low hedonic capacity (Hamburg, 1998; Meehl, 1975), reduced responsiveness to reward (D.F. Klein, 1987), and appetitive and approach deficits (Davidson, 1998; Depue & Iacono, 1989), are primary etiological factors involved in the development of depression.

A number of studies have examined the relations between PE and NE and depression in adults. Most of these studies, using both clinical (e.g., Brown, Chorpita, & Barlow, 1998; Watson, Clark, & Carey, 1998) and community (Krueger, Caspi, Moffitt, Silva, & McGee, 1996; Trull & Sher, 1994) samples, have supported the hypothesis that PE has a relatively specific association with depressive disorders while NE is a non-specific factor related to psychopathology in general. There is also a literature examining the relations between PE and NE and depressive symptoms in children and adolescents (Chorpita, 2002; Joiner, Catanzaro, & Laurent, 1996; Lonigan, Hooe, David, & Kistner, 1999; Lonigan, Philips, & Hooe, 2003) that generally supports the tripartite model. In addition, the specificity of the low PE-depression link has been further supported by findings showing that low PE-related behaviors have been observed in the infants of

depressed mothers (Cohn, Campbell, Matias, & Hopkins, 1990; Field, 1992) and were linked to left frontal hypoactivation in the infants, which is hypothesized to reflect an approach system deficit characteristic of depression (Dawson et al., 1999). Lastly, in a community sample of 100 children, low PE at age 3 was associated with maternal lifetime mood disorder, but was not related to other forms of psychopathology in mothers (Durbin, Klein, Hayden, Buckley, & Moerk, 2005).

Although the majority of research linking temperament and depression has consisted of cross-sectional studies, a few longitudinal studies have found that lower levels of PE-related behaviors in childhood predict the development of depressive disorders (Caspi, Moffitt, Newman, & Silva, 1996; van Os, et al., 1997; Block, Gjerde, & Block, 1991). For example, van Os et al. (1997) found that childhood behavioral apathy predicted both childhood onset and chronic depression in adulthood. In addition, low extraversion in childhood has shown links to depressive symptoms at age 18 (Block et al., 1991). One longitudinal study has found associations between low PE at age 3 and other factors implicated in risk for later depression, including EEG asymmetry at age 5 (Shankman, Tenke, Bruder, Durbin, Hayden, & Klein, 2005), depressive cognitive schemas at age 7 (Hayden, Durbin, Klein, & Olino, 2006), and depressive symptoms at age 10 (Dougherty, Klein, Durbin, Hayden, & Olino, 2007). Longitudinal studies have also suggested that NE may be a general risk factor for the development of internalizing disorders (Clark et al., 1994). For instance, NE in infancy and early childhood has been related to maternal reports of anxiety and depression when children were 7 years old (Rende, 1993). Finally, NE in youth predicted changes in both anxiety and depressive symptoms over a 7-month follow-up whereas PE uniquely predicted changes in

depressive symptoms over time (Lonigan Philips, & Hooe, 2003). Thus, these longitudinal studies support the hypothesis that PE represents a specific risk for the development of depression whereas NE represents a general risk factor for mood, anxiety, and possibly other disorders.

In addition to PE and NE, behavioral inhibition (BI) has also been related to risk for mood disorders. The construct of BI, though central to most child temperament models, has not been well integrated into adult temperament models. BI, which is characterized by fear, wariness and low exploration (Kagan, 1997), has been linked to depression; however, the results are less consistent (Biederman et al., 1990; Biederman et al., 1993; Kochanska, 1991) than the link between BI and risk for anxiety disorders (Goldsmith & Lemery, 2000; Schwartz, Snidman, & Kagan, 1997). BI, in certain contexts, can appear very similar to low PE, but is distinguished by different underlying processes. While children with high BI may exhibit low approach behavior and social withdrawal, this is limited to unfamiliar contexts and normalizes with familiarity (Laptook, Klien, Durbin, Hayden, Olino, & Carlson, 2008). In contrast, children with low PE exhibit low positive affect, low approach behavior, and social withdrawal on a stable basis and across contexts and over time. Thus, the correlation between laboratory measures of BI and PE is relatively low (Durbin et al., 2005; Pfeifer, Goldsmith, Davidson, & Rickman, 2002). In sum, BI is conceptually and empirically different from PE and may be more highly associated with risk for anxiety than depressive disorders. Nevertheless, given that BI may also play a role in the development of depression, that anxiety often preceeds depression, and the high comorbidity between depression and anxiety, we believe that BI provides an interesting comparison to PE. Furthermore, BI is

particularly important as social anxiety disorder significantly increased risk for subsequent depression, and BI uniquely predicted subsequent depression among individuals with social anxiety disorder (Beesdo, Bittner, Pine, Stein, Stein, Hofler et al., 2007).

Temperament and HPA axis functioning. Some personality traits have been associated with individual differences in physiological reactivity to stressors which, in turn, could increase susceptibility to depression. It should be noted, however, that the direction of the relationship between temperament and neuroendocrine stress reactivity may be difficult to discern, and it is conceivable that temperament and physiological stress reactivity reflect similar processes at different levels of analysis.

Over the past decade, there has been a proliferation of studies examining the relation between physiological systems and children's emotional development (Fox, 1994; Kagan, 1998). Negative emotionality/distress has been associated with elevated cortisol levels (Ahnert,, Gunnar, Lamb, & Barthel, 2004; Buchanan, Absi, & Lovallo, 1999; Buss et al., 2003; Davis, Donzella, Krueger, & Gunnar, 1999), and this relation has been found in infants (Lewis & Ramsay; 2005; van Bakel, & Riksen-Walraven, 2002) and children (Dettling, Parker, Lane, Sebanc, & Gunnar, 2000; Gunnar, Tout, de Haan, Pierce, et al., 1997). Similarly, high levels of neuroticism in adults are associated with increased salivary cortisol levels (Kunz-Ebrecht, Kirschbaum, Marmot, & Steptoe, 2004).

In addition to NE, researchers have focused on the identification of predictors of individual differences in biobehavioral processes as they relate to the tendency to withdraw and approach in novel or challenging circumstances (Gunnar 1994; Harmon-Jones & Allen, 1998; Kagan, Reznick, & Snidman, 1987). Much attention has been given

to the link between the withdrawal behaviors (e.g., BI) and HPA axis functioning. BI has been associated with elevated cortisol levels (Kagan et al., 1987), as has shyness (de Haan, Gunnar, Trout, Hart, & Stransbury, 1998) and social wariness (Schmidt et al., 1997; Smider et al., 2002) in children. However, not all studies report such associations (Davis et al., 1999; Schmidt, Fox, Schulkin, & Gold, 1999).

Surprisingly, however, little research has examined the relation between PE, which is characterized by approach-type behaviors, and HPA axis functioning in children. One study reported that parent-reported approach motivation of children between the ages of 3 to 5 years tended to be associated with decreases in cortisol across the assessment session (Blair, Peters, & Granger, 2004), which suggests that high PE may serve a protective function against HPA axis hyperactivity. In contrast, a few studies have reported a positive relation between constructs that overlap with PE, such as surgency, extraversion and impulsivity, and cortisol levels in children (e.g., Davis, Donzella, Krueger, & Gunnar, 1999). Although these constructs are related to PE, they also overlap with activity level and sociability, and it may be the level of arousal that is related to increases in cortisol rather than the expression of positive emotions (Pressman & Cohen, 2005).

Some studies have examined the relation between positive affect and HPA axis functioning in adults; however, these studies are limited to self-reported positive moods. Cortisol has been shown to typically decrease following the experimental induction of positive moods (e.g., Hubert & de Jong-Meyer, 1990) and with increasing levels of trait positive affect (Cohen, Doyle, Turner, Alper, & Skoner, 2003; Polk, Cohen, Doyle, Skoner, & Kirschbaum, 2005). One study found that cortisol levels for men low in trait

positive affect did not decrease in the afternoon, resulting in a relatively high, flat rhythm (Polk et al., 2005), which is similar to the rhythm dysregulation observed in depression (e.g., Deuschle et al., 1997; Weber, Lewicki, Deushle, Colla, Vecsei, & Heuser, 2000).

Not all studies reported associations between positive affect and HPA axis functioning in adults (Pressman & Cohen, 2005). It is possible that positive emotions interact with other intra-individual factors or environmental factors to predict HPA axis functioning. For example, Moskowitz and Epel (2006) found that healthy daily cortisol rhythms were predicted by the joint presence of positive emotions and positive cognitions. This is especially interesting given the finding that low PE at age 3 predicted the development of depressive cognitive styles at age 7 (Hayden et al., 2006).

Despite the limited developmental research in this area, there is indirect evidence for an association between low PE and HPA axis functioning from neurophysiological studies. A substantial body of research has suggested that relatively greater left than right frontal hemisphere activity is associated with approach-related behaviors and positive affect, and relatively greater right than left frontal activity is associated with withdrawal-related behaviors and negative affect (Davidson, 1995). Thus, the link between PE/approach and HPA-reactivity is supported by evidence that both decreased left frontal activity and increased right frontal activity are associated with cortisol levels during a stranger approach task (Buss et al., 2003). In addition, Luby et al. (2003) found that preschoolers with an anhedonic form of depression, which is characterized by low PE, displayed greater cortisol reactivity than depressed and non-depressed comparison groups. Similarly, adults with melancholic depression, which is characterized by low PE,

exhibit higher cortisol levels than adults with non-melancholic depression (Turkcapar et al., 1999).

Factors influencing HPA axis functioning: maternal history of depression, parenting and life stress. Developmental studies have established that offspring of depressed mothers exhibit greater elevations in cortisol in response to a psychosocial stressor very early in life (Dawson, Hessl, & Frey, 1994), in addition to increased waking salivary cortisol levels (Mannie, Harmer, & Cowen, 2007). In addition, the observed increased waking salivary cortisol levels in young people at familial risk for depression was not accounted by differences in self-reported parenting relationships, life events, or neuroticism (Mannie et al., 2007). Overall, these findings support a genetic risk for depression on the HPA axis.

Nevertheless, it is difficult to distinguish the effects of the environment, such as poor parenting or early maltreatment, from genetic risk for depression on the HPA axis. Therefore, it remains unclear whether the HPA axis dysfunction found in the offspring of depressed individuals is due to the direct effects of a genetic vulnerability for depression or to insufficient care provided by the depressed parent (e.g., Ashman, Dawson, Panagiotides, Yamada, & Wilkinson, 2002; Cohn et al., 1986; Dawson & Ashman, 2000; Halligan, Herbert, Goodyer, & Murray, 2004). For instance, children with MDD parents showed higher cortisol levels, and this effect occurred predominantly in children whose parents were currently depressed (Young, Vazquez, Jiang, & Pfeffer, 2006). These findings support an environmental effect of MDD in a parent and are in line with studies that have found associations between poor parental care and cortisol functioning in both animals (Coplan, Andrews, Rosenblum, Owens, Friedman, Gorman, & Nemeroff, 1996)

and infants (Bugental, Martorell, & Barraza, 2003; Gunnar, 1998; Spangler, Schieche, Maier, & Ackerman, 1994).

There is also evidence that both a familial (possibly genetic) vulnerability for depression and the effects of depression on parenting independently contribute to children's HPA axis functioning. For instance, Azar, Paquette, Zoccolillo, Baltzer, and Tremblay (2007) found that both maternal lifetime depression and maternal overcontrol were independently associated with children's increased cortisol reactivity, and the relation between maternal lifetime depression and children's increased cortisol reactivity was not accounted by maternal depression during the child's life. Nevertheless, it is possible that maternal rearing practices, combined with the possible genetic risk in the offspring of affectively ill mothers, influence the stress responses of offspring (Granger et al., 1998). However, this is an area of research that needs more attention.

Life stress within the child's environment has also been examined in relation to children's HPA axis functioning. Numerous retrospective studies of adults and children have reported associations between increased reactivity of the HPA system and stressful life events, including early trauma such as child abuse and neglect (Cicchetti & Rogosch, 2001; DeBellis et al., 1999; Heim, Newport, et al., 2000; Kaufman et al., 1997), as well as less severe concurrent stressors, such as family socioeconomic status (Lupien et al., 2000). For instance, preschoolers who were exposed to high levels of concurrent maternal stress and had a history of high maternal stress exposure in infancy evidenced elevated cortisol levels (Essex, M. J. Klein, Cho, & Kalin, 2002). While a number of studies have found life stress within the child's environment to be associated with hypercortisolism (Gunnar 2000; Gunnar, Morison, Chisholm & Schuder, 2001), other studies have found a

link between life stress and hypocortisolism (e.g., Kaufman et al., 1997; Heim, Newport, Bonsall, Miller, & Nemeroff, 2001; Ronsaville, Municchi, Laney, Cizza, Meyer, Haim, et al. 2006), similar to the hypocortisolism found in patients with Post Traumatic Stress Disorder (PTSD) and the offspring of individuals with PTSD (Yehuda, 2000; Yehuda et al., 1993). It has been suggested that the differences in findings may be due to certain characteristics of the stressor (Gunnar & Donzella, 2002; Miller, Chen, & Zhou, 2007), such as duration (Gunnar 2000; Gunnar, Morison, et al., 2001) and timing (Essex et al., 2002; Hessl et al., 1998) or whether the maltreated children also suffered from an affective illness (Hart, Gunnar, & Cicchetti, 1996).

Current study. The current study aims to test the hypothesis that low PE, a possible temperamental precursor to depression, is associated with HPA axis functioning in children prior to the onset of a depressive disorder. Very little research has examined the relation between PE and cortisol, and no studies to date have examined the independent and joint effects of familial risk for depression, parenting, and life stress, along with early temperament, in predicting children's stress reactivity and basal cortisol levels. Furthermore, we are aware of only one study that has examined the effects of parenting on HPA axis functioning in children beyond toddlerhood (Smeekens, Riksen-Walraven, & van Bakel, 2007). Thus, we aim to add to this small literature base and to tease apart these complex and related constructs in examining their unique and joint effects in their relation to early HPA axis functioning. For instance, early temperament will be assessed separately from child psychopathology, and we will examine life stress separately from other overlapping constructs, such as strained parent-child relationships, maternal depression during the child's life, marital discord, and low socioeconomic

status. Overall, we aim to set a foundation for longitudinal studies to examine developmental pathways from neuroendocrine dysfunction to the development of depression.

This study will examine these predictors in relation to children's cortisol in response to environmental stimuli during a laboratory visit, as well as under basal conditions, both of which appear to be determined by different underlying processes. These different underlying processes are important to consider in extrapolating the study's hypotheses.

Basal activity follows a circadian rhythm with the highest levels typically occurring in the morning and then dropping throughout the day. Afternoon reflects a more quiescent period of the circadian cycle. Bartels, de Geus, Kirscbaum, Sluyter, and Boomsma (2003) found significant genetic contribution to cortisol levels after waking in the morning, a moderate genetic contribution around noon, and no genetic contribution during early afternoon (see also Wust et al., 2000; Kupper et al., 2005; Schreiber, Shirtcliff, Van Hulle, Lemery-Chalfant, M. H. Klein, Kalin et al., 2006). In addition, one study found genetic control over baseline in late afternoon cortisol at the home and at the laboratory but failed to find a significant genetic component to the response to a social stressor (Kirschbaum et al., 1992). However, the sample size for this study was small (13 monozygotic twin pairs and 11 dizygotic twin pairs), suggesting that it remains unclear as to the role hereditary plays in the adrenocortical response to a social stressor. Overall, these findings support the assertion that morning cortisol levels are under the most genetic control, whereas cortisol levels later in the day and in response to stressors are potentially under less genetic control and greater environmental control.

Due to these differences in genetic and environmental contributions to cortisol in response to a stressor and basal cortisol levels throughout the day, we hypothesize that the predictors of cortisol in response to the laboratory stressors will differ somewhat from predictors of children's basal cortisol levels. First, we hypothesize that the predictors of children's cortisol reactivity to the lab stressors will include direct effects of both intrinsic and/or biological (i.e., temperament, maternal history of depression) and environmental (i.e., parenting, life stress) factors. As most salivary cortisol samples were collected prior to late afternoon when environmental factors appear to exert more control, it is probable that some genetic control over cortisol reactivity is present. In addition, as some studies have reported that environmental factors exert the most control later in the day and in response to stressors, we hypothesize that environmental factors will predict children's stress reactivity and evening cortisol levels but have less of an impact on children's basal morning cortisol levels.

In considering this complex interplay between genetic and environmental factors on HPA axis functioning, we hypothesize that the intrinsic characteristics of the child, such as temperamental low PE, high NE and high BI, and maternal history of depression will be associated with greater cortisol levels in response to laboratory stressors. We also hypothesize that environmental factors will predict children's cortisol reactivity, but the direction of the relation will change depending on the qualitative characteristics of the environmental demand. Specifically, we predict that parental hostility will be associated with greater stress reactivity, and maternal supportive presence will be associated with less stress reactivity, as previous studies have reported similar findings in infants (e.g., Bugental, Martorell, & Barraza, 2003; Gunnar, Larson, Hertsgaard, Harris, & Brodersen,

1992). In addition, we hypothesize that children who experienced high levels of family stress in the 6 months prior to the laboratory visit will evidence dampening of the cortisol stress response, similar to that observed in patients with PTSD. This is supported by a recent study that reported chronic family stress was negatively correlated with children's cortisol levels (Ronsaville et al., 2006).

In addition to the hypothesized direct effects, we also predict several moderated effects. Given the inconsistent findings in the literature between temperament, familial risk for depression and cortisol reactivity (Ellenbogen et al., 2006; Gunnar & Donzella, 2002), it is possible that these vulnerabilities interact with environmental risk factors to predict cortisol reactivity. In support of this contention, both child temperamental NE and BI have been associated with increased cortisol in response to a stressor when quality of care was poor (e.g., Gunnar, Brodersen, Nachmian, Buss, & Rigauso, 1996; Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996). No research to date has examined any of the other possible moderating effects between the study variables. However, as individuals with a vulnerability to depression are more susceptible to the depressive episode-triggering effect of adversity (Kendler et al., 2002; Kendler et al., 2004), we predict that the risk factors selected for this study, including child temperament, maternal history of depression, parenting and life stress, will interact to predict greater stress reactivity.

Next, the basal morning salivary cortisol sample provides an interesting comparison to the lab coritisol reactivity data. Research has shown that morning cortisol levels are under the most genetic control and that elevated morning cortisol levels are specifically implicated in risk for depression. Therefore, it appears that morning cortisol

levels may capture genetic/familial or trait-like factors that put individuals at risk for developing mood disorders. As such, we hypothesize that child temperamental PE and maternal history of depression will be associated with children's morning cortisol levels. Specifically, given the longitudinal findings supporting early temperamental low PE in the development of depression and the increased incidence of depression in children of mothers with a lifetime history of depression, we hypothesize that children low in temperamental PE and children of mothers with a lifetime history of depression will exhibit elevated morning cortisol levels.

This hypothesis is supported by Ellenbogen et al. (2006)'s findings that at risk offspring evidence higher daytime levels of cortisol in their natural environment, but did not show differences in the cortisol response to the laboratory psychosocial stressor. These findings suggest that predictors of elevated morning cortisol might be part of an endophenotype predisposing the individual to the development of depression. Furthermore, we hypothesize that the relation between maternal history of depression and elevated offsprings' cortisol levels will be most pronounced in the offspring of mothers with melancholic depression, given the findings between hypercortisolism and melancholic depression in both depressed children and adults.

We also predict that parenting behaviors and life stress will have less of an influence on morning cortisol levels and a greater influence on evening cortisol levels, as cortisol collected in the morning appears to be less dependent on environmental factors, whereas cortisol collected later in the day appears to be more dependent on environmental factors. Lastly, in addition to environmental factors, we predict that temperament and maternal history of depression will be associated with elevated evening

cortisol levels as depression has been linked to disturbances in the temporal pattern of secretion including a flattened circadian curve (Deuschle et al., 1997), a reduced duration of nocturnal quiescent period (Halbreich et al., 1985), and an earlier (Pfohl et al., 1985) or elevated nadir (Yehuda et al., 1996).

In sum, we aim to examine the unique and joint effects of child temperament, maternal history of depression, parenting behaviors and life stress on children's cortisol levels in response to a laboratory stressor and children's basal cortisol levels.

METHOD

Participants

Recruitment. One hundred and sixty-six participants from a consecutive series of 210 children were recruited during their first laboratory visit for a larger study examining temperamental risk for mood disorders in preschoolers. Participants from the larger project were recruited from a commercial mailing list. Children between the ages of 3 and 4 who live with at least one biological parent were eligible. From the subset of children who participated in the cortisol assessments, one child was excluded due to a significant medical or developmental disability.

As a result, the final sample of participants included 166 (83 females and 83 males) children between the ages of 3 years, 0 months and 4 years, 1 month and their biological parents. One-hundred sixty children (80 females, 80 males) completed all 4 salivary cortisol samples during the lab visit. Fifty additional participants declined to participate or were excluded due to reasons pertaining to the cortisol assessments; 27 children refused to participate; 16 children did not complete all 4 salivary cortisol assessments; 4 parents did not want their children to participate in the salivary cortisol assessments; 2 children were sick and one child was taking antibiotics.

Ninety-four children provided a morning salivary cortisol sample, and 93 children provided an evening salivary cortisol sample. Ninety-two children provided both morning and evening cortisol samples; 87 children provided the morning and evening cortisol samples on the same day, and 5 children provided the samples on two separate days. Six children participated in the home cortisol assessment but since they did not complete all 4

laboratory cortisol assessments, they were not included in the laboratory cortisol analyses.

No children met criteria for a mood disorder diagnosis as assessed using the Preschool Age Psychiatric Assessment (PAPA; Egger et al., 1999). All families were compensated financially.

T-tests comparing laboratory cortisol participants to non-participants yielded significant differences on several variables. Non-participants exhibited significantly higher temperamental NE and BI and significantly lower interest during the laboratory assessment of temperament compared to participating children. In addition, parents of non-participants were rated as higher on parental hostility during the parent-child interaction battery compared to parents of participating children. There were no significant differences in rates of maternal psychopathology, parental supportive presence, or stressful life events. T-tests comparing home cortisol participants to non-participants yielded significant differences on maternal hostility and stressful life events. Parents of children who did not complete the home cortisol assessment were rated as higher on parental hostility during the parent-child interaction battery compared to parents of participating children. In addition, children who did not complete the home cortisol assessments experienced more stressful life events in the six months prior to the home cortisol assessment compared to participating children. There were no significant differences in rates of maternal psychopathology, parental supportive presence, or child temperament.

Descriptive Characteristics. The demographic characteristics of the sample were consistent with the 2000 census data from Suffolk County, New York. Participants

identified themselves as Caucasian (N = 155; 93.3%), African American (N = 2; 1.2%), Asian American (N = 2; 1.2%), or other race (N = 7; 4.4%); 16 (10.0%) participants identified themselves as Hispanic. Over half of families (58.1%) reported a family income ranging from $40,000-$100,000; 39.3% of families reported a family income greater than $100,001, and 2.6% of families reported a family income ranging from $20,000-$40,000. Approximately half of the mothers (48.4%) and fathers (49.7%) either graduated from high school (or received a GED), attended some college, or received a 2-year degree; and, approximately half of the mothers (51.6%) and fathers (49.7%) received a 4-year college degree or beyond. The mean age of parents was 36.6 years (*SD* = 3.9) for mothers and 38.8 years (*SD* = 4.8) for fathers. The mean age of child participants was 3.6 years (*SD* = 0.2), and 50% were female. The vast majority (98.1%) of the children came from two-parent homes, and 53.2% of the mothers worked outside the home part- or full-time; 15.7% worked more than 35 hours per week. Children were administered the Peabody Picture Vocabulary Test (PPVT; Dunn & Dunn, 1997) and the Expressive One-Word Picture Vocabulary Test (EOWPVT; Brownell, 2000) to screen for gross cognitive impairment (*M* = 105.04, *SD* = 14.13; *M* = 100.48, *SD* = 12.34, respectively).

T-tests comparing boys and girls on temperament traits did not show significant differences on PE, NE or BI. Boys and girls did not differ on parental supportive presence or hostility. However, girls experienced significantly more stressful life events in the 6 months prior to the lab visit ($t(164) = -2.23$, $p = .03$), and parents reported that girls had marginally significantly higher scores on the CBCL Affective Problems scale ($t(155) = -1.93$, $p = .06$) than boys.

Laboratory Assessment of Child Temperament

The laboratory assessment of child temperament lasted approximately 2.5 hours, during which children were videotaped while participating with a female experimenter in 12 standardized tasks selected from the Laboratory Temperament Assessment Battery (Lab-TAB; Goldsmith, Reilly, Lemery, Longley & Prescott, 1995). The tasks were designed to elicit behavioral expressions of a broad range of emotional and other temperamental traits. Episodes were ordered so as to prevent carry-over effects in that no episodes presumed to evoke similar affective responses occurred consecutively, and each was followed by a brief play break to allow the child to return to a neutral state. Mothers were present in the room for all episodes, with two exceptions noted below. Episodes are described in the order in which they were conducted.

Risk Room (behavioral inhibition and activity level). In this episode, which has been used in studies of behavioral inhibition (Kagan, 1997), the child was allowed to play freely by him/herself with a set of novel and ambiguous stimuli (e.g., a cloth tunnel, small staircase, mattress, Halloween mask, balance beam, and a large, black cardboard box). After 5 min. the experimenter returned and asked the child to approach each object.

Tower of Patience (inhibitory control, interest). The child and the experimenter took turns building a tower of cardboard blocks. The experimenter adhered to a schedule of delays of increasing length before placing her block on the tower (5, 10, 15, 20, and 30 secs.), forcing the child to wait longer each time to take his or her turn.

Arc of Toys (PE, interest, NE). The child was allowed to play freely by him or herself in a room full of toys. After a few minutes, the experimenter returned to ask the child to clean up the play area by putting the toys in a box.

Stranger Approach (behavioral inhibition). At the beginning of this episode, the child was left alone in the main experimental area. After a few moments, a friendly male research assistant entered the room and spoke to the child while gradually walking closer.

Make That Car Go (PE, interest). In this episode, the experimenter and child raced with two remote-controlled racecars.

Transparent Box (persistence, interest, NE). The experimenter locked an appealing toy in a transparent box. The child was then left with a set of inoperable keys to use to open the box. After several minutes, the experimenter returned, explaining that she had accidentally given the child the wrong keys. The child was then allowed to play with the toy.

Pop-Up Snakes (PE). The experimenter showed the child what appeared to be a can of potato chips, actually containing coiled spring snakes. The child was then encouraged to surprise his or her mother with the snakes.

Impossibly Perfect Green Circles (NE, persistence). The child was asked by the experimenter to draw a circle on a piece of paper. The experimenter responded by mildly criticizing the child's work and asking the child to draw another circle. This process was repeated for 2 min.

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

Exploring New Objects (BI, NE). The child is presented with the opportunity to explore new objects, including a tent, a small pet carrier, "gooey" toys, a remote controlled spider, and a plastic head covered with a red cloth. The mother is present, but is told to remain neutral.

Snack Delay (inhibitory control). The child was instructed to wait for the experimenter to ring a bell before eating a piece of candy. The experimenter waited a systematic series of delays before ringing the bell (10, 20, and 30 secs.).

Box Empty (NE). The child was given a wrapped empty box to open, under the pretense that an appealing toy was inside. After a brief interval in which the child was left alone to discover that the box was empty, the experimenter returned with several small toys for the child to keep, explaining that she had forgotten to place the toys inside.

Coding of laboratory data. Two different methods of coding the videotape data were employed. The first method was based on a global coding system (developed by and described in Durbin et al., 2005), which allowed raters to use their knowledge of child behavior and contextual influences to derive ratings to summarize observed behavior across the entire task. This method allows for all episodes to be coded for the same set of emotions and behaviors. The second approach only pertains to the coding of BI and involved making ratings at discrete time intervals based on highly specific behavioral codes. This microcoding method is similar to the vast majority of previous studies that have examined BI and was used to code the three episodes specifically designed to assess BI: *Risk Room, Stranger Approach,* and *Exploring New Objects.*

The global coding system considered facial, bodily and vocal indicators of positive affect (PA), fear, sadness and anger. A single rating was made per episode, which was based on all behaviors that were relevant to each dimension during that episode. Ratings of PA were made with consideration of the qualitative and quantitative aspects of displays of joy and enthusiasm. Overall, PA ratings were computed as the average standardized weighted sum of instances of low, moderate, and high displays of

facial, vocal, and bodily PA across all episodes. Similarly, overall ratings of sadness, anger, and fear were computed as the average standardized weighted sum of instances of low, moderate, and high displays of facial, vocal, and bodily of the respective form of affect from all episodes. In addition, included in the aggregate measure of PE, child anticipatory PA and interest were coded. Instances of anticipatory PA, which tap the approach/motivational and reward seeking aspects of PA were coded separately. Anticipatory PA is defined as the expression of PA in anticipation of a positive event/reward (e.g., the snack in *Snack Delay*, waiting to surprise mom with snakes in *Pop-up Snakes*). Interest ratings were based on the child's comments about the activity and how engaged the child was in play. The following scales were used to create the study's aggregate temperament variables (described below), and internal consistency estimates, as measured by alpha, were adequate: positive affect (α = .86), anticipatory positive affect (α = .64), interest (α = .66), sadness (α = .73), anger (α = .65), and fear (α = .64).

For micro-level coding of episodes eliciting BI, ratings were made for each epoch, which spanned 20-30 seconds depending on the particular episode. Affective codes were based on a system developed for the Lab-TAB by Goldsmith (Goldsmith et al, 1995), which draws from the Affex system of deriving affective meaning from facial muscle movements (Izard, Dougherty, & Hembree, 1980). Within each epoch, a maximum intensity rating of facial, bodily, and vocal fear was coded on a scale of 0 (absent) to 3 (highly present and salient). The definition of BI in the present study was largely based on Durbin et al. (2005), and was computed as the average standardized ratings of latency to fear (reversed); facial, vocal, and bodily fear (*Risk Room*, *Stranger Approach*, and

Exploring New Objects); latency to touch objects; total number of objects touched (reversed); tentative play; reference parent; proximity to parent; reference experimenter; time spent playing (reverse) (*Risk Room* and *Exploring New Objects*); startle (*Exploring New Objects)*; sad facial affect (*Exploring New Objects* and *Stranger Approach*); latency to vocalize; approach towards the stranger (reverse); avoidance of the stranger; gaze aversion; and verbal/nonverbal interaction with the stranger (reverse) (*Stranger Approach*).

Data reduction. Aggregate constructs capturing PE, NE and BI were created by averaging standardized scores of facial, bodily, and vocal ratings across episodes. The construct of BI was created as described above. The PE variable consists of the average of facial, bodily, and vocal PA, including anticipatory PA, and interest across all episodes. Similarly, the NE composite consisted of averaging ratings of facial, bodily, and vocal anger, sadness, and fear. Internal consistencies for PE, NE, and BI scales were .71, 78, and .81 respectively. Interrater reliability, as indexed by the intraclass correlation (ICC) and based on a subsample of 17 cases, was adequate for the composite scales of PE (ICC = .79), NE (ICC = .78), and BI (ICC = .87).

Child depressive symptoms. Primary caregivers (149 mothers; 8 fathers) completed the Child Behavior Checklist/1½-5 (CBCL/1½-5; Achenbach & Rescorla, 2000), which measures children's emotional and behavioral problems. Parents rated each item on a scale from 0 (*not true*) to 2 (*very or often true*) for their child's behavior in the past 6 months. The CBCL/1½-5 yields two broadband factors assessing internalizing and externalizing problems, as well as several subscales, which include withdrawn, attention, aggression, somatic complaints, anxious/depressed, emotionally reactive, and sleep

problems. The original CBCL scales did not distinguish between anxiety and depressive problems. However, the newer version of the CBCL/1½-5 has scales that are based on the DSM-IV. For the purpose of this study, we used the CBCL/1½ -5 Affective Problems scale, which consists of 10 items (Cronbach's $\alpha = .59$) covering sadness, lack of interest, loss of energy, and sleep and eating problems. As expected, parents reported few symptoms on the CBCL Affective Problems scale ($M = 2.65$, $SD = 2.25$). The CBCL was not available for 9 children due to missing questionnaire data.

Maternal depression. Semi-structured diagnostic interviews, using the Structured Clinical Interview for DSM-IV (SCID; First, Spitzer, Gibbon, & Williams, 1996), were conducted with 94% of the biological mothers. The SCID is among the most widely used diagnostic interviews. The diagnostic interviews were conducted by two Masters-level clinicians. The primary interviewer who conducted the majority of the interviews (69.3%) completed a formal training course on the SCID and had several years of experience conducting SCIDS in other studies. The interrater reliability between the primary interviewer and the secondary interviewer was based on 30 audiotaped interviews (20 mothers and 10 fathers) conducted by the primary interviewer for the larger project. The 30 tapes were selected to oversample subjects with a range of diagnoses. Interrater reliability was very good for all diagnoses used in this study (Lifetime MDD and/or DY, Kappa = .93; Lifetime Mood Disorder (including NOS diagnoses), Kappa = .87; Lifetime Anxiety Disorder, Kappa = .91; Lifetime Substance Use Disorder, Kappa = 1.00). Both interviewers were not involved in collecting, and did not have access to any of the data on the children.

Rates of lifetime diagnoses of mood disorders in mothers are similar to those from recent epidemiological studies (Kessler, Berglund, & Demler, 2005): lifetime Major Depressive Disorder (MDD; N = 37, 23.7%); lifetime Dysthymic Disorder (DD; N = 20, 12.8%); lifetime Depression Not Otherwise Specified (Dep-NOS; N = 25, 16.0%); lifetime MDD or DD (N = 47, 30.1%); lifetime MDD, DD, or Dep-NOS (N = 70; 44.9%); lifetime melancholic depression (N = 14; 8.9%). No mother met criteria for Bipolar I or Bipolar II disorder. One mother met criteria for current MDD, 4 mothers met criteria for current DD, and 2 mothers met criteria for current Dep-NOS. Rates of lifetime diagnoses of non-mood disorders in mothers are also consistent with epidemiological studies: any lifetime anxiety disorder (N = 55, 35.3%) and lifetime substance abuse/dependence (N = 34, 21.8%).

Parenting. Parent-child relationship quality was assessed using an observational measure of parent-child interactions for preschool aged children. Based on the Teaching Tasks battery (Egeland, Weinfield, Hiester, Lawrence, Pierce, & Chippendale, 1995), it consisted of six tasks (book reading, block building, naming objects with wheels, matching shapes, completing a maze using an etch-sketch, and gift presentation), which the primary caregiver and child work on together. The parent-child interaction assessment was completed during a second laboratory visit. The battery, which takes approximately 25 minutes, was videotaped for subsequent coding on 16 five-point scales tapping parent, child, and dyadic variables. The present study used two scales based on Weinfield, Egeland, and Ogawa (1998). The parental supportive presence scale (range = 1–5) captures parental emotional support and encouragement of the child during the tasks. Higher scores indicate greater supportiveness. Internal consistency and interrater

reliability of the parental supportive presence scale were adequate (α = .86 and ICC = .81, respectively). The parental hostility scale (range = 1–5) captures punitive, coercive, and hostile behavior during the tasks, including the parent's expression of anger, frustration, and annoyance, and discounting or rejecting the child. Higher scores indicate greater harshness. Internal consistency and interrater reliability (N = 53) of the hostility scale were also adequate (α = .81 and ICC = .72, respectively). One-hundred and fifty-one parents (144 mothers and 7 fathers) and children completed the Teaching Task battery; parenting data was not available for 14 families who did not participate in the second laboratory visit. One parent-child interaction was not videotaped due to technical problems.

Life events. Life events were assessed using the Preschool Age Psychiatric Assessment (PAPA; Egger et al., 1999) interview. Parents (158 Mothers, 8 Fathers) were asked whether 27 major life events (e.g., parents split up or divorced; parental arrest; child was seriously ill, injured, or hospitalized for more than 24 hours) occurred during the child's life, the extent to which the child was affected by the event, and the date of the event. The interviewer determined whether or not the event/condition matched with the defined criteria. For the purposes of this study, we summed the total number of events that occurred 6 months prior to the laboratory visit and 6 months prior to the home cortisol sampling. If the date of the home cortisol sampling occurred after the PAPA interview, we summed the total number of life events that occurred 6 months from the time of the PAPA interview. The interview typically occurred after the home cortisol assessment, but when it was before it usually occurred within two weeks from the time of the home cortisol assessment. For the present analyses, we examined only the life events

during the 6 months prior to each cortisol assessment in order to examine the effect of recent major life events on current HPA functioning.

Parental marital satisfaction and maternal current depressive symptoms. Mothers completed the Dyadic Adjustment Scale (DAS; Spanier, 1976; $\alpha = .95$) to measure relationship discord ($M = 111.79$, $SD = 18.95$) and the Diagnostic Inventory for Depression (DID; Zimmerman, Sheeran, & Young, 2004; $\alpha = .89$) to measure current depressive symptoms ($M = 4.88$, $SD = 4.69$); 103 mothers completed the DAS, and 114 mothers completed the DID. Higher scores on the DAS indicate greater marital satisfaction. Higher scores on the DID indicate greater depression symptomatology.

Socioeconomic status. In order to examine the relation between socioeconomic status (SES) and HPA functioning, a variable based on parental education level was created as a proxy for SES: 0 = Both parents had less than a 4-year college degree (28%); 1 = At least one parent received a 4-year college degree or beyond (39.1%); and 2 = Both parents received a 4-year college degree or beyond (32.9%).

Laboratory Salivary Cortisol Collection. Salivary cortisol was collected 4 times at specifically designated intervals during the assessment. Saliva for cortisol determination was obtained by having the children dip 2 in. long cotton dental rolls into small cups containing approximately .025 g of sugar-sweetened cherry Kool-Aid drink mix. Children then placed the cotton rolls in their mouths until saturated. These procedures are known to have little-to-no effect on cortisol concentrations given the assay procedures used (Schwartz, Granger, Susman, Gunnar, & Laird, 1998; Talge, Donzella, Kryzer, Grierens, & Gunnar, 2005). The procedures took approximately 5 minutes. The wet cotton was collected and then expressed into vials for storage at -20 C until assayed.

Salivary cortisol assessments were taken on the day of the laboratory visit and on a separate day at home (described below).

The timing of the laboratory cortisol samples was determined based on the presumed stress of the episodes, previous studies using a similar paradigm (Luby et al., 2003), and the principle that cortisol levels are believed to reflect the level of stress experienced about 20-40 minutes prior (Dickerson & Kemeny, 2004). Based on this, the first sample (baseline) was taken upon arrival to the laboratory after parents gave consent. It reflected the pre-assessment time with a parent, which was not hypothesized to be stressful. However, during this time, children were most likely on route to the laboratory, a situation that is new and unfamiliar to the children, which may have increased cortisol levels in some children. The second sample was collected 30 minutes following the Stranger Approach task of the Lab-TAB, the most stressful episode in the battery, during which the child was separated from his/her parent and a stranger entered the room. The third salivary cortisol sample was taken 30 minutes later after qualitatively different frustration-inducing laboratory stressors, with some positive episodes interspersed. This second set of frustrating and distressing episodes was structured play tasks designed to produce transient and mild frustration or anger in the child. Typically, the third sample was taken 30 minutes after the *Transparent Box* episode, a frustration-inducing task in which the child is unable to unlock a box with a desirable toy inside. The final (4^{th}) sample was collected 20 minutes after the completion of all Lab-TAB tasks. The final saliva sample aimed to assess the entire recovery process from all Lab-TAB tasks. To control for non-stress related elevations of cortisol, assessments were conducted at either 10 AM (68.8% of the sample) or 2 PM. Time of day (assessment time) was considered as

a potential confounding variable in all analyses using the laboratory assessments of cortisol. Families were instructed prior to coming to the laboratory that the child should not eat a meal within one hour before their scheduled lab visit, and to avoid caffeine for at least two hours and dairy for at least 15 minutes prior to arrival.

Home Salivary Cortisol Collection. Each parent was given a home salivary cortisol collection kit in order to collect the child's saliva 30 minutes after wakening and 30 minutes before falling asleep on a typical day for the child. The saliva samples were collected with reference to the child's usual time of wakening and sleep rather than at a fixed clock time. This methodology was utilized because the cortisol response to awakening has been found to have more test-retest stability (Edwards, Clow, Evans, & Hucklebridge, F., 2001; Pruessner, Hellhammer, & Kirschbaum, 1999; Wust et al., 2000) than single point measures yoked to clock time (Coste, Strauch, Letrait, & Bertagna, 1994), especially in populations with greater variability in their sleep/wake cycles, such as young children. Parents were instructed to select a typical day for sampling, rather than an especially exciting or troublesome day; to avoid school/daycare days unless the child is in school/daycare almost every weekday; to avoid feeding their child a meal within one hour before the evening sample; to avoid caffeine for at least two hours and dairy for at least 15 minutes prior to sampling; and to refrain from brushing the child's teeth before sampling. Parents refrigerated the samples and mailed them back to our laboratory where they were stored at -20 C until assayed.

Once all laboratory and home samples were collected, they were assayed using a time-resolved fluorescence immunoassay with flourometric end point detection (DELFIA). All samples were assayed in duplicate. Samples yielding values above 44

nanomoles per liter (nmol/L) were excluded, which applied to 4 laboratory samples from 4 different individuals and one home evening sample. The correlation between duplicates was .99 for both the laboratory and home samples. The inter- and intra-assay coefficients of variation (CV) for the laboratory and home samples were between 7.1% - 9.0% and 4.0% - 6.7%, respectively.

Laboratory data analysis strategy. Because the 4 laboratory cortisol samples are nested within individuals, we estimated the effects of children's temperament, maternal psychopathology, parenting, and life stress with multilevel or hierarchical linear analysis, a variant of multiple regression appropriate for nested data (Singer & Willet, 2003). Mixed effects models allow an assessment of individual-level cortisol change (level-1) and prediction of individual-level differences in change (level-2), if they exist. The multilevel model was estimated using the Hierarchical Linear Modeling statistical program version 6 (SSI Inc., Lincolnwood, IL).

A log10 transformation of the raw cortisol values yielded an unskewed response variable. To address the hierarchical structure of the data, two levels of equations were estimated. On the first level, we estimated each individual's baseline cortisol level (i.e., intercepts) and cortisol reactivity (i.e., slope and curvature). For all analyses, log 10 transformed cortisol values were treated as the dependent variable. Both linear and quadratic growth models were used to examine a within-subjects regression of an individual's HPA reactivity onto the time of each assessment. On the second level, we estimated equations to predict differences in level-1 intercepts, slopes, and curvature from the following level-2 predictors: child sex, child temperament, child depressive symptoms, maternal psychopathology, parenting, marital discord, SES, and stressful life

events. Finally, we examined whether any of the between-subject variables (i.e., temperament, maternal depression, parenting, and life stress) interacted to predict children's HPA axis reactivity.

Three adjustments were made to the data to ease interpretation of the results. First, time was anchored at baseline (at baseline, time = 0) so that the cortisol intercepts ($ß_{00}$) would reflect the average individual's cortisol level at baseline. Second, all level-2 between-person variables were centered at their grand mean. Third, we used a pairwise missing data procedure to handle any missing data at level-1, and any cases with missing data at level-2 were excluded from the analyses.

Home data analysis strategy. The purpose of the home analyses was to examine predictors of children's morning and evening cortisol levels. Similar to the laboratory analyses, raw cortisol values were log10 transformed, which yielded unskewed response variables for both morning and evening cortisol values. We examined the associations between morning and evening basal cortisol levels and the following variables: child sex, child temperament, child depressive symptoms, maternal psychopathology, maternal depressive symptoms during the child's life, parenting, marital discord, SES, and stressful life events. Lastly, we examined whether any of the between-subject variables (i.e., temperament, maternal depression, parenting, and life stress) interacted to predict basal cortisol levels.

RESULTS

Laboratory Cortisol Results

Cortisol Reactivity. In order to investigate whether there was any systematic change over time, baseline trajectory models were estimated. Models of linear and quadratic change were examined. These models can be understood as a within-subjects regression of an individual's cortisol values onto the time of each assessment. To evaluate these models, the following function was specified to describe the data from each individual:

$$\text{Level 1: } Y_{ij}=\beta_{0j}+\beta_{1j}(\text{Time})+\beta_{2j}(\text{Time}^2)+r_{ij}$$

Level 2

$$\text{Intercept: } \beta_{0j} = \gamma_{00} + u_{0j}$$

$$\text{Slope: } \beta_{1j} = \gamma_{10} + u_{1j}$$

$$\text{Curvature: } \beta_{2j} = \gamma_{20} + u_{2j}$$

(Equation 1)

where Y_{ij} is cortisol values of individual j at time i; β_{0j} is the cortisol value of individual j at Time 0, (i.e., the baseline cortisol value of individual j); β_{1j} is the rate of the linear change in cortisol for individual j over the course of the laboratory visit; β_{2j} is the rate of the curvature in cortisol; and r_{ij} is the residual variance in repeated measurements for individual j, assumed to be independent and normally distributed across subjects.

As seen in Table 2 and Figure 1, the baseline trajectory model demonstrated that there was a significant linear decrease in cortisol values from baseline (20 minutes prior to the laboratory visit) to the children's cortisol levels following the separation stressor (sample 2). The observed linear decrease in cortisol from sample 1 to sample 2 was

unexpected. We had expected a linear increase in response to the separation stressor; however, similar decreases in response to stressors have been observed in other studies (Gotlib, Joorman, Minor, & Cooney, 2006; Luby et al., 2003; Talge, Bruce, Donzella, & Gunnar, 2003). Following the separation stressor, children's cortisol levels began to rise for sample 3 (typically in response to a frustrating task) and sample 4 (20 minutes after the laboratory visit), as indicated by a significant positive quadratic effect (i.e., concave up). The growth curve measuring children's cortisol reactivity, obtained in this investigation, was similar to another study of preschoolers using analogous stress-inducing laboratory tasks (Luby et al., 2003).

Nevertheless, the random error terms associated with the intercept (variance component = .08, $df = 159$, $\chi^2 = 1164.25$, $p < .001$), linear (variance component = .03, $df = 159$, $\chi^2 = 523.60$, $p < .001$), and quadratic (variance component = .001, $df = 159$, $\chi^2 = 500.65$, $p < .001$) components were significant, suggesting that it might be useful to model between-person predictors of each of these components. Children's individual differences in their cortisol reactivity varied across the visit. From the first cortisol assessment to the second assessment, 36.7% of children had 10% or more increase in salivary cortisol and 51.9% had a 10% or more decrease in salivary cortisol. From the second cortisol assessment to the third assessment, 50.0% had a 10% or more increase in salivary cortisol and 27.5% had a 10% or more decrease in salivary cortisol. From the third cortisol assessment to the final assessment, 79.1% of children had 10% or more increase in salivary cortisol and 10.1% had a 10% or more decrease in salivary cortisol. As seen through examining the individual differences in laboratory cortisol changes, there was a subgroup of children who conformed to the a priori reactivity hypothesis.

In order to interpret the growth curve of children's cortisol levels across the laboratory visit, it is first important to address the nature of the baseline sample. Gunnar and Talge (2005) reported that research from their laboratory and others suggests that samples obtained at lab arrival do not match samples obtained at home at the same time of day as the lab visit, which suggests that the initial lab sample may reflect a response to coming to the lab. If this is the case, each sample may reflect a continuous reactivity process across the laboratory visit. As such, a greater reactivity across the lab visit appears to be evidenced in higher baseline levels, less of a decline in slope or a positive increase in slope from baseline, or a slower, flatter rate of the curvature. However, this is only an assumption as the data cannot accurately distinguish reactivity to the lab experience from the child's true baseline. We also examined the relation between mean cortisol levels across the visit and the growth curve and found that higher mean cortisol levels were significantly associated with higher baseline cortisol levels (unstandardized coefficient = .050, *SE* = .006, *t* = 8.067, *df* = 157, $p < .001$), less of a decline in slope (unstandardized coefficient = .010, *SE* = .005, *t* = 1.995, *df* = 157, $p < .05$), and a slower rate of the curvature (unstandardized coefficient = -.003, *SE* = .001, *t* = -2.495, *df* = 157, $p < .05$). This demonstrates that the combination of less of a decline in slope and a slower rate of curvature is correlated with greater mean cortisol levels across the visit.

Main Effects

In Table 1 we present correlations between mean laboratory cortisol levels and all major variables. Higher mean laboratory cortisol levels were significantly correlated with higher ratings of temperamental BI in children, less parental support, greater parental hostility, and less marital satisfaction (as assessed by the DAS). In addition, females had

marginally significantly lower mean laboratory cortisol levels. Mean laboratory cortisol levels were marginally correlated with home morning cortisol and significantly correlated with home evening cortisol levels; however, these positive correlations were only small in magnitude, suggesting that the laboratory and home cortisol assessments may be tapping separate processes in children's HPA axis functioning.

Prior to examining the substantive predictors of the growth curve, we examined potential confounds, such as time of the laboratory visit, food intake prior to the lab visit, gender, race, and children's depressive symptoms, as assessed by parental reports on the CBCL Affective Problems Scale (see Table 2). Food intake prior to the lab visit, gender, race, and children's depressive symptoms were not significantly associated with the growth curve. Nevertheless, children's depressive symptoms were marginally significantly associated with a steeper rise in the curvature. Food intake prior to the lab visit was likely not related to the growth curve as families were instructed to avoid feeding their child within one hour prior to the lab visit. However, time of lab visit (10 AM vs. 2 PM) was significantly correlated with the growth curve, reflecting the diurnal rhythm in cortisol levels across the day (i.e., higher levels after awakening and lower levels later in the day). Morning laboratory visits were associated with significantly greater baseline cortisol levels, a marginally significant steeper decline in slope, and a significantly steeper rise in the curvature; therefore, we made an adjustment for this rhythm by controlling for time of visit in all analyses that follow.

Temperament and cortisol reactivity. We examined the main effects of PE, NE, and BI on the trajectory of change in cortisol over time. First, the univariate effects of PE, NE and BI on cortisol reactivity were examined, after controlling for the time of visit

(Table 2). PE was not significantly associated with the intercept, slope, or the curvature of the change in cortisol.

NE was significantly associated with the curvature, but was not significantly associated with the intercept or the slope. The relation between NE and the curvature of the growth curve demonstrates that children high in NE evidence a slower rate of positive curvature. BI was significantly associated with higher baseline cortisol levels, but was not significantly associated with the slope or curvature of the change in cortisol. The higher intercept together with no effects for slope and curvature seem to imply overall higher laboratory cortisol levels, which is supported by the significant positive correlation between BI and higher mean levels of cortisol across the 4 laboratory assessments (see Tabel 1).

Next, we examined the unique effects of NE and BI, controlling for children's current depressive symptoms. The level-2 predictors were entered simultaneously to control for the effects of one variable on the other. The univariate findings for NE and BI did not change when they were examined simultaneously and after controlling for children's depressive symptoms. Children's depressive symptoms, as assessed by parental reports on the CBCL Affective Problems scale, were not significantly associated with the intercept, slope or curvature of the growth curve.

Maternal depression and cortisol reactivity. We examined the main effects of maternal lifetime history of MDD or DD on the trajectory of change in cortisol over time. All univariate findings are presented in Table 2. Maternal MDD/DD was not significantly associated with the intercept, slope, or the curvature of the change in cortisol. In addition, we examined the main effects of maternal lifetime history of melancholic depression on

children's cortisol reactivity, given the literature on melancholia and hypercortisolemia in adult depressives. We created a three-level variable for maternal lifetime melancholic depression: 0 = no lifetime depressive disorder; 1 = lifetime non-melancholic depressive disorder, including non-melancholic MDD, DY and Dep-NOS; 2 = lifetime melancholic depressive disorder. We did not find main effects for maternal lifetime melancholic depression on children's cortisol reactivity. Furthermore, neither lifetime anxiety disorders nor lifetime substance abuse/dependence in mothers predicted children's cortisol reactivity.

Maternal depression during the child's life and cortisol reactivity. As seen in Table 2, we examined the main effect of mothers' current depressive symptoms at the time of the laboratory visit as assessed with the DID. Mothers' current depressive symptoms were not associated with the intercept, slope, or the curvature of the change in cortisol. In addition, we examined the main effects of maternal depression during the child's life (including MDD, DD, Dep-NOS or MDD with partial recovery) on the trajectory of change in cortisol over time. The presence of maternal depression during the child's life was not significantly associated with the intercept, slope, or the curvature of the change in cortisol.

Parenting and cortisol reactivity. We examined the main effects of parental supportive presence and hostility on the trajectory of change in cortisol over time (see Table 2). First, the effects of parental supportive presence and hostility were each entered separately as level-2 predictors. Parental supportive presence was not significantly associated with children's intercept, slope or curvature. In contrast, parental hostility was significantly associated with higher baseline cortisol levels and greater stress reactivity as

parental hostility marginally predicted the slope and significantly predicted the curvature of the growth curve. The growth curve for children of parents high in hostility demonstrates greater baseline cortisol levels, an increase in cortisol in response to the first stressor and a slight decrease following the first stressor on (i.e., concave down). Next, the Level-2 predictors (supportiveness and hostility) were entered simultaneously to control for the effects of one variable on the other as they were moderately correlated (r = -.55). After controlling for parental supportiveness, parental hostility continued to marginally predict the slope (unstandardized coefficient = 0.20, *SE* = .102, *t* = 1.93, *df* = 142, *p* = .055) and significantly predict the curvature (unstandardized coefficient = -.05, *SE* = .020, *t* = -2.49, *df* = 142, *p* < .05) of the change in cortisol. Parental supportiveness did not predict the intercept, slope or curvature of the growth curve.

Environmental factors and cortisol reactivity. Marital discord, as assessed by maternal reports on the DAS, was not significantly associated with the intercept, slope or the curvature of cortisol reactivity. SES was marginally significantly associated with the curvature, but was not significantly associated with the intercept or slope of cortisol reactivity. Life stress in the 6 months prior to the lab visit was significantly associated with lower baseline cortisol levels, but was not significantly associated with the slope or the curvature of the change in cortisol. As life stress in the 6 months prior to the lab visit was also marginally significantly associated with lower mean laboratory cortisol levels (see Table 1), this suggests that severe life stressors may dampen children's HPA axis reactivity.

Unique Effects

Temperamental NE, BI, parental hostility and life stress in the 6 months prior to the laboratory visit were significantly associated with components of the trajectory of cortisol during the lab visit. In order to examine their unique effects, these level-2 predictors were entered simultaneously to control for the effects of one variable on the other, in addition to controlling for the time of visit. The univariate findings, as presented in Table 2, did not change when these level-2 predictors were examined simultaneously. As seen in Figure 2, child temperamental BI continued to predict an elevated intercept; child temperamental NE predicted a flatter, slower rate of curvature; parental hostility predicted an elevated intercept and a flatter, slower rate of curvature; and life stress predicted a lower intercept.

Moderator Effects

Next, we conducted exploratory analyses examining whether any of the between subjects variables (i.e., temperament, maternal depression with and without melancholia, parenting, and life stress) interacted in predicting HPA reactivity. Both within-domain interactions (e.g., PE X BI) and between-domain interactions (e.g., PE X maternal depression) were examined. For each analysis, we conducted a multilevel regression in which the direct effects of the two level-2 between-person predictors along with the cross-product of the two predictors were entered. Each predictor variable was initially centered (converted into deviation score form) to minimize multicollinearity, and interaction terms were formed as the product of the two centered predictors (Aiken & West, 1991). Several significant interactions emerged in the following domains: temperament X maternal depression; temperament X parenting; and maternal depression X parenting.

Interactions between temperament and lifetime history of maternal depression. We examined whether PE, NE or BI interacted with maternal lifetime history of MDD or DD to predict cortisol reactivity. BI interacted with maternal lifetime history of MDD or DD to predict the slope (unstandardized coefficient = .040, *SE* = .020, *t* = 2.046, *df* = 139, $p < .05$) and curvature (unstandardized coefficient = -.010, *SE* = .004, *t* = -2.237, *df* = 139, $p < .05$) of the change in cortisol. In order to probe the interaction, we examined the relation between BI and cortisol reactivity in mothers with a lifetime history of MDD or DD and those with no lifetime history of MDD or DD. As seen in Figure 3, for mothers with a lifetime history of MDD or DD, BI significantly predicted the slope (unstandardized coefficient = .071, *SE* = .030, *t* = 2.368, *df* = 40, *p* = .023) and curvature (unstandardized coefficient = -.019, *SE* = .007, *t* = -2.879, *df* = 40, *p* = .007) of the change in cortisol, whereas for mothers with no history of MDD or DD, BI did not significantly predict the slope or curvature of the growth curve. However, in both groups BI was significantly associated with a higher intercept at baseline. Thus, the combination of child high BI and maternal lifetime history of MDD or DD exhibited less of a decline in the linear slope and a slower rate of positive curvature, which demonstrate elevated levels of cortisol across the visit.

In addition, we examined whether child PE, NE, and BI interacted with maternal melancholic depression to predict children's cortisol reactivity. It is possible that the effects for MDD might be due to melancholia, given the literature on melancholia and cortisol in adult depressives. Child PE interacted with maternal melancholic depression to predict the slope (unstandardized coefficient = -.048, *SE* = .019, *t* = -2.493, *df* = 139, *p* = .014) and curvature (unstandardized coefficient = .011, *SE* = .005, *t* = 2.251, *df* = 139, *p* =

.026) of the change in cortisol; child NE interacted with maternal melancholic depression to predict the intercept (unstandardized coefficient = -.140, *SE* = .070, *t* = -2.01, *df* = 130, *p* = .046); and child BI interacted with maternal melancholic depression to predict the slope (unstandardized coefficient = .045, *SE* = .019, *t* = 2.327, *df* = 139, *p* = .021) of the growth curve.

Next, we examined a full model examining the three interactions with maternal melancholic depression simultaneously predicting the intercepts, slopes, and curvatures of cortisol reactivity. In this model, NE X melancholic depression no longer significantly predicted the intercept; therefore, it was dropped from the final model[1]. The final temperament X melancholic depression model is presented in Table 3. In this model, the main effects of PE, BI, and maternal melancholic depression were included as level-2 predictors, along with the interactions between PE and maternal melancholic depression and BI and maternal melancholic depression. In the full model, the interaction between PE and maternal melancholic depression significantly predicted the slope and curvature of the growth curve, and the interaction between BI and melancholic depression significantly predicted the slope of the growth curve.

In order to probe the interactions, we examined the relation between PE, BI, and cortisol reactivity in the three groups of maternal lifetime history of depression: mothers with a history of maternal melancholic depression, mothers with a history of non-melancholic depression, and mothers with no history of depression. Only in the mothers with a history of maternal melancholic depression did PE significantly predict the slope (unstandardized coefficient = -.191, *SE* = .030, *t* = -6.431, *df* = 11, *p* < .001) and curvature (unstandardized coefficient = .053, *SE* = .009, *t* = 5.887, *df* = 11, *p* < .001) of

the growth curve (see Figure 4), and did BI significantly predict the slope (unstandardized coefficient = .139, *SE* = .061, *t* = 2.288, *df* = 11, $p < .05$) of the growth curve (see Figure 5). These interactions demonstrated that the combination of child low PE and maternal lifetime history of melancholic depression was associated with a positive increase in cortisol in response to the separation stressor and a decrease in cortisol levels (concave down) following the separation stressor, and the combination of child high BI and maternal lifetime history of melancholic depression is associated with a positive linear increase in cortisol levels in response to the separation stressor. The lack of effects for non-melancholics suggests that this effect is specific to melancholia, rather than being due to depression in general.

Interactions between temperament and parenting. Child PE and BI interacted with parenting behaviors to predict children's cortisol reactivity. Child PE interacted with parental hostility to predict the curvature (unstandardized coefficient = .010, *SE* = .005, *t* = 2.128, *df* = 139, *p* = .035) of the change in cortisol; and child BI interacted with parental support to predict the intercept (unstandardized coefficient = -.101, *SE* = .039, *t* = -2.562, *df* = 139, *p* = .012).

The significant interactions were probed using the techniques outlined by Aiken and West (1991). In this procedure, the effect of parental hostility on cortisol reactivity was estimated at 1 SD below the mean (low) and 1 SD above the mean (high) on child PE. As seen in Figure 6, among children low in PE, parental hostility was significantly associated with a higher intercept (unstandardized coefficient = .112, *SE* = .055, *t* = 2.034, *df* = 139, *p* = .044), an increasing slope (unstandardized coefficient = .093, *SE* = .040, *t* = 2.323, *df* = 139, *p* = .022), and a flatter, slower rate in curvature (unstandardized

coefficient = -.023, *SE* = .007, *t* = -3.380, *df* = 139, *p* = .001) of the change in cortisol. Therefore, the combination of low PE and parental hostility is associated with high and increasing cortisol. In contrast, among children high in PE, parental hostility was not significantly associated with the intercept, slope or curvature of the growth curve.

Next, we probed the interaction between BI and parental support. The effect of parental support on cortisol reactivity was estimated at 1 SD below the mean (low) and 1 SD above the mean (high) on child BI. As seen in Figure 7, parental support interacted with child BI to predict children's baseline (intercept) cortisol levels. For children high in temperamental BI, parental support was significantly and negatively associated with children's baseline levels of cortisol (unstandardized coefficient = -.137, *SE* = .056, *t* = -2.473, *df* = 140, *p* = .015). In contrast, for children low in temperamental BI, parental support was not significantly associated with children's baseline (intercept) cortisol levels (unstandardized coefficient = .052, *SE* = .042, *t* = 1.226, *df* = 140, *p* = .223). These findings demonstrate that high levels of parental support can protect children high in BI from elevated baseline cortisol levels, and conversely, the combination of low parental support and child high temperamental BI evidence elevated baseline corisol levels.

Interactions between lifetime history of maternal depression and parenting. As seen in Table 4, the interaction between maternal lifetime history of MDD or DD and parental hostility was significantly associated with the slope and curvature of the growth curve. In order to probe the interaction, we examined the relation between parental hostility and cortisol reactivity in mothers with a lifetime history of MDD or DD and those with no lifetime history of MDD or DD (see Figure 8). For mothers with a lifetime history of MDD or DD, parental hostility was significantly associated with a higher

intercept (unstandardized slope coefficient = .081, *SE* = .037, *t* = 2.209, *df* = 40, *p* = .033), an increasing slope (unstandardized coefficient = .107, *SE* = .029, *t* = 3.647, *df* = 40, *p* = .001) and a flatter, slower rate in curvature (unstandardized coefficient = -.024, *SE* = .005, *t* = -4.490, *df* = 40, *p* < .001), whereas for mothers with no history of MDD or DD, parental hostility was not significantly associated with the intercept, slope or curvature of the growth curve. Thus, the combination of maternal MDD/DD and high parental hostility leads to high and increasing cortisol reactivity.

Home Cortisol Results

The average time of the morning assessment took place at 8:16 AM (*SD* = 58 min) and the average time of the evening sample took place at 8:15 PM (*SD* = 49 min). Times of morning and evening samplings were not significantly correlated with cortisol levels (*r* = -.13, *p* = .20 and *r* = -.18, *p* = .08, respectively). A diurnal rhythm in cortisol was observed in the sample (*t* (86) = 46.12, *p* < .001), indicating that morning cortisol levels were significantly higher than evening cortisol levels for those individuals who provided both morning and evening samples on the same day of assessment (N = 87). Gender was not significantly associated with either morning or evening cortisol levels. One-way Analysis of Variance (ANOVA) tests yielded no significant differences among races in regard to morning (*F*(4, 79) = 2.18, *p* = .08) and evening (*F*(4, 78) = .99, *p* = .42) salivary cortisol levels.

Morning and evening cortisol levels were minimally and not significantly correlated, suggesting that they each capture distinct processes of HPA functioning. In addition, evening cortisol levels were not significantly associated with any variables in Table 1. Given the lack of significant correlations between evening cortisol and the

study's major variables, below we will focus on morning cortisol levels. Nevertheless, interaction effects will be examined for both morning and evening cortisol levels.

Child temperament and morning cortisol. As seen in Table 1, home morning cortisol was significantly associated with PE but was not significantly associated with NE or BI. As predicted, higher morning cortisol levels were associated with lower levels of PE in children. In order to examine the relative associations between the three temperament factors and morning cortisol levels after controlling for children's current depressive symptoms, we entered children's depressive symptoms in the first step, and PE, NE, and BI in the second step. Neither children's depressive symptoms (ß = -.08, *SE* = .01), NE (ß = .03, *SE* = .05), nor BI (ß = -.05, *SE* = .06) was associated with morning cortisol. Only child PE (ß = -.26, *SE* = .03, *p* = .02) was uniquely associated with morning cortisol levels.

Maternal depression and morning cortisol levels. The correlations between home cortisol levels and maternal depression are shown in Table 1. Maternal lifetime history of depression (i.e., MDD or DD) was not significantly associated with children's morning cortisol levels. We also examined the relation between maternal melancholic depression and morning cortisol, given the literature on melancholic depression and hypercortisolemia (Post & Ballenger, 1984; Wong et al., 1999). As indicated in Table 1, child morning cortisol was significantly and positively correlated with maternal melancholic depression. In order to explore this further, the same 3-level variable for melancholic depression used in the laboratory cortisol results was used here[2]. A one-way ANOVA yielded a main effect for group, $F(2, 91) = 3.38$, $p < .05$. Tukey's HSD post-hoc tests revealed that mothers with melancholic depression ($M = .71$, $SD = .25$) had

significantly higher morning cortisol levels than mothers with no lifetime history of depression (M = .52, SD = .19, $p < .05$), but did not significantly differ from mothers with non-melancholic depression (M = .55, SD = .16, p = .11). In addition, mothers with non-melancholic depression did not significantly differ from mothers with no lifetime history of depression.

To examine the specificity of the effects of maternal depression, we examined the relations between morning cortisol and other lifetime diagnoses in mothers. Child morning cortisol levels were not correlated with anxiety or alcohol/drug abuse/dependence disorder in mothers.

Although morning cortisol was not significantly associated with maternal non-mood disorders, it is important to rule out the possibility that the relation between morning cortisol and maternal melancholic depression association was due to the presence of comorbid psychopathology in mothers. A hierarchical multiple regression revealed that after controlling for maternal lifetime anxiety and substance abuse disorders (entered in the first block), maternal melancholic depression (entered in the second block) continued to predict morning cortisol levels. As a set, maternal anxiety (ß = .04, SE = .04) and substance abuse (ß = .02, SE = .05) did not predict morning cortisol levels, F (2, 91) = .077, p = .93. Maternal melancholic depression (ß = .25, SE = .02, $p < .05$) accounted for a significant increment in variance explained, F change (1, 90) = 5.00, $p < .05$.

Child exposure to maternal depression and morning cortisol. While many mothers with a history of mood disorder in this sample had not experienced a depressive episode during their child's lifetime, some children had been exposed to full syndromal or subthreshold depressive episodes in their mothers. Children's morning cortisol levels

were not significantly correlated with mothers' self-reported depressive symptoms at the time of the laboratory assessment, as assessed by the DID (r = -.01, *n.s.*). In addition, as in the laboratory cortisol analyses, we created a variable that indicated whether mothers met criteria for a mood disorder (i.e., MDD, DD, Dep-NOS) or experienced partial recovery during the child's lifetime (0 = no mood disorder during child's life, 1 = mood disorder present during the child's life). The correlation between child morning cortisol and whether the child was exposed to maternal depressive symptoms was small and not significant (r = .09, *n.s.*). Therefore, it appears that the relation between child morning cortisol and maternal melancholic depression was not due to the child's exposure to maternal depressive symptoms.

Nevertheless, due to the small to moderate correlations between maternal melancholic depression and maternal current depressive symptoms (r = .21, p < .05) and child exposure to maternal depression during the child's life (r = .51, p < .001), we conducted three analyses to determine whether the relation between morning cortisol and maternal melancholic depression was due to child exposure to maternal depression. First, we conducted a hierarchical regression analysis in which self-reported depressive symptoms at the time of the assessment, as assessed by the DID, were entered into the first step and maternal melancholic depression was entered into the second step. Maternal current depressive symptoms (ß = -.01, *SE* = .01) was not significantly associated with children's morning cortisol levels, F change (1, 80) = .01, p = .94. However, maternal lifetime melancholic depression (ß = .22, *SE* = .03, p = .05) entered on the second step, was marginally associated with children's morning cortisol, F change (1, 79) = 3.95, p < .05. (N = 82, due to missing data on the DID). Then, we conducted a hierarchical

regression analysis in which child exposure to maternal depression was entered into the first step, and maternal melancholic depression was entered into the second step. Maternal depression during the child's life (ß = .09, *SE* = .05) did not significantly predict morning cortisol levels, *F* change (1, 92) = .82, *p* = .37. However, maternal lifetime melancholic depression (ß = .23, *SE* = .02, *p* = .04) entered on the second step, continued to account for significant variance in morning cortisol, *F* change (1, 91) = 4.04, $p < .05$. Lastly, we recomputed the correlations between morning cortisol and maternal melancholic depression after eliminating all families in which mothers met criteria for a serious mood disorder (MDD or DD) or a subthreshold depressive disorder (Dep-NOS) or partial recovery during the child's lifetime. After eliminating these participants (N = 21), the magnitude of the effect sizes between morning cortisol and melancholic depression (r = .23, *p* = .05) did not substantially change. These results suggest that the relationship between morning cortisol and maternal melancholic depression is not attributable to the effects of depressive symptoms in mothers during the child's life.

Maternal parenting behavior and child morning cortisol. As seen in Table 1, maternal supportive presence and hostility were not significantly associated with child morning cortisol levels.

Environmental factors. As seen in Table 1, child morning cortisol was not significantly correlated with marital discord, as assessed by maternal reports on the DAS, family SES, or with number of severe life stressors in the 6 months prior to the home cortisol assessment. The correlation between child morning cortisol and DAS was based only on 71 cases due to missing data on the DAS.

Unique effects of child temperamental PE and maternal melancholic depression on morning cortisol. Given that child low PE was significantly (albeit a small effect) associated with maternal lifetime melancholic depression as seen in Table 1, we examined the unique effects of child low PE and maternal melancholic depression on children's morning cortisol levels. We conducted a multiple regression analysis in which both child PE and maternal melancholic depression were entered simultaneously. Child PE (pr = -.22, $p < .05$) continued to uniquely predict morning cortisol levels over an above maternal melancholic depression. In addition, maternal melancholic depression (pr = .20, p = .06) marginally predicted children's morning cortisol levels. It is important to note that the magnitude of the effect sizes for morning cortisol and child PE and maternal melancholic depression are comparable, suggesting that they are independent factors contributing to elevated morning cortisol levels.

Interactions predicting children's basal morning and evening cortisol. Finally, we conducted exploratory analyses examining interactions between the following variables: PE, NE, BI, maternal lifetime history of depression, maternal supportive presence, maternal hostility, and life stress (including within- and between-domain interactions). For each analysis, we conducted a hierarchical multiple regression in which the direct effects of the two predictors along with the cross-product of the two predictors were entered. Each predictor variable was initially centered (converted into deviation score form) to minimize multicollinearity, and interaction terms were formed as the product of the two centered predictors (Aiken & West, 1991). Three significant interactions emerged predicting children's morning cortisol: (1) behavioral inhibition X maternal supportive presence ($ß$ = -.26, SE = .03, p = .02); (2) negative emotionality X maternal lifetime

depression (*ß* = -.24, SE = .04, *p* = .03); and (3) negative emotionality X maternal lifetime melancholic depression (*ß* = -.26, SE = .05, *p* = .01). There were no significant interactions predicting children's evening cortisol.

The significant interactions were probed using the techniques outlined by Aiken and West (1991). For the first interaction, the effect of BI on morning cortisol was estimated at 1 SD below the mean (low) and 1 SD above the mean (high) on maternal supportive presence. Maternal supportive presence interacted with BI to predict morning cortisol levels such that for supportive mothers, higher BI was related to lower morning cortisol levels (ß = -.32, *SE* = .04, *p* < .05). In contrast, among mothers exhibiting lower levels of supportiveness, high BI was marginally correlated with higher morning cortisol levels (ß = .25, *SE* = .04, *p* = .099).

In probing the next interactions, we examined the relation between NE and morning cortisol levels in individuals without any history of depression, with a lifetime history of MDD or DD, and with a lifetime history of melancholic depression. Both depression groups yielded the same results so we will only report data comparing mothers with a history of MDD or DD and those without any history of depression. For mothers with a history of depression, higher NE was marginally significantly associated with lower morning cortisol (ß = -.29, *SE* = .05, *p* = .06), and for mothers without a history of depression, NE was not significantly associated with higher morning cortisol levels (ß = .21, *SE* = .07, *p* = .14).

DISCUSSION

We found evidence for direct and moderated effects of temperamental, familial, and environmental risk factors for depression on children's early HPA axis functioning. In addition, we found support for our hypothesis that child low PE, a possible temperamental precursor to depression, is associated with HPA axis functioning in young children prior to the onset of any mood disorder. Overall, our findings suggest that the factors contributing to children's early HPA system functioning are complex. In addition, it appears that the processes involved in cortisol reactivity are somewhat different from the processes involved in basal cortisol levels throughout the day, given the small intercorrelations between laboratory and home cortisol levels and previous findings suggesting different genetic and environmental determinants. In the sections that follow, we will discuss our findings separately for children's laboratory cortisol reactivity and home basal cortisol levels.

Laboratory Cortisol

Laboratory cortisol reactivity. We assessed children's salivary cortisol levels four times over the course of the laboratory visit. As seen in Figure 1, we observed a significant linear decrease in cortisol from sample 1 to sample 2 in response to the separation stressor, followed by a rise in cortisol, whereas we had expected a linear increase in response to the separation stressor. Nevertheless, there was significant variation across the change in cortisol, and a subset of children did conform to the predicted pattern (i.e., a linear increase from sample 1 to sample 2). Similar decreases in response to stressors have been observed in other studies with children (Gotlib, Joorman, Minor, & Cooney, 2006; Luby et al., 2003; Talge, Bruce, Donzella, & Gunnar, 2003).

Reasons for this phenomenon are only speculative at this point, and no studies directly address this issue. Some data demonstrate that the baseline laboratory cortisol sample does not capture a "true" baseline level, as time-matched home samples do not match lab baseline samples (Gunnar & Talge, 2005). It is possible that the decrease from sample 1 to sample 2 may reflect the negative feedback loop of the HPA-axis system working to restore equilibrium. Thus, we present our laboratory findings under the assumption that each sample reflects a continuous reactivity process across the laboratory visit. However, we acknowledge that this is only an assumption as the data cannot accurately distinguish reactivity to the laboratory experience from the child's true baseline cortisol levels.

Direct effects on children's laboratory cortisol reactivity. We found that child temperament, parenting behaviors and life stress were uniquely and directly associated with the growth curve of children's cortisol reactivity. Child temperamental PE was not directly associated with children's cortisol reactivity. However, both NE and BI, temperamental traits that have also been linked to the depressive disorders as well as other forms of psychopathology, were associated with HPA axis functioning in children. In response to laboratory stress, children high in NE evidence a slower, flatter rate of positive curvature, which indicates elevated cortisol levels, but less fluctuation in cortisol over the course of the laboratory visit. Child BI was significantly associated with higher baseline cortisol levels, but was not significantly associated with the slope or curvature of the change in cortisol. The higher intercept together with no effects for slope and curvature imply overall higher laboratory cortisol levels, which is supported by the significant positive correlation between BI and higher mean levels of cortisol across the 4 laboratory assessments. It appears that children high in BI did not evidence greater

reactivity to the stressors during the laboratory visit, but they evidenced greater reactivity to the laboratory visit overall, given that it is a novel situation in which they are confronted with new people and new stimuli, all of which are apt to evoke fear and withdrawal-related behaviors in children high in temperamental BI. These findings support prior research demonstrating associations between NE and BI and elevated salivary cortisol (e.g., Blair et al., 2004; Dettling et al., 2000), but differ from findings reported by Kagan et al. (1987) that report a relation between BI and higher morning cortisol levels (such main effects were not observed in our study) and no relation between BI and stress reactivity in response to laboratory stressors. However, these differences may be due in part to the interactions involving BI described below.

We also found that parental hostility was uniquely associated with children's cortisol reactivity. We did not find main effects for parental supportive presence on children's cortisol reactivity, which supports research suggesting that parental hostility and support represent distinct dimensions with differential associations with psychological well-being, rather than opposite ends of a single dimension (Barrera, Chassin, & Rogosch, 1993). Parental hostility was significantly associated with higher baseline cortisol levels and greater stress reactivity, as parental hostility marginally predicted the slope and significantly predicted a slower rate of curvature. These findings add to the one other study (i.e., Smeekens et al., 2007) that examined the relation between parenting and HPA functioning in children beyond toddlerhood. These findings also support previous research demonstrating associations between poor parenting practices and cortisol functioning in infants and animals (Bugental et al., 2003; Dawson & Ashman, 2000; Gunnar, 1998; Spangler et al., 1994). In addition, animal research has

provided evidence for nongenetic, intergenerational transmission of stress reactivity via quality of maternal care (Francis et al., 1999). This animal research has found support for a pathway from maternal care to children's HPA responses to stress via changes in DNA methylation within the glucocorticoid receptor gene promoter in the hippocampus of the offspring, and these effects on DNA methylation can be reversed by changes in maternal care (Meaney & Szyf, 2005; Weaver et al., 2004). These findings, along with our findings, suggest that the well documented link between hostile parent-child interactions and child depressive symptoms and disorder (Sheeber, Davis, Leve, Hops, & Tildesley, 2007) may be partially mediated by children's increased physiological reactivity to stressors and highlight the potential for early intervention programs targeting maternal care.

Previous research has also shown that the cortisol levels of preschoolers and older children are positively correlated with numerous concurrent stressors, ranging from maternal depression to low SES (Lupien et al., 2000). However, we did not observe a relation between cortisol reactivity and maternal current depressive symptoms, maternal depression during the child's life, marital discord, or family SES. Yet, we did find a main effect for major life stress occurring in the six months prior to the laboratory visit. Life stress was associated with lower baseline levels of cortisol and was marginally significantly associated with lower mean cortisol secretion during the lab visit.

Interestingly, the negative relation between major life stressors and cortisol levels has been observed in individuals with PTSD and in those at risk for PTSD (Yehuda et al., 2000), and in nondepressed women exposed to childhood physical or sexual abuse (Heim et al., 2000). Nevertheless, it is unlikely that many children in our sample suffer from

PTSD as our sample was recruited from a community sample of predominately middle-class families, rather than from high risk populations. Furthermore, even though we did not assess for physical or sexual abuse, the number of abused children in our sample is likely to be very small, if any at all. As argued in a recent meta-analytic review, it is likely that the negative relation between stress and cortisol is due to specific features of the stressors assessed, such as the controllability and duration of the stressor (Miller et al., 2007). For example, Ronsaville et al. (2006) found evidence for a link between chronic family stress and attenuated cortisol responses in youth. Unfortunately, we did not assess for duration of the stressors, and therefore, the life stressors assessed cannot be deemed *chronic*. Nevertheless, it is possible that the children experiencing greater stressors in the 6 months prior to the lab visit also experienced earlier chronic stressors, which had lasting effects on their developing HPA system.

We also found support for several interactions involving child temperament, maternal history of depression, and parenting behaviors to predict children's cortisol reactivity.

Moderator effects of child PE on children's cortisol reactivity. Even though child PE was not directly related to children's stress reactivity, we found that child temperamental low PE interacted with maternal lifetime history of melancholic depression and parental hostility to predict greater stress reactivity in response to the laboratory stressors. The combination of child low PE and maternal melancholic depression was associated with an increasing slope and less recovery from the laboratory stressors. This is especially noteworthy as individuals with melancholic depression are a subtype of depressed individuals who consistently show elevated cortisol levels. It is

possible that this interaction reflects the intergenerational transmission of hypercortisolism in children at temperamental risk for depression. Another possibility is that mothers with a lifetime history of melancholic depression transmit a temperamental predisposition (i.e., low PE/anhedonic traits) that renders these individuals more vulnerable to the depressogenic effects of stress. Therefore, the hypercortisolism seen in melancholic depression may be due the presence of low PE/anhedonic features, rather than to the other features of the depressive episode.

We also found that the combination of child low PE and high parental hostility was associated with high and increasing cortisol in response to the laboratory stressors. Interestingly, at the behavioral level, Lengua et al. (2000) found that parental rejection was more strongly related to adjustment problems for children low in PE than for children high in PE. Our findings add to Lengua's finding by demonstrating that the combination of child low PE and high parental hostility is associated with neuroendocrine reactivity to stress. This biological sensitivity to stressors may partially reflect a developmental pathway that leads to adjustment problems, given findings that preschoolers with high cortisol levels exhibited greater mental health problems in first grade (Essex et al., 2002).

Moderator effects of child BI on children's cortisol reactivity. We found that child temperamental BI interacted with maternal lifetime history of depression and parental supportive presence to predict children's stress reactivity. Specifically, for mothers with a lifetime history of depression, BI significantly predicted less of a decline in slope and a slower rate of positive curvature of the change in cortisol, whereas for mothers with no history of depression, BI did not significantly predict the slope or curvature of the growth

curve. Regardless of maternal lifetime depressive status, BI was significantly associated with a higher intercept. However, this interaction is qualified by the interaction between child temperamental BI and maternal melancholic depression. Child temperamental BI interacted with maternal history of melancholic depression to predict the slope of the growth curve, demonstrating a *positive* linear increase (rather than the typical linear decrease observed) in cortisol levels in response to the separation stressor, and this effect was not present for the children of mothers with non-melancholic depression. Thus, it appears that children with high BI and mothers with a history of melancholic depression were more reactive to laboratory stress than children with high BI and mothers with a history of depression and/or no history of depression. Our findings suggest that children high in temperamental BI who have mothers with a history of melancholic depression evidence greater physiological reactivity to stressors, which may put them at an increased risk for later adjustment problems and the development of depression. This interaction may explain why not all studies support the relation between BI and risk for depression.

We also found that the combination of high BI and low parental support was associated with an elevated baseline level of salivary cortisol during the laboratory visit. Conversely, the combination of high BI and high parental support was associated with a lower baseline level of salivary cortisol. Here, we see that parental support can protect high BI children from elevated cortisol levels. Similarly, Nachmias et al. (1996) found that fearful toddlers showed elevations in cortisol if they were insecurely attached to their caregiver and did not evidence elevations in cortisol if they were securely attached to their caregiver. Our findings are also in line with research indicating that the quality of the mother-child relationship mediates the relation between early and later forms of

behavioral inhibition, such that some parents of behaviorally inhibited children interact with their children in a way that exacerbates or maintains their child's fearful temperament. Fox et al. (2007) suggest that maternal caregiving behavior shapes the development of persistent behavioral inhibition by altering the neural systems that underlie reactivity to stress and novelty (for support of this hypothesis, see Ghera, Hane, Malesa, & Fox, 2006; Hane & Fox, 2006). As such, it is possible that high BI children whose parents do not provide adequate levels of support evidence elevated cortisol levels, which would put them at increased risk for more persistent forms of BI and the development of later psychopathology. Although our findings suggest that high levels of parental support may protect high BI children from adverse outcomes,it is important to note that maternal protectiveness, which may reflect an extreme and maladaptive attempt to be supportive, appears to have an adverse effect on the course of BI (Rubin, Burgess, & Hastings, 2002).

Moderator effects of child NE on children's cortisol reactivity. The relation between NE and cortisol levels was moderated by maternal history of depression. Briefly, we found that NE was marginally associated with lower laboratory baseline levels of cortisol in mothers with a lifetime history of maternal melancholic depression, but was not significantly associated with baseline cortisol levels in mothers with a lifetime history of non-melancholic depression or mothers with no lifetime history of depression. However, this interaction should be interpreted with caution as it was no longer significant when further probed. We will discuss this interaction further when we discuss the home cortisol data as a similar interaction was observed.

Moderator effects of maternal depression on children's cortisol reactivity. Our last significant interaction predicting children's cortisol reactivity involved maternal history of depression and parental hostility. Specifically, we found that the combination of maternal lifetime history of depression and parental hostility was associated with a higher intercept, an increasing slope, and a flatter, slower rate in curvature. Thus, the combination of a lifetime history of maternal depression and high parental hostility leads to high and increasing cortisol reactivity. In line with our findings, Brennan, Le Brocque, and Hammen (2003) reported that low levels of maternal warmth and high levels of maternal psychological control interacted with maternal depression to predict negative outcomes in youth. Conversely, high levels of maternal warmth and low levels of maternal psychological control interacted with maternal depression to predict resilient outcomes in youth. Similarly, maternal sensitivity moderated the effects of maternal depression on child outcomes at 36 months (NICHD Early Child Care Research Network, 1999). These findings support research with adults that show a familial risk for depression, in the presence of environmental adversities, may put individuals at greater risk for depression (Kendler et al., 2002; Kendler et al., 2004). However, our findings are the first, to our knowledge, to show that parental history of depression and parenting behaviors interact to predict children's neuroendocrine functioning, and it is possible that children's neuronendocrine deficits mediate the relation between early risk factors and the development of adverse child outcomes.

Home Cortisol

Direct effects on home basal cortisol levels. Both child low PE and maternal melancholic depression were significantly associated with higher morning cortisol levels.

The relation between low PE and elevated morning cortisol appears to be unique to low PE, as temperamental NE and BI, children's current depressive symptoms, mothers' current depressive symptoms, parenting behaviors, and life stress were not correlated with morning cortisol levels, and as the relation between low PE and morning cortisol persisted after controlling for the maternal melancholic depression. In addition, the relation between maternal melancholic depression and children's morning cortisol levels persisted after controlling for maternal lifetime anxiety and substance use disorders, mothers' current depressive symptoms and depressive symptoms during the child's life, parenting behaviors, and life stress. Furthermore, even though the relation between maternal melancholic depression and higher morning cortisol dropped to a trend level (p = .06) when child low PE was included in the model, the magnitude of the correlation between maternal melancholic depression and higher morning cortisol did not substantially change (.23 to .20). It is probable that the smaller sample size and the limited number of mothers with melancholic depression in the home analyses reduced power. Thus, our findings suggest that children's morning cortisol levels are uniquely associated with both temperamental PE and maternal melancholic depression.

These findings provide some support for the possibility that temperamental low PE and/or maternal melancholic depression are endophenotypes that predispose children to the development of depression via HPA axis functioning. These findings are especially noteworthy as morning cortisol levels are under the most genetic control (Bartels et al., 2003). We recognize that the direction of the relationship between temperamental and familial predispositions and neuroendocrine functioning may be difficult to discern. Nevertheless, a pathway(s) involving maternal melancholic depression, temperamental

low PE, and HPA axis dysfunction is plausible given the findings that low PE-related behaviors, maternal depression, and elevated morning cortisol levels have all been found to predict the subsequent development of depression (Block et al., 1991; Caspi et al., 1996; Goodman & Gotlib, 2002; Goodyer et al., 2000; Harris et al., 2000; van Os, et al., 1997).

Interestingly, both child low PE and maternal melancholic depression were associated with elevated waking cortisol, especially as both are characterized by anhedonia. In addition, child low PE was significantly associated (albeit small in magnitude) with maternal melancholia as seen in Table 1. It appears that children low in temperamental PE may be less resistant, and more sensitive, to the depressogenic effects of stress. And, it appears that for a subset of children, what is transmitted from mothers with a history of melancholic depression to offspring is deficits in reward sensitivity and the experience of pleasure, which make the offspring more sensitive the effects of stress. Therefore, the child may acquire these deficits in the experience of pleasure and reward sensitivity from their own temperamental vulnerability and/or a familial predisposition for melancholic depression. However, as supported by the multiple regression analysis in which both child low PE and maternal melancholia appear to provide two independent pathways to HPA axis dysregulation, in which one is due to the temperamental vulnerability and the other is due to a familial risk for potentially developing later anhedonic traits.

Our results support findings that the relation between familial risk for depression and elevated morning cortisol levels is independent of parenting and depression during the child's life (e.g., Mannie et al., 2007; Azar et al., 2007). Nevertheless, other studies

have found support for the environmental effects of having a depressed parent on children's elevated cortisol levels (e.g., Ashman et al., 2002; Essex et al., 2002; Halligan et al., 2004; Young et al., 2006). However, none of the studies supporting an environmental pathway, except Halligan et al. (2004), report associations with elevated *morning* cortisol, which appears to be a more genetic/familial marker of risk for depression. Halligan et al. (2004) did find a positive association between maternal postnatal depression and morning cortisol levels, but it was unclear whether this was due to environmental effects of having a depressed parent or a familial vulnerability for depression.

Moderator effects on children's morning cortisol. Similar to the findings of laboratory cortisol reactivity, we found that BI interacted with parental supportive presence to predict morning cortisol levels, such that for supportive mothers, higher BI was related to lower morning cortisol levels. In contrast, among mothers exhibiting lower levels of support, high BI was marginally correlated with higher morning cortisol levels. Therefore, in both the lab and home findings, we see that parental support can protect high BI children from elevated cortisol levels.

Similar to the lab findings on baseline cortisol levels, we found that NE was marginally significantly associated with lower morning cortisol levels for mothers with a history of depression and was not significantly associated with morning cortisol levels for mothers without a history of depression. We want to stress that when these interactions were probed, they were only marginally significant; however, given that we observed similar findings in the lab and home data, it is important to consider their significance. We had predicted that the combination of high temperamental NE and maternal history of

depression would be associated wtih greater cortisol levels; however, we observed the opposite (i.e., lower baseline or lower morning cortisol levels). It is possible that children who are high in temperamental NE evoke more maternal attention and subsequently maternal care from their mother with a history of depression. Conversely, children low in NE and whose mothers have a lifetime history of depression may experience a more neglectful maternal environment, which increases their risk for HPA axis dysfunction. Of course, this is only speculative, but our findings highlight the bidirectionality and complexity of the influences that child temperament, maternal history of depression, and parenting each have on one another.

We had hypothesized that early intrinsic and environmental risk factors for depression would be associated with elevated evening cortisol levels as depression has been linked to a flattened circadian curve (Deuschle et al 1997) and an elevated nadir (Yehuda et al 1996), and as recent life events have been associated with elevated evening cortisol (Strickland et al., 2002). Nevertheless, we did not find direct or moderator effects of any of the predictor variables on children's evening cortisol levels. It is possible that elevated evening cortisol levels are present only in individuals currently in a depressive episode and not in individuals at risk for depression. In addition, the life events in our study included events occurring in the six months prior to the cortisol assessments, and it is possible that only stressors that are currently affecting the individual increase evening cortisol levels. This supports the idea that evening cortisol levels are altered only when the HPA system is currently affected by a depressive illness or by current environmental demands.

Summary. It has been hypothesized that temperamental emotionality, familial psychopathology and environmental factors, including parenting and life stress, contribute to the development of HPA axis dysregulation in children, which in turn leads to increased risk for depression. However, the direct and moderator effects on HPA axis functioning remain unclear. Therefore, this study aimed to elucidate these direct and joint processes on HPA functioning. For instance, we were interested in determining which factors are associated with *risk* in children's developing HPA axis system? Conversely, which factors are associated with *resilience* in HPA axis functioning? Finally, which factors *potentiate* or amplify the effects of other risk factors on HPA axis functioning? Our findings are summarized in Table 5.

Risk. Several potential risk factors of deviations in HPA functioning were identified. High child temperamental BI and NE, parental hostility and more life stress were uniquely associated with parts of the growth curve of children's laboratory cortisol reactivity. In addition, child temperamental low PE and maternal melancholic depression conferred increased risk for higher morning basal cortisol levels. Conversely, the flip side of the risk factors identified above would identify children at less risk for potential disruption of the HPA axis system. However, the effects of these potential risk factors on children's emotional and behavioral development via HPA axis functioning is not clear at this time. It is also important to note that research in the area of HPA axis functioning tends to use the term "dysfunction" to describe significantly higher or lower levels of cortisol. However, we acknowledge that statistical deviance is not necessarily the same as dysfunction, and we encourage future research to determine at what level increased or decreased cortisol levels leads to adverse outcomes and whether the levels must be

maintained over a certain period of time to incur significant risk. These are critical questions for future research in the area of neuroendocrine and developmental psychopathology research.

Resilience. We use the terms *resilience* to refer to outcomes that are better than expected given significant risk exposure. Therefore, our findings identify at least two interactions that characterize resilience. First, children high in temperamental PE did not evidence greater stress reactivity in the face of high levels of parental hostility or in the presence of a familial predisposition for melancholic depression. These finding support recent evidence showing that positive emotions help buffer against stress (Folkman & Moskowitz, 2000), possibly by broadening the array of subsequent thoughts and actions that can allow for more adaptive coping in the face of stress (Tugade & Fredrickson, 2004). In addition, high levels of parental supportive presence attenuated the effects of child high temperamental behavioral inhibition on children's laboratory baseline and home morning cortisol levels. We acknowledge that the resilience effects can also be viewed as potentiation effects, depending on which level of the independent variable is examined (e.g., high PE or low PE). Both are included in Table 5.

Potentiation. The term *potentiator* is used to describe all influences that amplify the likelihood of psychopathology or adverse outcomes. The presence of maternal history of depression served as a potentiator in the relation between BI and cortisol reactivity and between parental hostility and cortisol reactivity, yielding greater cortisol reactivity when lifetime history of maternal depression was present along with either child high temperamental BI or greater parental hostility. Similarly, the presence of maternal melancholic depression further potentiated the relation between BI and laboratory cortisol

reactivity by yielding an increasing and positive slope in response to laboratory stress. In addition, the presence of child low PE served as a potentiator in the relation between parental hostility and cortisol reactivity. And, lastly, we identified an interaction between low PE and maternal melancholic depression, despite neither of them inferring any direct risk on children's laboratory cortisol reactivity. The combination of child low PE and maternal melancholic depression was associated with greater cortisol reactivity, demonstrating an interactive effect between temperamental and familial vulnerabilities of depression.

Strengths and Limitations. This study had several strengths. First, our sample was large enough to ensure adequate power to test our moderator analyses. Second, we used observational measures of child temperament and parenting, and maternal psychiatric diagnoses were derived from structured clinical interviews. Third, we examined the interrelations among temperamental, familial, and environmental risk factors of depression in a young sample prior to the onset of a depressive diagnosis in order to ensure that these factors occur prior to any depressive illness. This is especially important as this study is embedded in a longitudinal study. Lastly, we assessed both children's cortisol reactivity in response to laboratory stressors and children's home basal cortisol levels.

This study also had several limitations. First, our sample was somewhat biased. Those who did not participate in the laboratory cortisol assessments were significantly higher on parental hostility and temperamental NE and BI, and significantly lower on interest compared to children who were included in the final sample. Those who did not participate in the home cortisol assessment were significantly higher on parental hostility

and experienced more stressful life events in the six months prior compared to children who completed the home cortisol assessment. However, this should reduce the range of these variables and make the analyses more conservative. In addition, the sample was recruited from a commercial mailing list, which may have been biased in unknown ways, and the sample lacked ethnic and racial diversity.

Second, we conducted many exploratory analyses, which increased our Type II error. Third, the study was cross sectional, which did not allow us to test the hypothesis that these factors contribute to the development of depression. Fourth, the temperament variables were assessed at the same time as the lab cortisol levels, so their temporal association is unclear. Fifth, our assessment of child life stress only assessed whether a severe life stressor occurred in the six months prior to salivary cortisol collection. We did not assess for the duration of the stressor or whether past stressors that had earlier onsets were continuing into the six-month time period; therefore, we were unable to determine the role of *chronic* versus *acute* stressors on children's HPA axis functioning.

Sixth, even though we collected both laboratory and home cortisol samples, each was done on one occasion. This is especially problematic as cortisol tends to vary considerably depending on many factors, such as sleep, eating and exercise. Therefore, it has been recommended to collect samples over several days (Gunnar & Talge, 2005). Unfortuantely, this extra burden was not possible given the other demands of the larger project in which this study was embedded. Seventh, as mentioned above, we assumed that the lab cortisol assessments were tapping cortisol reactivity but the data cannot accurately distinguish reactivity to the lab experience from the child's true baseline. Eighth, we observed a mean decrease in cortisol after what was presumed to be the most

stressful part of the assessment, although a subset of respondents exhibited the expected increased, and this subset was identified by a number of the predictors we examined.

Lastly, although greater salivary cortisol reactivity and increased waking salivary cortisol might be a useful marker of vulnerability, it is not clear how far an increase in cortisol secretion at one point in the day can be regarded as clinically significant. Therefore, future research should determine at what *level* elevated and/or blunted cortisol cortisol levels, either in response to stressors or basal levels, become risk factors for depression

In conclusion, our findings underscore the complexity of factors that relate to early HPA axis functioning and risk for depression in young children. They also suggest several potential avenues for future research. First, research needs to examine these cross-sectional relations over time and determine how they uniquely and jointly contribute to specific pathways to different subtypes of depression and to other forms of psychopathology. In addition, other neuroendocrine factors have been implicated in risk for depression and should be examined in future research. For instance, altered secretion of the adrenal steroid dehydroepiandrosterone (DHEA) may also be a risk factor for depression in young people (Goodyer, Park, Netherton, & Herbert, 2001) and the ratio of salivary cortisol to DHEA might provide a more sensitive measure of functional hypercortisolemia than a measure of cortisol alone (Young, Gallagher, & Porter, 2002). Third, it has been proposed that cortisol hypersecretion and early temperament are involved in the development of depressive cognitive styles (Campbell & MacQueen, 2004; Hayden et al., 2006), which in turn leads to the development of depression. Therefore, future longitudinal studies should examine pathways to the depressive

disorders involving HPA dysfunction, early temperament, and depressogenic cognitions. Fourth, an emerging area of research that needs more attention in developmental psychopathology research is the examination of genetic polymorphisms as distal causal influences and moderators. Polymorphisms that could influence the HPA response to stress are being identified, such as functional variants in the glucocorticoid receptor, the mineralocorticoid receptor, and the serotonin transporter (Barr et al., 2004; DeRijk & de Kloet, 2005). Fifth, it has been suggested that exposure to adversity in the early years of life, when the nervous system is still developing, may result in a distinct and stable pattern of dysregulation that remains altered into adulthood (Liu et al., 1997; Meaney & Szyf, 2005). Therefore, it is important to consider the role of development in our findings as our research is embedded within changing biological and environmental systems.

Lastly, the results of this study may have implications for intervention and prevention with children and parents. For instance, our findings suggest that children of depressed mothers and/or children low in temperamental PE fare better if their mothers are less hostile. In addition, children high in temperamental BI fare better with a supportive parenting style. Therapeutic or preventive interventions that focus on these parenting qualities in at risk populations could be beneficial. Furthermore, recent work in the area of positive psychology has shown that interventions can increase positive emotions and overall happiness in adults (Emmons & McCullough, 2003; Seligman, Steen, Park, & Peterson, 2005; Sheldon and Lyubomirsky, 2006). This research is especially noteworthy as we have found that children high in PE had lower morning cortisol levels and were more resilient to the effects of environmental adversity.

REFERENCES

Achenbach, T. M., & Rescorla, L. A. (2000). *Manual for the ASEBA Preschool Form & Profiles*. Burlington VT: University of Vermont Department of Psychiatry.

Akiskal, H. S. (1989). Validating affective personality types. In L. Robins & 1. Barrett (Eds.), *The validity of psychiatric diagnosis* (pp. 217-227). New York: Raven Press.

Ahnert, L., Gunnar, M.R., Lamb, M.E., & Barthel, M. (2004). Transition to childcare: Associations with infant-mother attachment, infant negative emotion and cortisol elevations. *Child Development, 75, 639-650.*

Aiken, L. S., & West, S. G. (1991). Multiple regression: Testing and interpreting interactions. Newbury Park, CA: Sage.

Akin, D., Manier, D. H., Sanders-Bush, E., & Shelton, R. C. (2004). Decreased serotonin 5-HT(2A) receptor-stimulated phosphoinositude signaling fibroblasts from melancholic depressed patients. *Neuropsychopharmacology, 29,* 2081-2087.

Arborelius, L., Owens, M.J., Plotsky, P.M., Nemeriff, C.B. (1999). The role of corticotrophin-releasing factor in depression and anxiety disorders. *Journal of Endocrinology, 160,* 1-12.

Ashman, S.B., Dawson, G., Panagiotindes, H., Yamada, E., & Wilkinson, C.W. (2002). Stress hormone levels of children of depressed mothers. *Development and Psychopathology, 14,* 333-349.

Aubry, J. M., Gervasoni, N., Osiek, C., Perret, G., Rossier, M. F., Bertschy, G., & Bondolfi, G. (2007). The DEX/CRH neuroendocrine test and the prediction of depressive relapse in remitted depressed outpatients. *Journal of Psychiatric Research, 41,* 290-294.

Azar, R., Paquette, D., Zoccolillo, M., Baltzer, F., & Tremblay, R. E. (2007). The association of major depression, conduct disorder, maternal overcontrol with a failure to show a cortisol buffered response in 4-month-old infants of teenage mothers. *Biological Psychiatry, In Press, Corrected Proof, Available online 6 March 2007*.

Barr, C. S., Newman, T. K., Shannon, C., Parker, C., Dyoskin, R. L., Becker, M. L. et al. (2004). Rearing condition and rh5-HTTLPR interact to influence limbic-hypothalamic-pituitary-adrenal axis response to stress in infant macaques. *Biological Psychiatry, 55,* 733-738.

Barrera, M., Chassin, L., & Rogosch, F. Effects of social support and conflict on adolescent children of alcoholic and nonalcoholic fathers. *Journal of Personality and Social Psychology, 64*, 602-612.

Bartels, M., de Geus, E. J. C., Kirschbaum, C., Sluyter, F., & Boomsma, D. I. (2003). Heritability of daytime cortisol levels in children. *Behavioral Genetics, 33,* 421-433.

Beauchaine, T. P. (2003). Taxometrics and developmental psychopathology. *Development and Psychopathology, 15,* 501-527.

Beauchaine, T. P., & Marsh, P. (2006). Taxometric methods: Enhancing early detection and prevention of psychopathology by identifying latent vulnerability traits. In D. Cicchetti, & D. Cohen (Eds.) *Developmental Psychopathology*, 2nd Ed. (pp. 931-967). Hoboken, NJ: Wiley.

Biederman, J., Rosenbaum, J. F., Bolduc-Murphy, E. A, Faraone, S. V., Chaloff, J., Hirshfeld, D. R., & Kagan, J. (1993). A 3-year follow-up of children with and without behavioral inhibition. *Journal of the American Academy of Child & Adolescent Psychiatry*, 32, 814-821.

Biederman, J., Rosenbaum, J. F., Hirshfeld, D. R., Faraone, S. V., Bolduc, E. A, Gersten, M., Meminger, S. R., Kagan, J., Snidman, N., & Reznick, J. S. (1990). Psychiatric correlates of behavioral inhibition in young children of parents with and without psychiatric disorders. *Archives of General Psychiatry,* 47, 21-26.

Birmaher, B., Dahl, R.E., Ryan, N.D., Rabinovich, H., Ambrosini, P., Al-Shabbout, M. et al. (1992). Dexamethasone suppression test in adolescents with major depressive disorder. *American Journal of Psychiatry, 149*, 1040-1045.

Birmaher, B., Dahl, R. E., Perel, J., Williamson, D. E., Nelson, B., Stull, S., Kaufman, G., Waterman, S., Rao, U., Nguyen, N., Puig-Antich, J., & Ryan, N. D. (1996). Corticotropin-releasing hormone challenge in prepubertal major depression. *Biological Psychiatry, 39,* 267-277.

Blair, C., Peters, R., & Granger, D. (2004). Phsyiological and neuropsychological correlates of approach/withdrawal tendencies in preschool: Further examination of the behavioral inhibition system/behavioral activation system scales for young children. *Developmental Psychobiology, 45,* 113-124.

Block, J.H., Gjerde, P.F., & Block, J.H. (1991). Personality antecedents of depressive tendencies in 18-year-olds: A prospective study. *Journal of Personality and Social Psychology, 60,* 726-738.

Bradbury, M. J., Akana, S. F., & Dallman, M. F. (1994). Roles of Type I and Type II corticosteroid receptors in regulation of basal activity in the HPA axis during the

diurnal trough and the peak: Evidence for a nonadditive effect of combined receptor occupation. *Endocrinology, 134*, 1286-1296.

Brennan, P. A., Le Brocque, R., & Hammen, C. (2003). Maternal depression, parent-child relationships, and resilient outcomes in adolescence. *Journal of the American Academy of Child and Adolescent Psychiatry, 42*, 1469-1477.

Brown, T. A., Chorpita, B. F., & Barlow, D. H. (1998). Structural relationships among dimensions of the DSM-IV anxiety and mood disorders and dimensions of negative affect, positive affect, and autonomic arousal. *Journal of Abnormal Psychology, 107*, 179-192.

Brownell, R. (2000). *Expressive One-Word Picture Vocabulary Test.* Novato, CA: Academic Therapy Publications.

Buchanan, T.W., al'Absi, M., & Lovallo, W.R. (1999). Cortisol fluctuates with increases and decreases in negative affect. *Psychoneuroendocrinology, 24*, 227-241.

Bugental, D. B. (2004). Thriving in the face of adversity. *Journal of Social Issues, 60*, 219-235.

Bugental, D.B., Martorell, G.A., & Barraza, V. (2003). The hormonal costs of subtle forms of infant maltreatment. *Hormones and Behavior, 43*, 237-244.

Buss, K.A., Schumacher, J.R.M., Dolski, I., Kalin, N.H., Goldsmith, H., & Davidson, R.J. (2003). Right frontal brain activity, cortisol, and withdrawal behavior in 6-month-old infants. *Behavioral Neuroscience, 117*, 11-20.

Caspi, A, Moffitt, T. E., Newman, D. L., & Silva, P. A (1996). Behavioral observations at age 3 years predict adult psychiatric disorders. *Archives of General Psychiatry, 53*, 1033-1039.

Chorpita, B. F. (2002). The tripartite model and dimensions of anxiety and depression: An examination of structure in a large school sample. *Journal of Abnormal Child Psychology, 30*, 177-190.

Cicchetti, D., & Rogosch, F.A. (2001). Diverse patterns of neuroendocrine activity in maltreated children. *Development and Psychopathology, 13*, 677-693.

Clark, L.A, Watson, D., & Mineka, S. (1994). Temperament, personality, and the mood and anxiety disorders. *Journal of Abnormal Psychology, 103*, 103-116.

Cohen, S. Doyle, W. J., Turner, R. B., R. B., Alper, C. M., & Skoner, D. P. (2003). Emotional style and susceptibility to the common cold. *Psychosomatic Medicine, 65*, 652-657.

Cohn, J. F., Matias, R., Tronick, E. Z., Connell, D., et al., (1986). Face-to-face interactions of depressed mothers and their infants. *New Directions for Child Development, 34*, 31-45.

Cohn, J.F., Campbell, D.T., Matias, R., & Hopkins, J. (1990). Face-to-face interactions of postpartum depressed and nondepressed mother-infant pairs at 2 months. *Developmental Psychology, 26*, 15-23.

Compas, B. E., Connor-Smith, J., & Jaser, S. S. (2004). Temperament, stress reactivity, and coping: Implications for depression in childhood and adolescence. Journal of Clinical Child and Adolescent Psychology, 33, 21-31.

Connor-Smith, J. K., & Compas, B. E. (2002). Vulnerability to social stress: Coping as a mediator and moderator of sociotropy and symptoms of anxiety and depression. Cognitive Therapy and Research, 26, 39-55.

Coplan, J. D., Andrews, M. W., Rosenblum, L. A., Owens, M. J., Friedman, S., Gorman, J. M., & Nemeroff, C. B. (1996). Persistent elevations of cerebrospinal fluid concentrations of corticotrophin-releasing factor in adult nonhuman primates exposed to early-life stressors: Implications for the pathophysiology of mood and anxiety disorders. *Procededing of the National Academy of Sciences of the United States of America, 93,* 1619–1623.

Coste, J., Strauch, G., Letrait, M., & Bertagna, X. (1994). Reliability of hormonal levels for assessing the hypothalamic-pituitary-adrenocortical system in clinical pharmacology. *British Journal Clinical Pharmacology, 38*, 474–479.

Davidson, R. J. (1995). Cerebral asymmetry, emotion and affective style. In R. J. Davidson & K. Hugdahl (Eds.), *Brain asymmetry* (pp.361-387). Cambridge, MA: MIT Press.

Davidson, R. J. (1998). Affective style and affective disorders: Perspectives from affective neuroscience. *Cognition & Emotion,* 12,307-330.

Davis, E.P., Donzella, B., Krueger, W.K., & Gunnar, M.R. (1999). The start of a new school year: Individual differences in salivary cortisol response in relation to child temperament. *Developmental Psychobiology, 35*, 188-196.

Dawson, G., & Ashman, S.B., (2000). On the origins of a vulnerability to depression: the influence of the early social environment on the development of psychobiological systems related to risk for affective disorder. In: Nelson, C.A. (Ed.), *The Effects Of Adversity On Neurobehavioral Development: Minnesota Symposia On Child Psychology, vol. 31*. Lawrence Erlbaum Assoc, Mahwah, NJ, pp. 245–280.

Dawson, G., Frey, K., Self, J., Panagiotides, H., Hessl, D., Yamada, D., & Rinaldi, J. (1999). Frontal brain electrical activity in infants of depressed and nondepressed

mothers: Relation to variations in infant behavior. *Development and Psychopathology, 11*, 589-605.

Dawson, G., Hessl, D., & Frey, K. (1994). Social influences of early developing biological and behavioral systems related to risk for affective disorders. *Development and Psychopathology, 6,* 759-779.

DeBellis, M.D., Dahl, R.E., Perel, J.M., Birmaher, B., al-Shabbout, M., Williamson, D.E., et al. (1996). Nocturnal ACTH, cortisol, growth hormone, and prolactin secretion in prepubertal depression. *Journal of the American Academy of Child and Adolescent Psychiatry, 35,* 1130-1138.

de Kloet, E. R. (1991). Brain corticosteroid receptor balance and homeostatic control. Frontiers in *Neuroendocrinology, 12,* 95-164.

de Haan, M., Gunnar, M.R., Tout, K., Hart, J., & Stansbury, K., (1998). Familiar and novel contexts yield different associations between cortisol and behavior among 2 year olds. *Developmental Psychobiology, 31*, 93-101.

DeRijk, R.H., Schaaf, M., & de Kloet, E. R. (2002). Glucocorticoid receptor variants: clinical implications. *Journal of Steroid Biochemistry and Molecular Biology, 81,* 103–122.

Depue, R. A, & Iacono, W. G. (1989). Neurobehavioral aspects of affective disorders. *Annual Review of Psychology, 40,* 457-492.

Dettling, A.C., Parker, S., Lane, S.K., Sebanc, A.M., Gunnar, M.R., 2000. Quality of care and temperament determine whether cortisol levels rise over the day for children in full-day childcare. *Psychoneuroendocrinology, 25,* 819–836.

Deuschle, M., Schweiger, U., Weber, B., Gotthardt, U., Korner, A., Schmider, J., et al. (1997). Diurnal activity and pulsatility of the HPA system in male depressed patients and healthy controls. *Journal of Clinical Endocrinology and Metabolism, 82,* 234-238.

Dickerson, S. S. & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin, 130,* 255-391.

Dougherty, L. R., Klein, D. N., Durbin, C. E., Hayden, E. P., & Olino, T. M. (2007). Temperamental positive and negative emotionality and children's depressive symptoms: A longitudinal prospective study from age three to age ten. *Development & Psychopathology.* Submitted for publication.

Dunn, L.M., & Dunn, L.M. (1997). *Peabody Picture Vocabulary Test (3rd edition).* Circle

Pines, Minnesota: American Guidance Service.

Durbin, C. E., Hayden, E. P., Klein, D. N., & Olino, T. M. (2007). Stability of laboratory assessed temperamental emotionality traits from ages 3 to 7. *Emotion, 7,* 388-399.

Durbin, C. E., Klein, D. N., Hayden, E. P., Buckley, M. E., & Moerk, K. C. (2005). Temperamental emotionality and familial risk for mood disorders in preschoolers. *Journal of Abnormal Psychology,114,* 28-37.

Edwards, S., Clow, A., Evans, P., & Hucklebridge, F. (2001). Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity. *Life Scienc, 68*, 2093–2103.

Egger, H.L., Ascher, B.H., & Angold, A (1999). *The Preschool Age Psychiatric Assessment (PAPA).* Durham, NC: Center for Developmental Epidemiology, Department of Psychiatry and Behavioral Sciences, Duke University Medical Center.

Egeland, B., Weinfield, N., Hiester, M., Lawrence, C., Pierce, S., & Chippendale, K. (1995). *Teaching tasks administration and scoring manual.* University of Minnesota.

Ellenbogen, M. A., Hodgins, S., Walker, C., Couture, S., & Adam, S. (2006). Daytime cortisol and stress reactivity in the offspring of parents with bipolar disorder. Psychoneuroendocrinology, 31, 1164-1180.

Emmons, R. A., & McCullough, M. E. (2003). Counting blessings versus burdens: An experimental investigation of gratitude and subjective well-being in daily life. *Journal of Personality and Social Psychology, 84*, 377-389.

Essex, M.J., Klein, M.H., Cho, E., & Kalin, N.D. (2002). Maternal stress beginning in infancy may sensitize children to later stress exposure: effects on cortisol and behavior. *Biological Psychiatry, 52*, 776-784.

Feder, A., Coplan, J.D., Goetz, R.R., Mathew, S.J., Pine, D.S., Dahl, R.E., et al. (2004). Twenty-four hour cortisol secretion in prepubertal children with anxiety or depressive disorders. *Biological Psychiatry, 56*, 198-204.

Field, T. (1992). Infants of depressed mothers. *Development and Psychopathology, 4*, 9-66.

First, M. B., Gibbon, M., Spitzer, R. L., & Williams, J. B. W. (1996). *Structured clinical interview for DSM-IV Axis I disorders: Non-patient edition (SCID-I, Version 2.0).* New York: Biometrics Research, New York State Psychiatric Institute.

Folkman, S., & Moskowitz, J. T. (2000). Positive affect and the other side of coping. *American Psychologist, 55*, 647-654.

Fox, N.A. (1994). Dynamic cerebral processes underlying emotion regulation. *Monographs of the Society for Research in Child Development, 59* (2-3, Serial No. 240).

Fox, N. A., Hane, A. A., & Pine, D. S. (2007). Plasticity for affective neurocircuitry. *Current Directions in Psychological Science, 16*, 1-5.

Francis, D. D., Diorio, J., Liu, D., & Meaney, M. J. (1999). Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science, 286*, 1155-1158.

Ghera, M. M., Hane, A. A., Malesa, E. M., & Fox, N. A. (2006). The role of infant soothability in the relation between infant negativity and maternal sensitivity. *Infant Behavior and Development, 29*, 289-293.

Gold, P. W., Calabrese, J. R., Kling, M. A., Avgerinos, P., Khan, I., Gallucci, W. T., et al. (1986). Abnormal ACTH and cortisol responses to ovine corticotrophin releasing factor in patients with primary affective disorder. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 10*, 57– 65.

Gold, P.W., & Chrousos, G.P. (1999). The endocrinology of melancholic and atypical depression: Relation to neurocircuitry and somatic consequences. *Proceedings of the Association of American Physicians, 111*, 22-34.

Gold, P. W., Goodwin, F. K., & Chrousos, G. P. (1988). Clinical and biochemical manifestations of depression: Relations to the neurobiology of stress. *New England Journal of Medicine, 319*, 348-353.

Gold, P. W., Licinio, J., Wong, M. L., & Chrousos, G. P. (1995). Corticotropin releasing hormone in the pathophysiology of melancholic and atypical depression and in the mechanism of action of antidepressant drugs. *Annals of the New York Academy of Sciences, 771*, 716–729.

Goldsmith, H. H., & Lemery, K. S. (2000). Linking temperamental fearfulness and anxiety symptoms: A behavior-genetic perspective. *Biological Psychiatry, 48*, 1199-1209.

Goldsmith, H. H., Buss, K. A., & Lemery, K. S. (1997). Toddler and childhood temperament: Expanded content, stronger genetic evidence, new evidence for the importance of environment. Developmental Psychology, 33, 891-905.

Goldsmith, H. H., Reilly, J., Lemery, K. S., Longley, S., & Prescott, A. (1995). *Laboratory Temperament Assessment Battery: Preschool version.* Unpublished Manuscript.

Goodman, S. H., & Gotlib, I. H. (Eds.) (2002). *Children of depressed parents: Mechanisms of risk and implications for treatment.* Washington, DC: American Psychological Association.

Goodyer, I.M., Herbert, J., Altham, P.M., Pearson, J., Secher, S.M., & Shiers, H.M. (1996). Adrenal secretion during major depression in 8 to 16 year olds, I: Altered diurnal rhythms in salivary cortisol and dehydroepiandrosterone (DHEA) at presentation. *Psychological Medicine, 26,* 245-256.

Goodyer, I.M., Herbert, J., Tamplin, A., & Altham, P.M.E. (2000). First episode major depression in adolescents: Affective, cognitive and endocrine characteristics of risk status and predictors of onset. *British Journal of Psychiatry, 176,* 142-149.

Goodyer, I.M., Herbert, J., Tamplin, A., & Altham, P.M.E. (2001). Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high-risk adolescents. *British Journal of Psychiatry, 177,* 499-504.

Goodyer, I.M., Park, R.J., & Herbert, J. (2001). Psychosocial and endocrine features of chronic first episode major depression in 8-16 year olds. *Biological Psychiatry, 50,* 351-357.

Goodyer, I. M., Park, R. J., Netherton, C. M., & Herbert, J. (2001). Possible role of cortisol and dehydropepiandrosterone in human development and psychopathology. *British Journal of Psychiatry, 179,* 243-249.

Gotlib, I., Joormann, J., Minor, K., & Cooney, R. (2006). Cognitive and Biological Functioning in Children at Risk for Depression. In T. Canli (Ed.). *Biology of personality and individual differences* (pp. 353-382). New York, NY, US: Guilford Press.

Gray, JA. (1991). The neuropsychology of temperament. In J. Strelau & A. Angleitner (Eds.), *Explorations in temperament: international perspectives on theory and measurement* (pp. 105-28). London: Plenum.

Gunnar, M.R. (1994). Psychoendocrine studies of temperament and stress in early childhood: Expanding current models. In J.E. Bates & T. Wachs (Eds.), *Temperament: Individual differences at the interface of biology and behavior* (pp. 175-198). New York: American Psychiatric Press.

Gunnar, M. R. (1998). Quality of early care and buffering of neuroendocrine stress reactions: Potential effects of the developing human brain. *Preventive Medicine, 27,* 208-211.

Gunnar, M. (2000). Early adversity and the development of stress reactivity and regulation. In C. A. Nelson (Ed.), The *Effects of Adversity on Neurobehavioral Development. The Minnesota Symposia on Child Psychology,* (Vol. 31, pp. 163-200). Mahwaah, NJ: Lawrence Erkbaum.

Gunnar, M.R., Brodersen, L., Nachmias, M., Buss, K., & Rigatuso, R., (1996). Stress reactivity and attachment security. *Developmental Psychobiology, 29,* 10–36.

Gunnar, M., R., & Donzella, B. (2002). Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology, 27,*199-220.

Gunnar, M.R., Larson, M., Hertsgaard, L., Harris, M., & Brodersen, L. (1992). The stressfulness of separation among 9-month-old infants: effects of social context variables and infant temperament. *Child Development, 63,* 290–303.

Gunnar, M.R., Morison, S.J., Chisholm, K., & Schuder, M. (2001). Salivary cortisol levels in children adopted from Romanian orphanages. *Developmental Psychopathology, 13,* 611-628.

Gunnar, M. R. & Talge, N. M. (2005). Neuroendocrine measures in developmental research. In L. A. Schmidt & S. J. Segalowitz (Eds.) *Developmental Psychophysiology*, Cambridge University Press.

Gunnar, M.R., Tout, K., deHaan, M., Pierce, S., & Stansbury, K., (1997). Temperament, social competence, and adrenocortical activity in preschoolers. *Developmental Psychobiology, 31,* 65–85.

Granger, D. A., Serbin, L. A., Schwartzman, A., Lehoux, P., Cooperman, J., & Ikeda, S. (1998). Children's salivary cortisol, internalising behavior problems, and family environment: Results from the Concordia Longitudinal Risk Project. *International Journal of Behavioral Development, 22,* 707–728.

Granger, D.A., Weisz, J.R., McCracken, J.D., Ideda, S.C., & Douglas, P. (1996). Reciprocal influences among adrenocortical activation, psychosocial processes, and the behavior adjustment of clinic-referred children. *Child Development, 67,* 3250-3262.

Greden, F., Gardner, R., King, D., Grunhaus, L., Carroll, J., Kronfol, Z. (1983). Dexamethasone suppression test in antidepressant treatment of melancholia. *Archives of General Psychiatry, 40,* 493-500.

Halbreich, U., Asnis, G. M., Shindledecker, R., Zumoff, B., & Nathan, R. S. (1985). Cortisolsecretion in endogenous depression. II. Time-related functions. *Archives of General Psychiatry,* 42, 909 –914.

Halligan, S.L., Herbert, J., Goodyer, I.M., & Murray, L. (2004). Exposure to postnatal depression predicts cortisol in adolescent offspring. *Biological Psychiatry, 55,* 376-381.

Hamburg , S.R. (1998). Inherited hypohedonia leads to learned helplessness: A conjecture updated. *Review of General Psychology, 2,* 384-403.

Hane, A. A., & Fox, N. A. (2006). Ordinary variations in maternal caregiving of human infants influence stress reactivity. *Psychological Science, 17,* 550-556.

Harmon-Jones, E., & Allen, J.J.B. (1998). Anger and frontal brain activity: EEG asymmetry consistent with approach motivation despite negative affective valence. *Journal of Personality and Social Psychology, 74*, 1310-1316.

Harris, T.O., Borsanyi, S., Messari, K., Stanford, K., Cleary, S.E., Shiers, H.M., et al. (2000). Morning cortisol as a risk factor for subsequent major depressive disorder in adult women. *British Journal of Psychiatry, 180,* 24-28.

Hart, J., Gunnar, M.G., & Cicchetti, D. (1996). Altered neuroendocrine activity in maltreated children related to symptoms of depression. *Development and Psychopathology, 8,* 201-214.

Hayden, E.P., Durbin, C.E., & Klein, D.N. (2006). Positive emotionality and cognitive risk for depression. *Development and Psychopathology, 18,* 409-234.

Heim, C., & Nemeroff, C. B. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biological Psychiatry, 49,* 1023-1039.

Hein, C., Newport, D. J., Bosnall, R., Miller, A. H., & Nemeroff, C. B.(2001). Altered pituitary-adrenal axis responses to provocative challenge tests in adult survivors of childhood abuse. *American Journal of Psychiatry, 158,* 575-581.

Heim, C., Newport, D. J., Heit, S., Graham, Y. P., Wilcox, M., Bosnall, R., et al. (2000). Pituitary-adrenal and autonomic responses to stress in women after sexual and psychical abuse in childhood. *Journal of the American Medical Association, 284,* 592-597.

Heim, C., Ehlert, U., & Hellhammer, D. H. (2000). The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychoneuroendocrinology, 25*, 1-35.

Hessl, D., Dawson, G., Frey, K., Panagiotides, H., Self, H., Yamada, E., & Osterling, J. (1998). A longitudinal study of children of depressed mothers: Psychobiological findings related to stress. In D. M. Hann, L. C. Huffman, K. K. Lederhendler, & D. Minecke, (Eds.) *Advancing Research on Developmental Plasticity: Integrating*

the Behavioral Sciences and the Neurosciences of Mental Health. Bethesda, MD: National Institutes of Mental Health, p 256.

Holsboer, F. (2000). The corticosteroid receptor hypothesis of depression. *Neuropsychopharacology, 23,* 477-501.

Holsboer, F., & Barden, N. (1996). Andtidepressants and HPA regulation. *Endocrinology Review, 17*, 187-203.

Holsboer, F., Liebl, R. & Hofschuster, E. (1982). Repeated dexamethasone suppression test during depressive illness. Normalization of test result compared with clinical improvement. *Journal of Affective Disorders, 4*, 93-101.

Holsboer, F., Vonbardeleben, U., Girken, A., Stalla, G. K., & Muller, O. A. (1984). Blunted corticotropin and normal cortisol response to human corticotropinreleasing factor in depression. *New England Journal of Medicine, 311*, 1127.

Hubert, W., & de Jong-Meyer, R. (1991). Autonomic, neuroendocrine, and subjective responses to emotion-inducing film stimuli. *International Journal of Psychophysiology, 11*, 131-140.

Johnson, E.O., Kamilaris, T. C., Chrousos, G. P., & Gold, P. W. (1992). Mechanisms of stress: A dynamic overview of hormonal and behavioral homeostasis. *Neuroscience and Biobehavioral Reviews, 16,* 115-130.

Joiner, T. E., Catanzaro, S. J., & Laurent, J. (1996). Tripartite structure of positive and negative affect, depression, and anxiety in child and adolescent psychiatric inpatients. *Journal of Abnormal Psychology, 105*, 401-409.

Kagan, J. (1997). Temperament and the reactions to unfamiliarity. *Child Development, 68*, 139-143.

Kagan, J. (1998). Biology and the child. In N. Eisenberg (Ed.), *Handbook of child psychology: Vol.* 3: *Social, emotional and personality development* (5th ed., pp. 177-235). New York: John Wiley.

Kagan, J., Reznick, J.S., & Snidman, N. (1987). The physiology and psychology of behavioral inhibition in children. *Child Development, 58,* 1459-1473.

Kaufman , J., Birahmer, B., Perel, J., Dahl, R.E., Moreci, P., Nelson, B., et al. (1997). The corticotrophin-releasing hormone challenge in depressed abused, depressed nonabused, and normal control children. *Biological Psychiatry, 42*, 669-679.

Kendler, K. (1997). The diagnostic validity of melancholic major depression in a population-based sample of female twins. *Archives of General Psychiatry, 54,* 299-304.

Kendler, S., Gardner, C. O., & Prescott, C. A. (2002). Toward a comprehensive developmental model for major depression in women. *American Journal of Psychiatry, 159,* 1133-1145.

Kendler, K. S., Kuhn, J. W., & Prescott, C. A. (2004). Childhood sexual abuse, stressful life events and risk for major depression in women. *Psychological Medicine, 34,* 1475-1482.

Kendler, K. S., Thornton, L. M., & Gardner, C. O. (2001). Genetic risk, number of previous depressive episodes, and stressful life events in predicting onset of major depression. *American Journal of Psychiatry, 158,* 582-586.

Kessler, R. C., Berglund, P., & Demler, O. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry, 62,* 593-602.

Kirschbaum, C., Wust, S., Faig, H. G., & Hellhammer, D. H. (1992). Heritability of cortisol responses to human corticotropinreleasing hormone, ergometry, and psychological stress in humans. *Journal of Clinical Endocrinology & Metabolism, 75,* 1526 -1530.

Klein, D. F. (1987). Depression and anhedonia. In D.C. Clark & J. Fawcett (Eds.), *Anhedonia and affect deficit states* (pp. 1-14). New York: PMA Publishing.

Kochanska, G. (1991). Patterns of inhibition to the unfamiliar in children of normal and affectively ill mothers. *Child Development,* 62, 250-263.

Kunz-Ebrecht, S. R., Kirschbaum, C., Marmot, M., & Steptoe, A. (2004). Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. *Psychoneuroendocrinology, 24,* 516-528.

Kupfer, D. J. (1991). Biological markers of depression. In *The Diagnosis of Depression,* J. F. Feighner, & W. F. Boyer, (Eds). John Wiley: Chichester, 79-98.

Kupper, N., de Geus, E. J., van Berg, M., Kirschbaum, C., Boomsma, D. I., Willemsen, G., (2005). Familial influences on basal salivary cortisol in an adult population. *Psychoneuroendocrinology, 30,* 857–868.

Kutcher, S., Malkin, D., Silverberg, J., Marton, P., Williamson, P. Malkin, A. et al. (1991). Nocturnal cortisol, thyroid stimulating hormone, and growth hormone secretory profiles in depressed adolescents. *Journal of the American Academy of Child and Adolescent Psychiatry, 30,* 407-414.

Kraepelin, E. (1921). *Manic depressive insanity and paranoia.* Edinburgh: E. & S. Livingstone.

Krueger, R. F., Caspi, A., Moffitt, T. E., Silva, P. A., & McGee, R. (1996). Personality traits are differentially linked to mental disorders: A multitrait-multidiagnosis study of an adolescent birth cohort. *Journal of Abnormal Psychology, 105,* 299-312.

Laptook, R., Klein, D. N., Durbin, C. E., Hayden, E. P., Olino, T. M., & Carlson, G. (2007). Differentiation Between Low Positive Affectivity and Behavioral Inhibition in Preschool-Age Children: A Comparison of Behavioral Approach in Novel and Non-Novel Contexts. *Personality and Individual Differences, 44,* 756-765.

Lengua, L. J., Wolchick, S. A., Sandler, I. N., & West, S. G. (2000). The additive and interactive effects of parenting and temperament in predicting adjustment problems of children of divorce. *Journal of Clinical Child Psychology, 29, 232-244.*

Levitan, R. D., Vaccarino, F. J., Brown, G. M., & Kennedy, S. H. (2002). Low-dose dexamethoasone challenge in women with atypical major depression: pilot study. *Journal of Psychiatry & Neuroscience, 27,* 47-51.

Lewis, M., & Ramsay, D. (2005). Infant emotional and cortisol responses to goal blockage. *Child Development, 76,* 518-530.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A. et al. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stresses. *Science, 277,* 1659–1662.

Lonigan, C. J., Carey, M. P. & Finch, A. J. (1994). Anxiety and depression in children and adolescents: Negative affectivity and the utility of self-reports. Journal of Consulting and Clinical Psychology, 62, 1000-1008.

Lonigan, C. J., Hooe, E. S., David, C. F. & Kistner, J. A. (1999). Positive and negative affectivity in children: Confirmatory factor analysis of a two-factor model and its relation to symptoms of anxiety and depression. Journal of Consulting and Clinical Psychology, 67, 374-386.

Lonigan, C. J., Philips, B. M., Hooe, E. S., & Eric, S. (2003). *Relations of positive and negative affectivity to anxiety and depression in children : Evidence from a latent variable longitudinal study. Journal of Consulting and Clinical Psychology, 71, 465-481.*

Luby, J.L., Heffelfinger, A., Mrakostsky, C., Brown, C., Hessler, M., & Spitznagel, E. (2003). Alterations in stress cortisol reactivity in depressed preschoolers relative to psychiatric and no-disorder comparison groups. *Archives of General*

Psychiatry, 60, 1248-1255.

Lupien, S. J., King, S., Meaney, M.J., & McEwen, B.S. (2000). Child's stress hormone levels correlate with mother's socioeconomic status and depressive state. *Biological Psychiatry, 48*, 976-980.

Mannie, Z. N., Harmer, C. J., & Cowen, P. J. (2007). Increased waking salivary cortisol levels in young people at familial risk of depression. *American Journal of Psychiatry, 164*, 617-621.

Mazure, C. M. (1998). Life stressors as risk factors in depression. *Clinical Psychology: Science and Practice, 5*, 291-313.

McEwen, B. S. (1998). Stress, adaptation, and disease: Allostasis and allostatic load. *Annals of the New York Acadamy of Sciences, 840,* 33-44.

Meaney, M. J., & Szyf, M. (2005). Maternal care as a model for experience for experience-dependent chromatin plasticity? *Trends in Neurosciences, 28,* 456-463.

Meehl, P.E. (1975). Hedonic capacity: Some conjectures. *Bulletin of the Menninger Clinic,* 39, 295- 306.

Meyer S. E., Chrousos G. P., Gold P. W. (2001). Major depression and the stress system: A lifespan perspective. *Development and Psychopathology 13,* 563-578.

Miller, G. E., Chen, E., & Zhou, E. S. (2007). If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenal axis in human. *Psychological Bulletin, 133,* 25.45.

Moskowitz, J. T., & Epel, E. S. (2006). Benefit finding and diurnal cortisol slope in maternal caregivers: A moderating role for positive emotion. *The Journal of Positive Psychology, 1,* 83-91.

Nachmias, M., Gunnar, M., Mangelsdorf, S., Parritz, R. H., & Buss, K. (1996). Behavioral inhibition and stress reactivity: the moderating role of attachment security. *Child Development, 67*, 508-522.

Nelson, J.C., & Davis, J.M. (1997). DST studies in psychotic depression: A meta-analysis. *American Journal of Psychiatry, 154*, 1497-1503.

Nemeroff, C. B. (1996). The corticotrophin-releasing factor (CRF) hypothesis of depression: New findings and new directions. *Molecular Psychiatry, 1*, 336-342.

NICHD Early Child Care Research Network (1999). Chronicity of maternal depressive symptoms, maternal sensitivity, and child functioning at 36 months. *Developmental Psychology, 35*, 1297-1310.

Olino, T.M. (2007). Associations Between Parental Psychopathology and Personality and Offspring Temperament: Implications for the Conceptualization of Temperament. Unpublished doctoral dissertation, Stony Brook University.

Peeters, F., Nicolson, N.A., & Berkhof, J. (2003). Levels and variability of daily life cortisol secretion in major depression. *Psychiatry Research, 126*, 1-13.

Pfeifer, M., Goldsmith, H. H., Davidson, R. J., & Rickman, M. D. (2002). Continuity and change in inhibited and uninhibited children. *Child Development*, 73, 1474-1485.

Pfohl, B., Sherman, B., Schlechte, J., & Stone, R. (1985). Pituitary–adrenal axis rhythm disturbances in psychiatric depression. *Archives of General Psychiatry, 42,* 897–903.

Polk, D. E., Cohen, S., Doyle, W. J., Skoner, D. P., & Kirschbaum, C. (2005). State and trait affect as predictors of salivary cortisol in healthy adults. *Psychoneuroendocrinology, 30,* 261-272.

Posener, J.A., DeBattista, C., Williams, G. H., Kraemer, H.C., Kalehzan, B.M., & Schatzberg, A.F. & (2000). Twenty-four hour monitoring of cortisol and Corticotrophin secretion in psychotic and nonpsychotic major depression. *Archives of General Psychiatry, 57*, 755-760.

Pressman, S. D., & Cohen, S. (2005). Does positive affect influence health? *Psychological Bulletin, 131*, 925-971.

Pruessner, J. C., Hellhammer, D. H., & Kirschbaum. C (1999). Burnout, perceived stress, and cortisol responses to awakening. *Psychosomatic Medicine, 61,*197–204.

Puig-Antich, J., Dahl, R.E., Ryan, N.D., Novacenko, H., Goetz, D., Twomey, J., & Klepper, T. (1989). Cortisol secretion in prepubertal children with major depressive disorder: Episode and recovery. *Archives of General Psychiatry, 46,* 801-809.

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

Roberts, B. W., & DelVecchio, W. F. (2000). The rank-order consistency of personality traits from childhood to old age: A quantitative review of longitudinal studies. *Psychological Bulletin,* 126, 3-25.

Ronsaville, D. S., Municchi, G., Laney, C., Cizza, G., Meyer, S. E., Haim, A., Radke-Yarrow, M., chrousos, G., Gold, P. W., & Martinez, P. E. (2006). Maternal and environmental factors influence the hypothalamic-pituitary-adrenal axis response

to corticotrophin-releasing hormone infusion in offspring of mothers with and without mood disorders. *Development and Psychopathology, 18,* 173-194.

Rothbart, M. K., & Bates, J. E. (2006). Temperament. In N. Eisenberg, W. Damon, & R. M. Lerner (Eds.), *Handbook of child psychology Vol 3: Social, emotional, and personality development (*6th ed., pp. 99-166). Hoboken, NJ: John Wiley & Sons Inc.

Rubin, K., Burgess, K., & Hastings, P. (2002). Stability and social-behavioral consequences of toddlers' inhibited temperament and parenting behaviors. *Child Development, 73,* 483-495.

Rush, A. J., Giles, D. E., Schlesser, M. A., Orsulak, P. J. Parker, C. R., Weissenburger, J. E., et al. (1996). The dexamethasone suppression test in patients with mood disorders. *Journal of Clinical Psychiatry, 57,* 470-484.

Rush, A. J., & Weissenburger, J. E. (1994). Melancholic symptom features and DSM-IV. *American Journal of Psychiatry, 151,* 489-498.

Seligman, M. E. P., Steen, T., Park, N., & Peterson, C. (2005). Positive psychology progress: Empirical validation of interventions. *American Psychologist, 60,* 410-421.

Sheldon, K. M., & Lyubomirsky, S. (2006). How to increase and sustain positive emotion: The effects of expressing gratitude and visualizing best possible selves. *The Journal of Positive Psychology, 1,* 73-82.

Schmidt, L.A., Fox, N.A., Rubin, K.H., Sternberg, E.M., Gold, P.W., Smith, C.C., & Schulkin, J. (1997). Behavioral and neuroendocrine responses in shy children. *Developmental Psychobiology, 30,* 127-140.

Schmidt, L.A., Fox, N.A., Schulkin, J., & Gold, P.W. (1999). Behavioral and psychophysiological correlates of self-presentation in temperamentally shy children. *Developmental Psychobiology, 35,* 119-135.

Schreiber, J. E., Shirtcliff, E., Van Hulle, C., Lemery-Chalfant, K., Klein, M. H., Kalin, N. H., Essex, M. J., & Goldsmith, H. H. (2006). Environmental influences on family similarity in afternoon cortisol levels: Twin and parent offspring designs. *Psychoneuronendocrinology, 31,* 1131-1137.

Schwartz, E. B. Granger, D. A., Susman, E. J., Gunnar, M. R., & Laird, B. (1998). Assessing salivary cortisol in studies of child development. *Child Development, 69,* 1503-1513.

Shankman, S. A., Tenke, C., Bruder, G. E., Durbin, C. E., Hayden, E. P., & Klein, D. N. *(*2005*).* Low positive emotionality as a risk marker for depression in young

children: Association with EEG asymmetry. *Development and Psychopathology, 17,* 85-98.

Shannon, K. E., Beauchaine, T. P., Brenner, S. L., Neuhaus, E., & Gatzke-Kopp, L. (2007). Familial and temperamental predictors of resilience in children at risk for conduct disorder and depression. *Development and Psychopathology, 19,* 701-727.

Sheeber, L. B., Davis, B., Leve, C., Hops, H., & Tildesley, E. (2007). Adolescents' relationships with their mothers and fathers: Associations with depressive disorder and subdiagnostic symptomatology. *Journal of Abnormal Psychology, 116*, 144-154.

Shiner, R., & Caspi, A. (2003). Personality differences in childhood and adolescence: Measurement, developments, and consequences. *Journal of Child Psychology and Psychiatry, 44*, 2-32.

Silberg, J., & Rutter, M. (2002). Nature-nurture interplay in the risks associated with parental depression. In S. H. Goodman and I. H. Gotlib (Eds.), *Children of depressed parents: Mechanisms for risk and implications for treatment* (pp. 13-36). Washington, DC: American Psychological Association.

Singer, J.D., & Willett, J.B. (2003). *Applied longitudinal data analysis: Modeling change and event occurrence.* New York, NY: Oxford University Press.

Smeekens, S., Riksen-Walraven, M., & van Bakel, H. J. A. (2007). Cortisol reactions in five-year-olds to parent-child interaction: The moderating role of ego-resiliency. *Journal of Child Psychology and Psychiatry, 48,* 649-656.

Smider, N.A., Essex, M.J., Kalin, N.H., Buss, K.A., Klein, M.H., Davidson, R.J., & Goldsmith, H.H. (2002). Salivary cortisol as a predictor of socioemotional adjustment during kindergarten: A prospective study. *Child Development, 73*, 75-92.

Spangler, G., Schieche, I. U., Maier, U., & Ackerman, C. (1994). Maternal sensitivity as an external organizer for biobehavioral regulation in infancy. *Developmental Psychobiology, 27,* 425-437.

Spanier, G. S. (1976). Measuring dyadic adjustment: New scales for assessing the quality of marriage and similar dyads. *Journal of Marriage and the Family,* 38, 15-28.

Stewart, J. W., Quitkin, F. M., McGrath, P. J., & Klein, D. F. (2005). Defining the boundaries of atypical depression: Evidence from the HPA axis supports course of illness distinctions. *Journal of Affective Disorders, 86*, 161-167.

Strickland, P.L., Deakin, J.F., Percival, C., Dixon, J., Gater, R.A., Goldberg, D.P. (2002). Bio-social origins of depression in the community: Interactions between social adversity, cortisol, and serotonin neurotransmission. *British Journal of Psychiatry, 180,* 168-173.

Talge, N. M., Bruce, J., Donzella, B., & Gunnar, M. R. (2003). *Stability of physiological measures in young children.* Poster presented at the International Society for Developmental Psychobiology, New Orleans, LA.

Talge, N. M., Donzella, B., Kryzer, E. M., Gierens, A., & Gunnar, M. R. (2005). It's not that bad: Error introduced by oral stimulants in salivary cortisol research. Developmental Psychobiology, 47, 369-376.

Thase, M.E., & Howland, R.H. (1995). Biological processes in depression: An updated review and integration. In Beckham, E.E., Leber, W.R. (Eds.), *Handbook of Depression*. Guilford Press, New York, pp. 213-279.

Trull, T. J., & Sher, K. J. (1994). Relationship between the five-factor model of personality and Axis I disorders in a nonclinical sample. *Journal of Abnormal Psychology, 103,* 350-360.

Tugade, M. M., & Fredrickson, B. L. (2004). Resilient individuals use positive emotions to bounce back from negative emotional experiences. *Journal of Personality and Social Psychology, 86,* 320-333.

Turkcapar, M.H., Akdemir, A., Orsel, S.D., Demirergi, N., Sirin, A., Kilic, E.Z., & Ozbay, M.H. (1999). The validity of diagnosis of melancholic depression according to different diagnostic systems. *Journal of Affective Disorders, 54,* 101-107.

van Bakel, H. J. A., & Riksen-Walraven, J. M. A. (2002). Stress reactivity in 15-month-old infants: Links with infant temperament, cognitive competence, and attachment security. *Developmental Psychobiology, 44,* 157-167.

van Os, J., Jones, P., Lewis, G., Wadsworth, M., & Murray, R. (1997). Developmental precursors of affective illness in a general population birth cohort. *Archives of General Psychiatry,* 54, 625-631.

Vythilingam, M., Vermetten, E., Anderson, G.M., Luckenbaugh, D., Anderson, E., Snow, J., et al. (2004). Hippocampal volume, memory, and cortisol status in major depressive disorder: Effects of treatment. *Biological Psychiatry,* 56, 101-112**.**

Watson, S., Gallagher, P., Del-Estal, D., Hearn, A., Ferrier, I.N., Young, A.H. (2002). Hypothalamic-pituitary –adrenal axis function in patients with chronic depression. Psychological Medicine, 32, 1021-1028.

Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, I. R., et al. (2004). Epigenetic programming by maternal behavior. *Nat Neuroscience, 7,* 847-854.

Weber, B., Lewicki, S., Deuschle, M., Colla, M., Vecsei, P., & Heuser, I. (2000). Increased diurnal plasma concentrations of cortisone in depressed patients. *Journal of Clinical Endocrinology and Metabolism, 85*, 1133-1136.

Weinfield, N. S., Egeland, B., & Ogawa, J. R. (1998). Affective quality of mother-child interaction. In M. Zaslow & C. Eldred (Eds.), *Parenting behavior in a sample of young mothers in poverty: Results of the New Chance Observational Study* (pp. 71-113). New York: Manpower Demonstration Research Corporation.

Winokur, G. (1997). All roads lead to depression: clinically homogeneous, etiologically heterogeneous. *Journal of Affective Disorders, 45*, 97-108.

Wong, M., Kling, M. A., Munson, P. J., Listwak, S., Licinio, J., Prolo, P., Karp, B., et al. (2000). Pronounced and sustained central hypernoradrenergic function in major depression with melancholic features: Relation to hypercortisolism and corticotrophin-releasing hormone. *Proceedings National Academy of Sciences, 97*, 325-330.

Wust, S., Federenko, I., Hellhammer, D. H., & Kirschbaum, C. (2000). Genetic factors, perceived chronic stress, and the free cortisol response to awakening. *Psychoneuroendocrinology, 25*,707–720.

Yehuda, R., Bierer, L. M., Schmeidler, J., Aferiat, D. H., Breslau, I., & Dolan, S. (2000). Low cortisol and risk for PTSD in adult offspring of Holocaust survivors. *American Journal of Psychiatry, 157*, 1252–1259.

Yehuda, R., Southwick, S. M., Krystal, J. H., Bremner, D., Charney, D. S., & Mason, J. W. (1993). Enhanced suppression of cortisol following dexamethasone administration in posttraumatic stress disorder. *American Journal of Psychiatry, 150*, 83–86.

Yehuda, R., Teicher, M.H., Trestman, R.L., Levengood, R.A., Siever, L.J. (1996). Cortisol regulation in posttraumatic stress disorder and major depression: A chronobiological analysis. *Biological Psychiatry, 40*, 79-88.

Young, E. A., Carlson, N. E., & Brown, M. B. (2002). 24 hour ACTH and cortisol pulsatility in depressed women. *Neuropsychopharmacology, 25*, 267-276.

Young, E. H., Gallagher, P. & Porter, R. J. (2002). Elevation of the cortisol-dehydroepiandrosterone ratio in drug-free depressed patiens. *American Journal of Psychiatry, 159*, 1237-1239.

Young, E. A., Vazquez, D., Jiang, H., & Pfeffer, C. R. (2006). Saliva cortisol and response to dexamethasone in children of depressed parents. *Biological Psychiatry, 60,* 831-836.

Zimmerman, M., Sheeran, T., & Young, D. (2004) The Diagnostic Inventory for Depression: A self-report scale to diagnose DSM-IV for major depressive disorder. J Clin Psychol. 60:87–110.

Footnotes

1. In order to probe the interaction between child NE and maternal melancholic depression, we examined the relation between NE and baseline cortisol levels in the three groups of maternal lifetime history of depression: mothers with a history of maternal melancholic depression, mothers with a history of non-melancholic depression, and mothers with no history of depression. NE was margninally associated with a lower baseline levels of cortisol in mothers with a lifetime history of maternal melancholic depression (unstandardized coefficient = -.581, *SE* = .312, *t* = -1.858, *df* = 11, *p* = .090), but was not significantly associated with baseline cortisol levels in mothers with a lifetime history of non-melancholic depression or mothers with no lifetime history of depression.
2. We combined the morning cortisol levels of individuals with non-melancholic MDD (M = .53, SD = .19), DY (M = .58, SD = .19) and Dep-NOS (M = .58, SD = .10) as all three groups exhibited similar cotisol values.

Table 1

Correlations among all major variables

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Lab Mean Cortisol	--														
2. Home AM Cortisol	.21†	--													
3. Home PM Cortisol	.23*	.07	--												
4. Positive Emotionality	-.07	-.25*	.12	--											
5. Negative Emotionality	.10	-.03	-.04	.14†	--										
6. Behavioral Inhibition	.24**	-.02	.02	-.09	.34**	--									
7. M. MDD or DD	-.01	.13	.10	-.13	.11	-.04	--								
8. M. Melancholic Depression	.02	.23*	-.01	-.18*	.07	-.02	.76**	--							
9. P. Supportiveness	-.18*	-.02	.13	.11	-.08	-.05	-.11	-.04	--						
10. P. Hostility	.27**	-.03	-.04	-.15†	-.07	.01	.09	.05	-.55**	--					
11. DAS	-.22*	-.19	.05	.33**	.08	-.11	-.25*	-.26**	.19†	-.34**	--				
12. Life Stress (Lab)	-.15†	--	--	-.03	.02	-.05	.00	.03	.01	.00	-.07	--			
13. Life Stress (Home)	--	.15	-.18†	.00	.02	-.01	-.09	-.03	.09	-.01	-.05	.92**	--		
14. SES	.03	-.02	.03	-.10	.04	.18*	.04	.16†	.06	-.01	.14	.06	.02	--	
15. Gender	-.14†	-.07	-.11	-.09	.06	.14†	.02	.01	.02	-.05	-.03	.17*	.15†	-.05	--
Mean	4.41	9.36	2.24	.12	-.10	-.03	--	--	4.49	1.17	111.8	.24	.22	--	--
SD	4.50	6.04	7.94	.66	.43	.30	--	--	.50	.27	18.96	.56	.52	--	--
Proportion	--	--	--	--	--	--	47/156	14/56/86							83/83

Table 1 continued.
$^{\dagger}p < .10$, $*p < .05$, $**p < .01$; Cortisol levels are measured in nanomoles per liter (nmol/L); M. MDD or DD = Maternal Lifetime Major Depressive Disorder or Dysthymic Disorder; M. Melancholic Depression = Maternal Lifetime Melancholic Depression; P. Supportiveness = PCI Parental Supportive Presence; P. Hostility = PCI Parental Hostility; DAS = Dyadic Adjustment Scale; SES is based on parental education; Gender: Male = 0 and Female = 1

Table 2

HLM exploratory univariate analyses: Children's laboratory cortisol reactivity predicted from children's temperament, maternal psychopathology, marital discord, SES and life stress

	Cortisol Intercept Coefficient (SE)	*t*-value	Cortisol Slope Coefficient (SE)	*t*-value	Cortisol Curvature Coefficient (SE)	*t*-value
Baseline Trajectory Model[a]	.465 (.024)	19.767***	-.085 (.016)	-5.184***	.030 (.004)	8.174***
Control Variables						
Time of visit[b]	-.111 (.047)	-2.383*	.068 (.039)	1.747†	-.016 (.008)	-2.009*
Food intake[c]	-.010 (.099)	-.099	-.135 (.083)	-1.610	.031 (.020)	1.590
Gender[b]	-.031 (.046)	-.680	-.001 (.034)	-.030	-.004 (.007)	-.481
Race[h]	-.006 (.096)	-.065	-.038 (.125)	-.300	.008 (.024)	.319
CBCL affective problems[d]	-.009 (.009)	-.995	-.008 (.006)	-1.167	-.002 (.002)	1.882†
Children's Temperament[b]						
Positive emotionality	-.004 (.020)	-.191	-.000 (.017)	-.008	.001 (.004)	.202
Negative emotionality	.076 (.065)	1.168	.043 (.031)	1.371	-.017 (.008)	-2.112*
Behavioral inhibition	.101 (.035)	2.912**	.010 (.022)	.455	-.005 (.005)	-.984
Maternal Psychopathology[d]						
Lifetime MDD or DD	-.032 (.053)	-.599	.007 (.037)	.193	.000 (.008)	.013
Lifetime melancholic depression	.004 (.042)	.101	.003 (.025)	.125	-.001 (.006)	-.197
Current depressive symptoms (DID)[e]	.005 (.005)	.821	.001 (.005)	.198	-.000 (.001)	-.254

Table 2 continued.

	Cortisol Intercept Coefficient (SE)	*t*-value	Cortisol Slope Coefficient (SE)	*t*-value	Cortisol Curvature Coefficient (SE)	*t*-value
MDD, DD or Dep-NOS during the child's lifetime	-.059 (.059)	-.997	.059 (.043)	1.368	-.009 (.010)	-.878
Lifetime anxiety	-.047 (.042)	-1.120	.004 (.032)	.113	-.000 (.007)	-.063
Lifetime substance	-.053 (.063)	-.844	.029 (.039)	.728	-.008 (.008)	-.871
Parenting[f]						
Parental supportiveness	-.066 (.048)	-1.378	-.020 (.048)	-.425	.005 (.010)	.479
Parental hostility	.186 (.081)	2.288*	.158 (.090)	1.748[†]	-.040 (.017)	-2.316*
Environmental Factors						
Marital discord (DAS)[g]	-.002 (.002)	-.880	-.000 (.001)	-.072	-.000 (.000)	-.023
SES[f]	-.002 (.031)	-.081	-.025 (.021)	1.210	-.008 (.005)	-1.752[†]
Life stress[b]	-.117 (.038)	-3.107**	.034 (.028)	1.217	-.006 (.006)	-1.018

[†]$p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$;
Time of visit (10 AM = 0 and 2 PM = 1) was controlled in all analyses except those examining the the control variables; Race: 0 = Non-Caucasian; 1 = Caucasian; MDD = Major Depressive Disorder; DD = Dysthymic Disorder; DID = Diagnostic Interview for Depression; Dep-NOS = Depressive Disorder – Not Otherwise Specified; Parental supportiveness = PCI parental supportive presence; Parental Hostility = PCI parental hostility; DAS = Dyadic Adjustment Scale; SES is based on parental education; Gender: Male = 0 and Female = 1. [a] N = 160, *df* = 159; [b] N = 160, *df* = 157; [c] N = 151, *df* = 149; [d] N = 151, *df* = 148; [e] N = 109, *df* = 106; [f] N = 145, *df* = 142; [g] N = 99, *df* = 96. [h] N = 142, *df* = 139.

Table 3

Interactions between child temperament and maternal melancholic depression in predicting children's laboratory cortisol reactivity

Fixed effect	Coefficient (*SE*)	*t*-value	Variance component (SD)	Chi-squared test of variance
Predicting cortisol intercept				
Intercept	.478 (.024)	19.873***	.075 (.273)	945.703***
Time of visit	-.131 (.049)	-2.670**		
Child positive emotionality	-.003 (.029)	-.106		
Child Behavioral inhibition	.088 (.033)	2.621*		
Maternal melancholic depression	.006 (.030)	.214		
PE X melancholic depression	-.006 (.041)	-.151		
BI X melancholic depression	-.028 (.034)	-.822		
Predicting cortisol slope				
Intercept	-.085 (.017)	-5.014***	.172 (.030)	430.101***
Time of visit	.058 (.039)	1.479		
Child positive emotionality	-.005 (.021)	-.235		
Child behavioral inhibition	.019 (.020)	.916		
Maternal melancholic depression	.003 (.015)	.209		
PE X melancholic depression	-.042 (.019)	-2.217*		
BI X melancholic depression	.039 (.019)	2.069*		

Table 3 continued.

Fixed effect	Coefficient (*SE*)	*t*-value	Variance component (SD)	Chi-squared test of variance
Predicting cortisol curvature				
Intercept	.030 (.004)	7.810***	.001 (.038)	416.960***
Time of visit	-.012 (.008)	-1.424		
Child positive emotionality	.002 (.005)	.440		
Child behavioral inhibition	-.006 (.005)	-1.262		
Maternal melancholic depression	-.001 (.004)	-.353		
PE X melancholic depression	.010 (.004)	2.086*		
BI X melancholic depression	-.007 (.004)	-1.587		

N = 144; for *t* tests and chi-square tests, *df* = 137.
For all analyses, SE = standard error; SD = standard deviation; Time of visit: 10 AM = 0 and 2 PM = 1; PE = Positive Emotionality; BI = Behavioral Inhibition; Melancholic Depression = maternal lifetime history of melancholic depression.
$^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$.

Table 4

Interaction between maternal lifetime history of depression and parental hostility in predicting children's laboratory cortisol reactivity

Fixed effect	Coefficient (*SE*)	*t*-value	Variance component (*SD*)	Chi-squared test of variance
Predicting cortisol intercept				
Intercept	.479 (.025)	19.521***	.077 (.277)	963.642***
Time of visit	-.125 (.055)	-2.265*		
Maternal lifetime MDD or DD	-.020 (.025)	-.782		
Parental hostility	.067 (.033)	2.015*		
Maternal lifetime MDD or DD X Parental hostility	.005 (.027)	.168		
Predicting cortisol slope				
Intercept	-.086 (.017)	-5.188***	.027 (.165)	414.255***
Time of visit	.047 (.039)	1.223		
Maternal lifetime MDD or DD	.002 (.016)	.108		
Parental hostility	.036 (.025)	1.432		
Maternal lifetime MDD or DD X Parental hostility	.048 (.021)	2.268*		
Predicting cortisol curvature				
Intercept	.030 (.004)	8.056***	.001 (.036)	395.722***
Time of visit	-.010 (.008)	-1.284		
Maternal lifetime MDD or DD	.001 (.004)	.154		
Parental hostility	-.010 (.005)	-2.008*		
Maternal lifetime MDD or DD X Parental hostility	-.010 (.004)	-2.268*		

Table 4 continued.
N = 144; for *t* tests and chi-square tests, *df* = 139.
For all analyses, *SE* = standard error; *SD* = standard deviation; Time of visit: 10 AM = 0 and 2 PM = 1; MDD = Major Depressive Disorder; DD = Dysthymic Disorder; P. Hostility = PCI parental hostility.
$^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$.

Table 5

Summary of study findings in terms of risk, resilience and potentiation as they relate to the development of neuroendocrine functioning in young children.

Risk	Resilience	Potentiation
low PE[a]	high PE X high parental hostility	low PE X high parental hostility
high NE	high BI X high parental support	low PE X maternal melancholic depression
high BI		high BI X low parental support
maternal lifetime melancholic depression[a]		high BI X maternal lifetime melancholic depression
high parental hostility		high BI X maternal lifetime MDD or DD
Life stress – 6 months prior		maternal lifetime MDD or DD X high parental hostility

[a] = variable was associated with higher morning basal cortisol levels; all other variables were associated with parts of the growth curve of children's cortisol reactivity in response to laboratory stress. Note: We acknowledge that the resilience effects can also be viewed as potentiation effects, depending on which level of the independent variable is examined; both are presented here.

Figure Captions

Figure 1. The growth curve trajectory model of children's salivary cortisol reactivity in response to laboratory stressors.

Figure 2. The unique effects of child temperamental negative emotionality and behavioral inhibition, parental hostility, and life stress on children's laboratory cortisol reactivity.

Figure 3. The interaction between maternal lifetime history of depression and children's temperamental behavioral inhibition to predict children's laboratory cortisol reactivity.

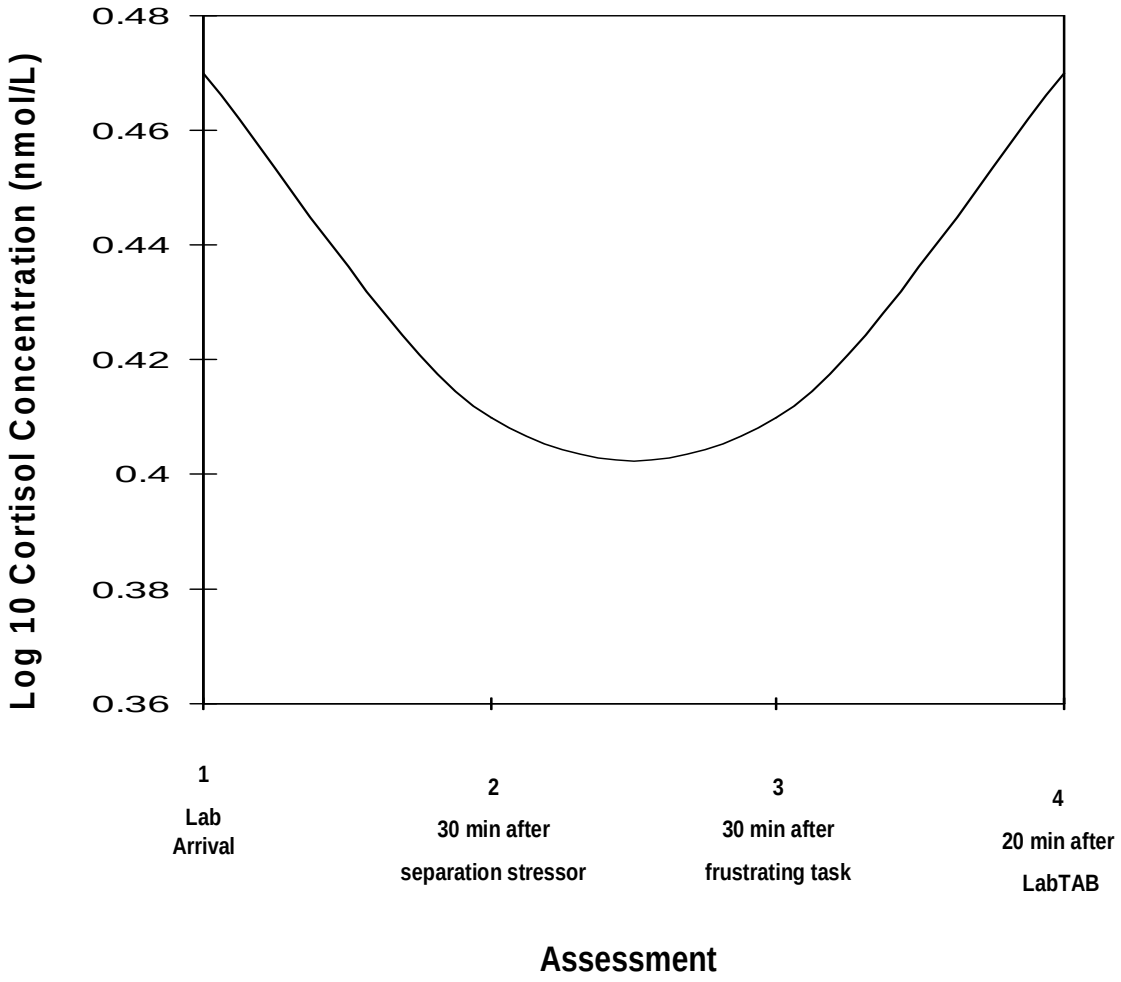
Figure 4. The interaction between maternal lifetime history of melancholic depression and child temperamental positive emotionality to predict children's laboratory cortisol reactivity.

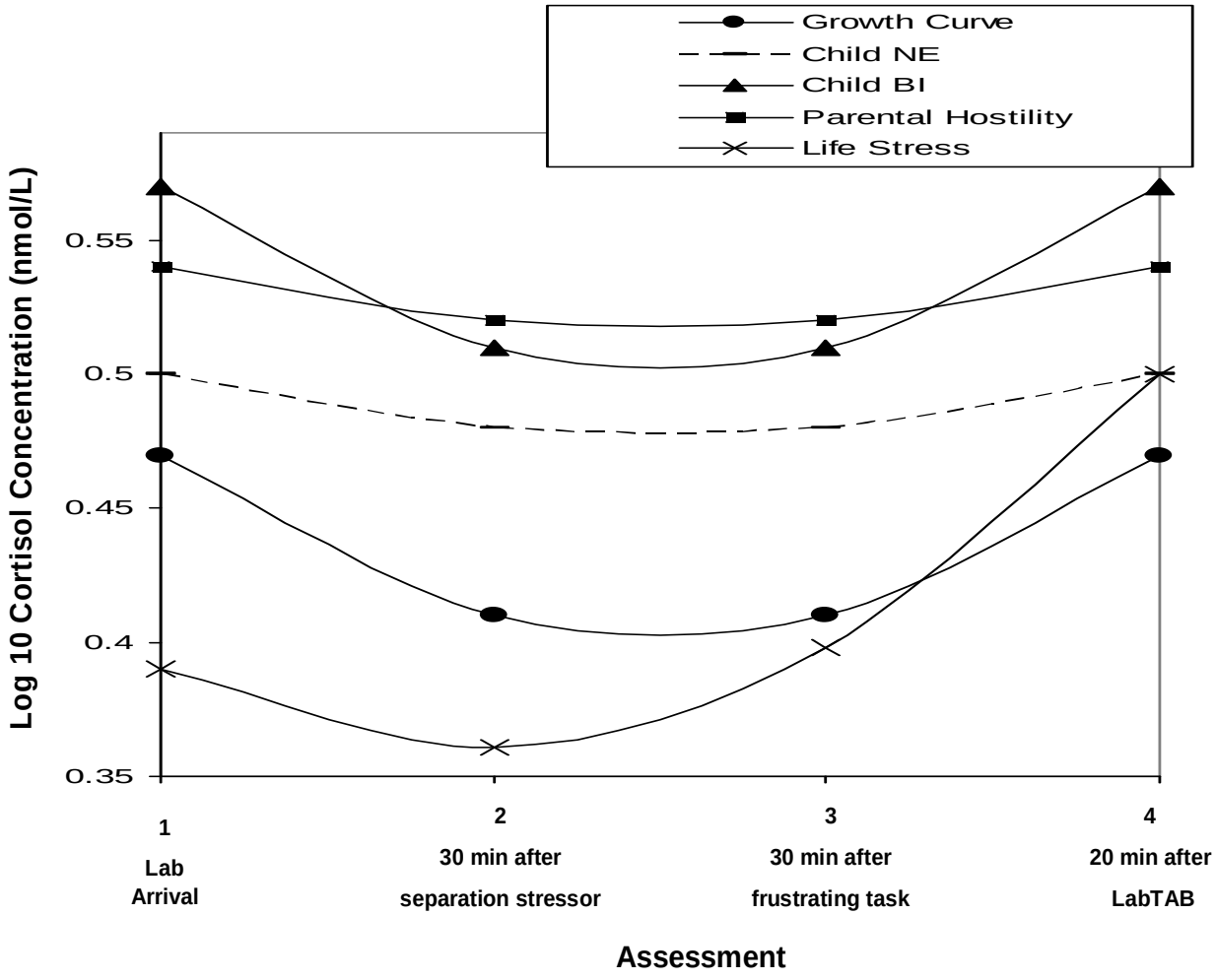
Figure 5. The interaction between maternal lifetime history of melancholic depression and child temperamental behavioral inhibition to predict children's laboratory cortisol reactivity.

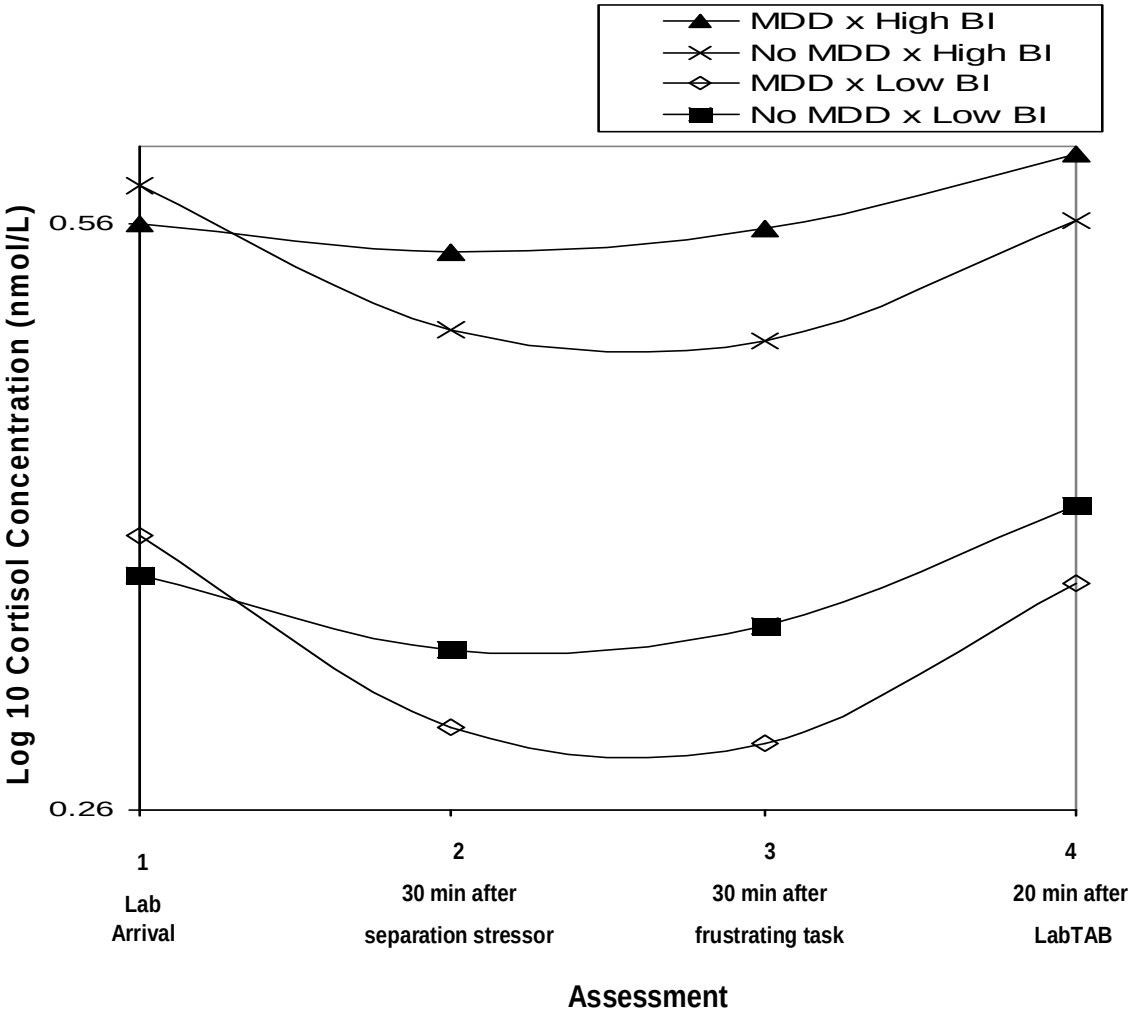
Figure 6. The interaction between child temperamental positive emotionality and parental hostility to predict children's laboratory cortisol reactivity.

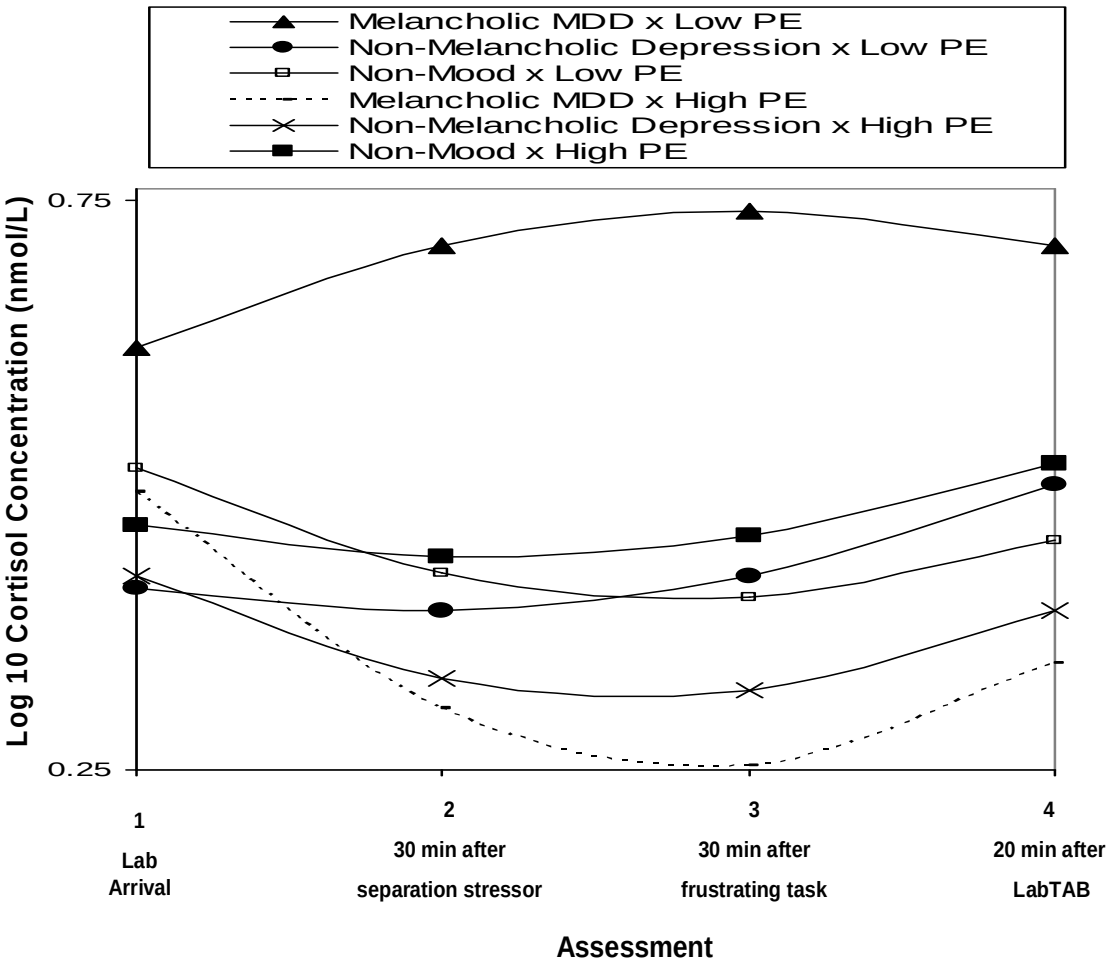
Figure 7. The interaction between child temperamental behavioral inhibition and parental support to predict children's laboratory cortisol reactivity.

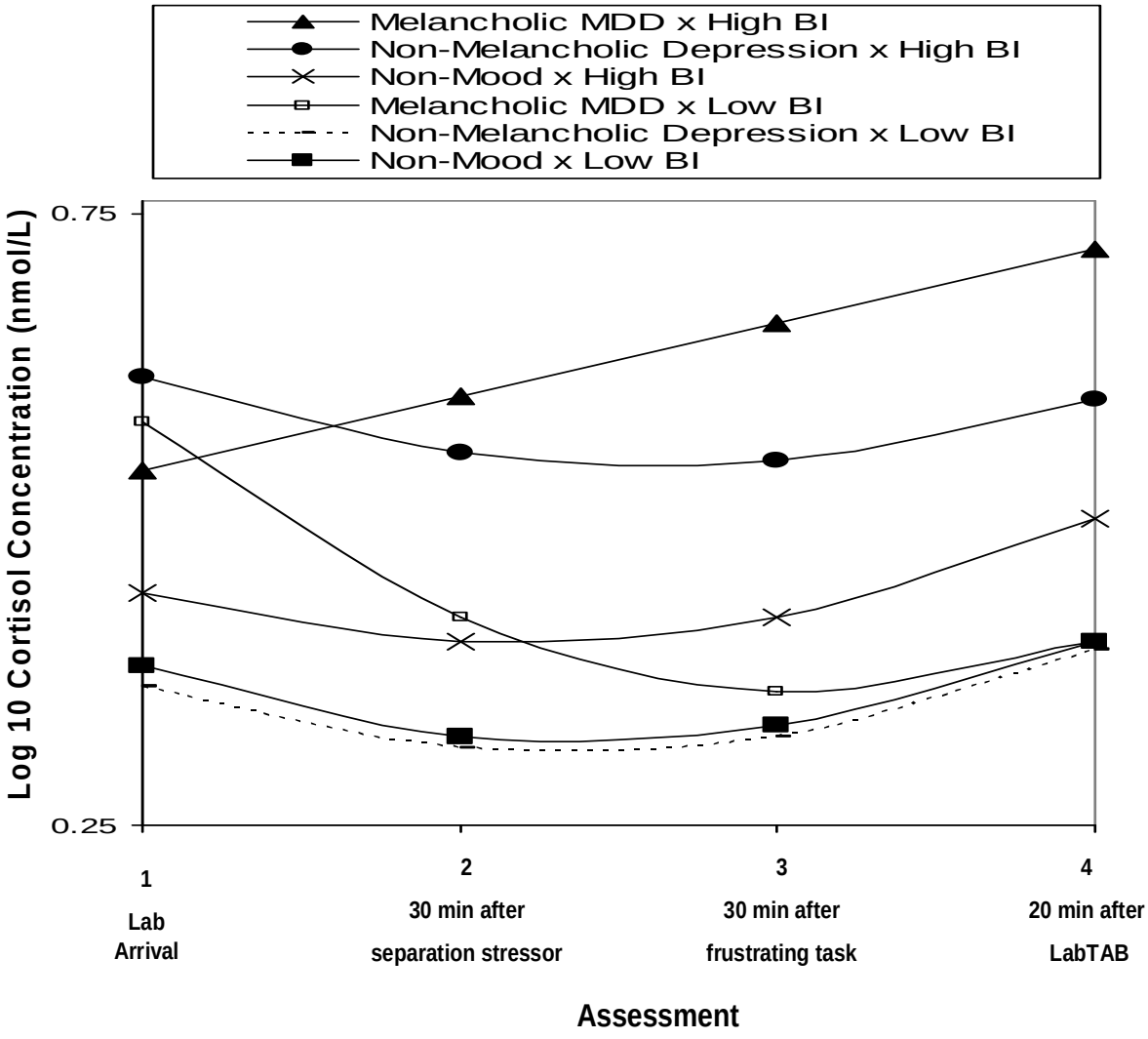
Figure 8. The interaction between maternal lifetime history of depression and parental hostility to predict children's laboratory cortisol reactivity.

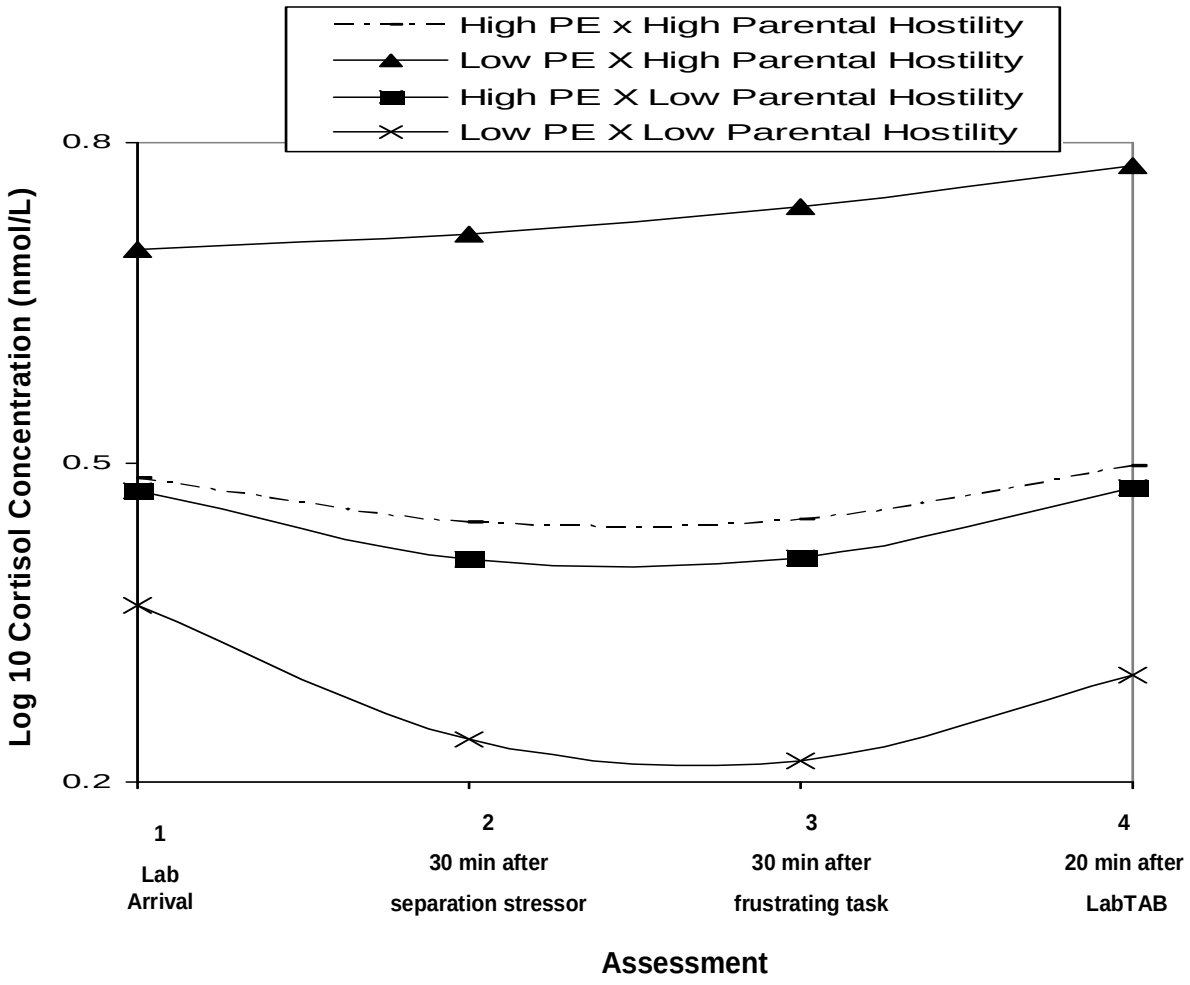

Log 10 Cortisol Concentration (nmol/L)
0.48
0.46
0.44
0.42
0.4
0.38
0.36
1
Lab
Arrival
2
30 min after
separation stressor
3
30 min after
frustrating task
4
20 min after
LabTAB
Assessment

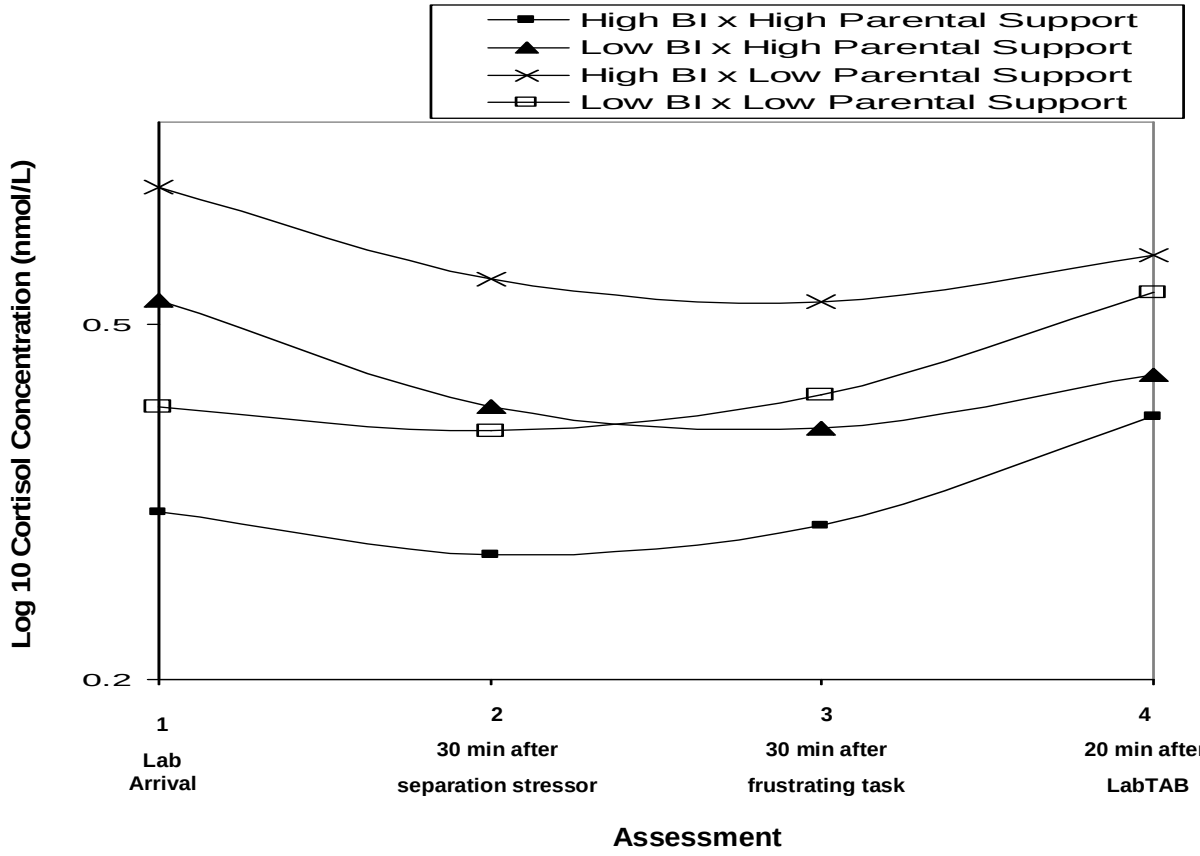

Growth Curve
Child NE
Child BI
Parental Hostility
Life Stress
Log 10 Cortisol Concentration (nmol/L)
0.55
0.5
0.45
0.4
0.35
1
Lab
Arrival
2
30 min after
separation stressor
3
30 min after
frustrating task
4
20 min after
LabTAB
Assessment

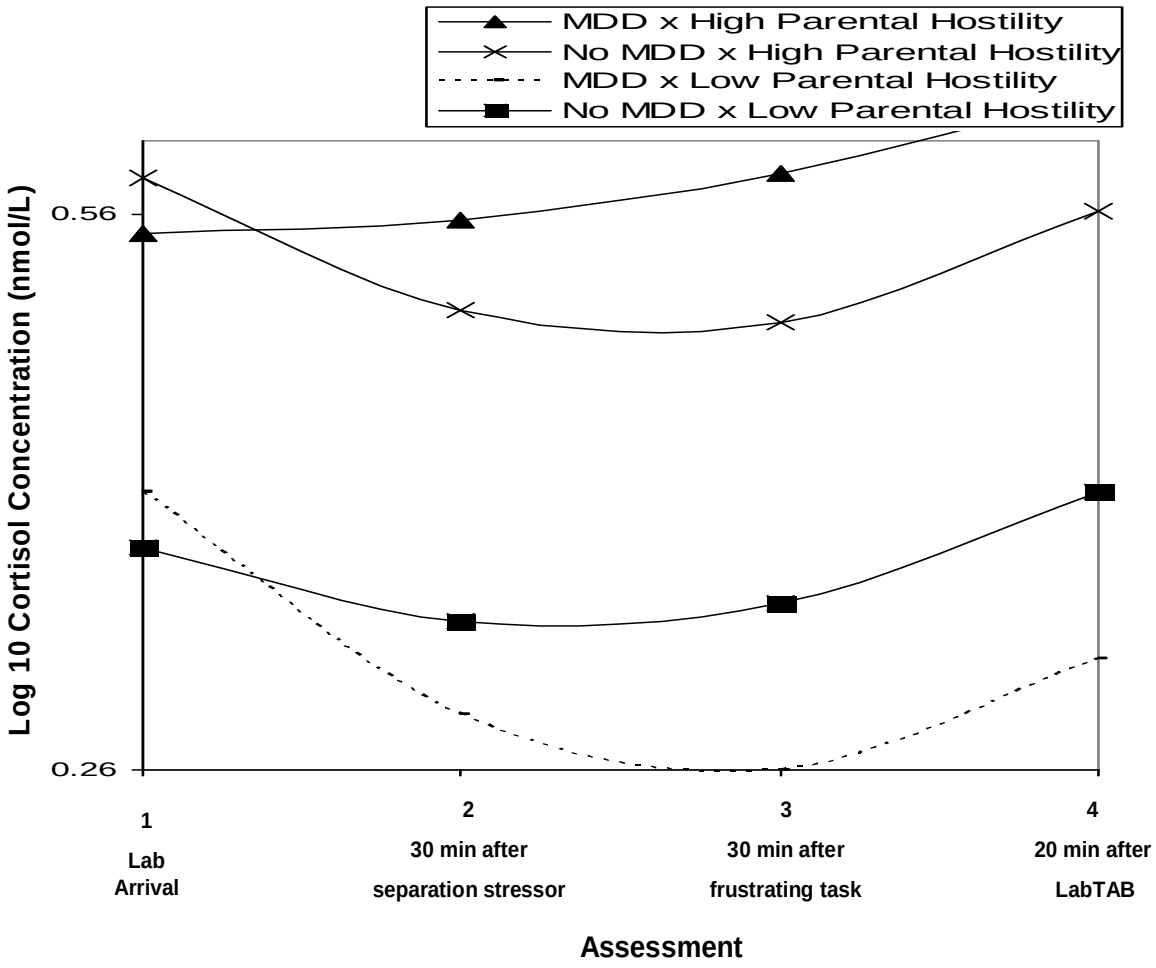

MDD x High BI
No MDD x High BI
MDD x Low BI
No MDD x Low BI
Log 10 Cortisol Concentration (nmol/L)
0.56
0.26
1
Lab Arrival
2
30 min after separation stressor
3
30 min after frustrating task
4
20 min after LabTAB
Assessment


Melancholic MDD x Low PE
Non-Melancholic Depression x Low PE
Non-Mood x Low PE
Melancholic MDD x High PE
Non-Melancholic Depression x High PE
Non-Mood x High PE
Log 10 Cortisol Concentration (nmol/L)
0.75
0.25
1
Lab Arrival
2
30 min after separation stressor
3
30 min after frustrating task
4
20 min after LabTAB
Assessment


Melancholic MDD x High BI
Non-Melancholic Depression x High BI
Non-Mood x High BI
Melancholic MDD x Low BI
Non-Melancholic Depression x Low BI
Non-Mood x Low BI
Log 10 Cortisol Concentration (nmol/L)
0.75
0.25
1
Lab Arrival
2
30 min after separation stressor
3
30 min after frustrating task
4
20 min after LabTAB
Assessment


High PE x High Parental Hostility
Low PE X High Parental Hostility
High PE X Low Parental Hostility
Low PE X Low Parental Hostility
Log 10 Cortisol Concentration (nmol/L)
0.8
0.5
0.2
1
Lab Arrival
2
30 min after separation stressor
3
30 min after frustrating task
4
20 min after LabTAB
Assessment


High BI x High Parental Support
Low BI x High Parental Support
High BI x Low Parental Support
Low BI x Low Parental Support
Log 10 Cortisol Concentration (nmol/L)
0.5
0.2
1
Lab Arrival
2
30 min after separation stressor
3
30 min after frustrating task
4
20 min after LabTAB
Assessment


MDD x High Parental Hostility
No MDD x High Parental Hostility
MDD x Low Parental Hostility
No MDD x Low Parental Hostility
Log 10 Cortisol Concentration (nmol/L)
0.56
0.26
1
Lab Arrival
2
30 min after separation stressor
3
30 min after frustrating task
4
20 min after LabTAB
Assessment