ANTENATAL PROGRAMMING OF THE HPA-AXIS: THE EFFECT OF MATERNAL DISTRESS DURING PREGNANCY ON THE DEVELOPMENT OF STRESS REACTIVITY AND HEALTH OUTCOMES IN INFANTS AND TODDLERS

By

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Abstract

The antenatal programming hypothesis proposes a fetal origin of adult diseases. Animal research has isolated the offspring's neuroendocrinological stress reactivity as the primary target of antenatal programming and the mediator of the antenatal effect on the offspring's behavioral and health outcomes. Evidence from humans is scarce, but consistent with the animal literature. It shows that antenatal maternal distress heightens behavioral (and in a few studies, neuroendocrinological) stress reactivity, and/or compromises physical or emotional health. Nevertheless, direct human evidence that supports the antenatal programming hypothesis is lacking. The antenatal programming hypothesis suggests that the programming of the offspring's HPA system serves as the mechanism that mediates the long-term effect of antenatal maternal distress on the offspring's adverse behavioral, physical and emotional outcomes.

In this dissertation, I report secondary analyses of data collected in 2 cohorts. The dissertation consists of 3 separate studies with non-overlapping samples examining evidence of antenatal programming in humans. In Study 1, I report analyses conducted to examine whether the offspring's stress reactivity, measured behaviorally, mediates the effect of antenatal maternal distress on physical and emotional health outcomes. In Study 2, I report analyses conducted to examine the stability of antenatal effects on the offspring's neuroendocrinological and behavioral stress reactivity, and the linkage between the behavioral and neuroendocrinological effects of antenatal maternal distress over time. Finally, in Study 3, I report analyses examining the mediating role of the offspring's perinatal neuroendocrinological stress reactivity as well as the moderating role of postnatal maternal distress in the relation between antenatal maternal distress and infant behavioral stress reactivity.

Together, these three studies provide preliminary evidence in humans that is consistent with the antenatal programming hypothesis: 1) antenatal maternal distress predicts the offspring's behavioral and health outcomes, 2) the effect of antenatal maternal distress is stable and persistent, and 3) the offspring's HPA system is the primary target and mediator of antenatal effects.

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Table of Contents

GENERAL INTRODUCTION

Fetal Origin of Adult Diseases

Literature Review and Definition of Terms

Recently, there has been a shift in research emphasis from postnatal to antenatal influences on child development (Huizink, Mulder, & Buitelaar, 2004). Non-human animal research has accumulated a substantiated body of evidence indicating that the experience of stress during gestation affects the development of the offspring's physiology, neurobiology, behavior and health (Weinstock, 2001). The interest in the effect of antenatal adversity on the development of health and disease originated from human epidemiological research, where a high prevalence of cardiovascular and metabolic diseases was observed among adults who had low birth weight (Seckl, Drake, & Holmes, 2005). More recently, developmental research has provided prospective evidence in support of the "antenatal programming" phenomenon as documented in the animal literature (Glover & O'Connor, 2005; Van den Bergh, Mulder, Mennes, & Glover, 2005b). The three developmental studies reported here attempt to contribute to this growing literature.

Infant birth weight is a key predictor of adult morbidity and mortality (Barker, 1990; Curhan et al., 1996a, Curhan, Willett, Rimm, Spiegelman, Ascherio, & Stampfer, 1996b). The link between low birth weight and the later development of cardiovascular and metabolic disorders, such as cardiovascular disease, hypertension, insulin resistance, and Type 2 diabetes, has been well-established (Barker, 1991; Barker et al., 1993a; Barker, Osmond, Simmonds, & Wield, 1993b; Curhan et al., 1996a,b; Fall et al., 1995; Forsen et al., 1997; Leon et al., 1996; Lithell et al., 1996; Rich-Edward et al., 1997; Yajnik et al., 1995). This association between birth weight and health outcomes appears

to be independent of lifestyle risk factors such as smoking, adult weight, social class, alcohol and lack of exercise (Barker et al., 1993a). Similarly, it has been observed that another form of compromised fetal growth, infant prematurity, also increases cardiovascular risk in adult life (Irving, Belton, Elton, & Walker, 2000). In fact, the health risk associated with prematurity is exacerbated rather than buffered by postnatal catch-up growth, suggesting that it is the restriction of intrauterine growth, rather than infant small size, which is key (Barker, 1991; Bavdekar et al., 1999; Forsen et al., 1997; Law et al., 2002; Leon et al., 1996; Levine, Berkenbosch, Suchecki, & Tilders, 1994; Osmond, Barker, Winter, Fall, & Simmonds, 1993). Further, twin studies have shown that the phenomenon cannot be solely explained by genetics. For example, the smaller of twins at birth is at greater risk for higher blood pressure in later life (Levine et al., 1994). These findings suggest that there may be a fetal origin of adult diseases, and many theories have been proposed to link together the events of the two ends of one's lifespan; the most prominent of such theories is known as the "antenatal programming hypothesis."

The Antenatal Programming Hypothesis

To explain the apparent association between fetal growth and later diseases, a number of authors have advanced the idea of early life physiological "programming" or "imprinting" (Barker et al., 1993a; Edwards, Benediktsson, Lindsay, & Seckl, 1993; Seckl, 1998). According to these authors, programming reflects the action of some physiological agents during sensitive periods or "windows" of development in promoting organizational effects on developing tissues that may persist throughout life. Literature on the increased health risk prevalence among adults with low birth weight suggests that the

relations between the antenatal condition and risk of adult diseases are generally continuous, where birth weight and gestation age are within the normal range, and is not exclusively associated with severe intrauterine growth retardation, multiple births or very premature babies (Barker, 1991; Barker et al., 1993a; Curhan et al., 1996a,b). It is, therefore, argued that low birth weight is only a marker of an adverse intrauterine environment, a more extreme case of the natural variation of antenatal adversity (see Phillips & Jones, 2006). For this reason, the antenatal programming hypothesis is not only applicable to adults with low birth weight, but also to the population at large.

The Nature of Antenatal Adversity

Non-human animal studies and human developmental research that examined the effect of stress (or, in case of humans, *distress*) during pregnancy on the offspring's development have provided prospective evidence on the antenatal programming hypothesis and its central mechanism. In the animal literature, maternal stress during pregnancy is experimentally induced by maternal restraint, adverse caging conditions, and pharmacological agents (e.g., ethanol or morphine injection, maternal infections, or injection of biochemical agents that release endogenous stress hormones; for review, see Weinstock, 2001, 2002). To achieve concordance with animal studies, the human literature has focused on measuring the levels and variations of distress experienced during pregnancy (Glover & O'Connor, 2005), such as self-report of daily hassles, anxiety/depressive symptomatology, or the experience of natural disaster (for a review, see Van den Bergh et al., 2005b).

While the approach to the study of maternal adversity during pregnancy differs between animal and human research, the physiological consequences of antenatal stress

are comparable (Davis, Hobel, Sandman, Glynn, & Wadhwa, 2005; Wadhwa, Dunkel-Schetter, Chicz-DeMet, Porto, & Sandman, 1996). Physiologically, adversity during pregnancy, whether of a physical and/or emotional nature, increases the exposure of the fetus to maternal glucocorticoids (the end product of the neuroendocrinological stress system), either through the weakening of the biochemical barrier that blocks maternal glucocorticoids from passing through the placenta to the fetus, or the heightening of maternal neuroendocrinological reactivity. As a result, it increases the net volume of glucocorticoids to which the fetus is exposed, which can strongly affect the developing organs of the fetus (Seckl, 1998; Seckl & Meaney, 2004; Seckl et al., 2005). Thus, maternal glucocorticoids have been identified as the programming agent of fetal antenatal adversity (Matthews, 2000, 2002).

The most well-documented effect of maternal glucocorticoids on the offspring is reduced birth weight. Research has consistently shown that glucocorticoid therapy during pregnancy results in reduced birth weight of the offspring in humans (Bloom, Sheffield, Mcintire, & Leveno, 2001; French, Hagan, Evans, Godfrey, & Newnham, 1999), as well as in animals (French et al., 1999; Ikegami, Jobe, & Newnham, 1997; Newnham et al., 1999; Newnham and Moss, 2001; Nyirenda, & Seckl, 1998; Reinisch, Simon, & Karwo, 1978). This is therefore consistent with the premises of epidemiological research, wherein low birth weights – and subsequent adverse health outcomes – are the results of antenatal adversity. The primary target of antenatal glucocorticoid programming is the fetal Hypothalamus-Pituitary-Adrenal (HPA) axis and its central regulators, the amygdala and the hippocampus (Matthews, 2002; Seckl et al., 2005).

The HPA axis: Physiology

Stress-induced activation of the HPA axis causes the synthesis and release of corticotrophin-releasing hormone (CRH) from the neurosecretory cells of the paraventricular nucleus (PVN) of the hypothalamus into the hypophyseal portal system to target corticotroph cells within the anterior lobe region of the pituitary gland. At the pituitary, CRH stimulates the synthesis of a polypeptide precursor pro-opiomelanocortin (POMC), which is then cleaved by processing enzymes to produce adrenocorticotrophic hormone (ACTH; Dallman et al., 1987; for a review, see Matthews & Challis, 1995). ACTH stimulates the synthesis and release of glucocorticoids from the zona fasciculate of the adrenal cortex (Dallman et al., 1987). In turn, glucocorticoids regulate their own release via the action of the positive and negative feedback systems through the central regulators. In terms of the positive feedback system, the amygdala activates the HPA axis, and consequently the production of glucocorticoids through its CRH-containing neurons, which is itself susceptible to activation by glucocorticoids through the glucocorticoids receptors (GR) at the amygdala. In terms of the negative feedback system, when glucocorticoids bind to the GR on the pituitary, PVN of the hypothalamus or hippocampus, it initiates a cascade of negative feedback to decrease the amount of CRH released from the PVN, and thus decreases ACTH release from the pituitary and the synthesis and secretion of glucocorticoids. Hence, the production of glucocorticoids by the HPA axis is a highly regulated process, where GR is responsible for initiating, down-regulating and up-regulating the production of glucocorticoids in response to stress. Since the expressions of GR at key locations along the HPA-axis are regulated by the exposure to stress-induced increase in maternal glucocorticoids, the result is a net up-regulation of the fetal HPA activity (to be reviewed below).

The HPA Axis: Target of Antenatal Programming

Evidence suggests that exposure of the fetal HPA axis to glucocorticoids during gestation 1) up-regulates the HPA axis' production of glucocorticoids, 2) heightens the reactivity of the HPA axis, 3) increases the feedforward drive of the axis, and 4) decreases the sensitivity of the negative feedback system. According to animal studies, antenatal stress 1) increases the expression and release of CRH at the PVN and, hence, up-regulates the production of glucocorticoids by the HPA axis (Welberg, Seckl & Holmes, 2001); 2) increases the expression and release of CRH at the amygdala, which results in enhanced stimulation from the amygdala to the HPA axis, and as a consequence heightens the reactivity of the HPA responses to stressors (Cratty, Ward, Johnson, Azzaro, & Birkle, 1995; Hsu, Chen, Takahashi, & Kalin, 1998; Makino, Gold, & Schulkin, 1994); 3) increases GR density at the amygdala, which enhances the sensitivity of the amygdala to the glucocorticoids and, consequently, increases the feedforward drive of the axis (Welberg et al., 2001); and 4) reduces the number of GR at the hippocampus, resulting in the decreased sensitivity of the feedback system (Dean, Lingas, & Matthews, 2001). As a result, the exposure of the fetus to maternal glucocorticoids permanently alters the "set point" of the HPA axis at the level of the amygdala, hippocampus, and the PVN. This results in a net increase in activity of the offspring's neuroendocrinological system, both at baseline as well as in reaction to stressors.

The HPA Axis: Mediator of Antenatal Programming

In non-human animal studies, it is observed that enhanced neuroendocrinological activity-reactivity of the antenatally stressed offspring is complemented by enhanced behavioral stress reactivity, heightened emotionality, and poor physical health (for

review, see Weinstock, 2001). Given the integrating, permissive, suppressive, stimulatory, and preparative effect of glucocorticoids on other systems (Sapolsky, Romero, & Munck, 2000), it has been argued that the offspring's neuroendocrinological stress reactivity mediates the effect of antenatal stress on the offspring's behavior and health (Matthews, 2002). While scant, there is direct evidence from animal research that supports the mediating role of the offspring's HPA system in the relation between antenatal maternal stress and the offspring's future behavioral outcomes (Ward, Johnson, Salm, & Birkle, 2000). However, only indirect evidence is available in human research. Findings from epidemiological research showed that not only was neuroendocrine activity more pronounced among adults who had low birth weight, enhanced neuroendocrine activity was also more likely to co-occur with increased incidences of cardiovascular and metabolic diseases among adults who had low birth weight (Phillips et al., 2000). These epidemiological findings suggest that enhanced neuroendocrinological reactivity may be the mechanism through which individuals with low birth weight develop greater risk for cardiovascular and metabolic diseases. In terms of prospective evidence, while most human developmental research measured stress reactivity behaviorally rather than neuroendocrinologically, results showed that heightened behavioral stress reactivity (arguably a proxy for neuroendocrinological stress reactivity) among the offspring whose mother experienced distress during pregnancy predicted future emotional and behavioral difficulty (Van den Bergh et al., 2005b).

In light of the scarcity of prospective human evidence that supports the antenatal programming hypothesis – evidence that indicates that the programming of the offspring's HPA system mediates the long-term effect of antenatal maternal distress on

the offspring's adverse behavioral, physical and emotional outcomes – three studies are therefore reported below, each exploring a different aspect of the antenatal programming hypothesis.

Thesis Objectives

As reviewed above, antenatal distress has profound and long-lasting effects on the offspring's stress reactivity and health outcomes. Animal research has isolated the offspring's neuroendocrinological stress reactivity as the primary target of antenatal programming and the mediator of antenatal effect on the offspring's behavioral and health outcomes. Evidence from human study, though scarce, is consistent with the animal literature in showing that behavioral and neuroendocrinological stress reactivity are targets of antenatal effect. Nevertheless, direct human evidence that supports the antenatal programming hypothesis is lacking. The antenatal programming hypothesis suggests that the programming of the offspring's HPA system is the mechanism that mediates the long-term effect of antenatal maternal distress on the offspring's adverse behavioral, physical and emotional outcomes. The current dissertation is a secondary analysis of archival data collected from two independent cohorts, reported in the following as three non-overlapping samples, each exploring a different aspect of the process and the effect of the antenatal programming on the HPA axis in human infants and toddlers.

While the three samples reported in the following studies are non-overlapping, Studies 1 and 2 nonetheless consist of participants recruited from the same population for the purpose of a bigger study. In the original study, data collection was conducted in one prenatal visit at the 3rd trimester and four postnatal visits at 6, 12, 18 and 24 months. All

participants were administered the Beck Depression Inventory at 3rd trimester and at 6, 12, and 18 months, the Impact of Event Scales at 3rd trimester and at 6 and 12 months, the Infant Characteristics Questionnaires at 6 and 24 months, and behavioral observation of infant reactivity in laboratory at 6 and 18 months. At 12 months, two non-overlapping samples of 30 were randomly selected to be administered two different sets of additional measures. One sub-sample of 30 was provided with an illness diary at 12 months and asked to record indicators of infant physical illness between 12 and 18 months. At 24 months, this sub-sample of 30 was also administered the Child Behavior Checklist. On the other hand, during the 12- and 18-month visits, baseline and post-challenge infant cortisol samples were collected from the other sub-sample of 30. In contrast, while everyone in the cohort from which Study 3 was sampled was a potential candidate for the full protocol, only the 33 dyads whose perinatal cortisol was collected were invited back at 10 months to receive the full protocol.

To examine different facets of the antenatal programming hypothesis, I partitioned the two cohorts into three non-overlapping samples. The aim was to (1) examine different research questions engendered by the unique constructs being measured, and to simultaneously (2) provide replication of core relations via measurement of the same set of constructs with different measures across independent samples of participants to establish convergent validity across samples. Unlike infant salivary cortisol, both the illness diary and the Child Behavior Checklist were based on maternal reports; therefore, laboratory behavioral observation (instead of maternal-report Infant Characteristics Questionnaires) was used in association with health outcomes measures to avoid reporter biases. On the other hand, since the video recording of infant

behavioral observation was only available at 6 but not 18 months, the Infant Characteristics Questionnaires was therefore the only infant reactivity measure administered repeatedly within the same time frame as infant salivary cortisol. Hence, in order to investigate the stability of antenatal effects across different, yet parallel dimensions, longitudinal infant behavioral (in terms of maternal reports) and cortisol data were analyzed together and reported in Study 2. As a result, the laboratory observation of infant behavioral reactivity became the default option in testing the reactivity-mediated antenatal effect on health outcomes in the other sub-sample of 30 from which health outcomes, rather than cortisol samples, were collected.

In contrast, while all participants in the larger study where Samples 1 and 2 were drawn received both the Beck Depression Inventory and the Impact of Event Scales, I specifically selected one maternal measure for use in each sample based on theoretical, rather than methodological or technical reasons. The Beck Depression Inventory was used in Study 1 in association with measures of the offspring's physical and mental health as previous literature identified strong relations between antenatal maternal depression and the offspring's poor physical and emotional health within the context of the offspring's developmental stress reactivity (Van den Bergh, Mulder, Mennes, & Glover, 2005). The Impact of Event Scale was used in Study 2 because its event-specificity and its sensitivity to change (Horowitz et al., 1979; Corcoran & Fischer, 1994) made it the more conservative measure in demonstrating the stability of antenatal maternal effect and a more suited measure in testing the co-evolution of its behavioral and neuroendocrinological effects over time.

Together, these three studies attempt to achieve the following objectives: 1) to explore the effect of antenatal maternal distress on the offspring's behavioral and neuroendocrinological stress reactivity, as well as physical and emotional health, 2) to provide preliminary evidence that is consistent with the hypothesis that the antenatal maternal effect on the offspring's behavioral and neuroendocrinological reactivity to stress is stable, and 3) to provide preliminary evidence that is consistent with the hypothesis that the offspring's HPA axis is the primary target of antenatal programming and a mediator of its effects.

Study 1 reports the examination of the following research questions using data collected for 30 dyads between the 3rd trimester and 2 years: 1) Does antenatal maternal distress predict prospective physical and emotional health outcomes in the offspring? 2) Does stress reactivity mediate the effects of antenatal distress on prospective physical and emotional health outcomes? According to the antenatal programming hypothesis, I hypothesized that 1) antenatal maternal distress predicts the offspring's physical and emotional health, and 2) the offspring's stress reactivity mediates the effect of antenatal maternal distress on poor physical and emotional health outcomes in toddlers.

Study 2 reports the examination of the following research questions using data collected for 30 dyads between the 3rd trimester and 24 months: 1) Does antenatal maternal distress predict 12- and 18-month neuroendocrinological stress reactivity and are antenatal maternal effects on neuroendocrinological stress reactivity stable over time? 2) Does antenatal maternal distress predict 6- and 24-month behavioral stress reactivity and are antenatal maternal effects on behavioral stress reactivity stable over time? 3) Does the change in antenatal maternal effects on neuroendocrinological stress reactivity

from 12 to 18 months correlate with the change in antenatal maternal effects on behavioral stress reactivity from 6 to 24 months? I hypothesized that: 1) antenatal maternal distress is positively associated with both 12- and 18-month neuroendocrinological stress reactivity and the effects are stable over time, 2) antenatal maternal distress is positively associated with both 6- and 24-month assessment of behavioral stress reactivity and the effects are stable over time, 3) the change in antenatal maternal effects on neuroendocrinological stress reactivity from 12 to 18 months is positively correlated with the change in antenatal maternal effects on behavioral stress reactivity from 6 to 24 months.

Study 3 reports the examination of the following research questions using data collected for 33 dyads between 24 hours after birth and 10 months: 1) Is the effect of antenatal distress on neuroendocrinological reactivity evidenced across different developmental stages, and stable over time? 2) Is the effect of antenatal distress on infant behavioral reactivity mediated by perinatal neuroendocrinological stress reactivity independent of the effects of postnatal maternal distress and infant neuroendocrinological reactivity? 3) Does postnatal maternal distress moderate the effect of antenatal distress on infant behavioral and neuroendocrinological stress reactivity? I hypothesized that: 1) the effect of antenatal maternal distress on the offspring's neuroendocrinological reactivity is stable between the perinatal period and infancy, 2) the effect of antenatal maternal distress is mediated by the offspring's perinatal neuroendocrinological reactivity, but independent of postnatal maternal distress and infant neuroendocrinological reactivity, and 3) postnatal maternal distress moderates the effect of antenatal maternal distress on infant behavioral and neuroendocrinological stress reactivity. Together, the three studies

explore the parallel between the antenatal programming hypothesis in animal and human research.

Animal studies have demonstrated that the offspring's neuroendocrinological stress reactivity is the primary target and mediator of antenatal programming. On the other hand, while human studies have demonstrated that antenatal maternal distress predicts the offspring's behavioral or neuroendocrinological stress reactivity, no direct evidence suggesting that stress reactivity, measured either behaviorally or neuroendocrinologically, mediates the effect of antenatal distress on the offspring's developmental outcomes. To this end, I explore the mediating effect of infant behavioral stress reactivity on the relation between antenatal maternal distress and infant physical and emotional health outcomes. Data were gathered from 30 dyads over 24 months, which include maternal self-report of distress, infant behavioral stress reactivity observed in the laboratory, and maternal-report physical and mental health outcomes. The results are reported in Study 1 below.

Secondary analyses reported in Studies 2 and 3 were conducted to bridge the gap between the model reported in Study 1, where behaviorally measured stress reactivity mediates the antenatal effect, and the model documented in the animal literature where neuroendocrinologically measured stress reactivity mediates the antenatal effect. Analyses reported in Studies 2 and 3 were conducted with the goal to provide evidence that are consistent with the following hypothesis: 1) the behavioral and neuroendocrinological effects of antenatal maternal distress parallel each other, and 2) maternal distress during pregnancy directly programs the offspring's antenatal HPA

system and indirectly shapes the development of infant behavioral reactivity through the programming of the offspring's HPA system during the antenatal period.

The stability of antenatal maternal affect is a necessary, though not sufficient, condition for antenatal programming. Findings from the animal literature have demonstrated that not only are antenatal effects on the offspring's behavioral and neuroendocrinological stress reactivity stable over time, the two effects are intertwined over the course of development. Only limited evidence is available in the human literature, demonstrating the stability of the antenatal effect on behavior. It remains to be shown that the antenatal effect on the offspring's neuroendocrinological stress reactivity is stable, or that the antenatal effects on the behavioral and the neuroendocrine dimensions of infant stress reactivity are linked over the course of development. Hence, the stability of antenatal effects on behavioral and neuroendocrinological stress reactivity, and the linkage between the evolution of behavioral and neuroendocrinological effects of antenatal maternal distress were explored using data on self-reported maternal distress, repeated measures of maternal-reported infant behavioral stress reactivity, and repeated measures of infant neuroendocrinological stress reactivity collected from 30 dyads over a 24-month period. The results are reported in Study 2 below.

The linkage between the behavioral and neuroendocrinological effects of antenatal maternal adversity over the course of development, according to findings from animal research, reflects a profound and long-lasting effect of antenatal maternal distress on the offspring's HPA system during the fetal period; and the antenatal programming of the offspring's HPA system in turn biases the offspring's behavioral reactivity to stress across the life span. However, human evidence that suggests a relation between the

behavioral and neuroendocrinological effect of antenatal maternal distress is lacking, and there is no available human evidence supporting the hypothesis that antenatal programming of the offspring's HPA system during the fetal period is responsible for the antenatal effect on the offspring's behavioral stress reactivity in the future. To this end, I explore the mediating role of the offspring's antenatally programmed neuroendocrinological stress reactivity in the relation between antenatal maternal distress and infant behavioral reactivity, using data from maternal self-report of distress, salivary cortisol collected before and after medical examination 24 hours after birth, and infant behavioral reactivity to novelty observed in the laboratory. Data were collected in 30 dyads over 10 months. The results are reported in Study 3 below.

The 3 studies are therefore complementary to each other, designed to provide preliminary evidence that is consistent with different aspects of the antenatal programming hypothesis. Specifically, the 3 studies explore the different dimensions of the relationship between antenatal maternal distress and the offspring's stress reactivity, from the presence of a global behavioral mediator, to the co-variation between the behavioral and neuroendocrinological effects of antenatal maternal distress, to finally the mediation of the effect of antenatal maternal distress by the offspring's antenatal neuroendicrinological function.

STUDY 1

Antenatal Programming of the Offspring's Stress Reactivity: The Mediation of Antenatal Effects on the Development of Physical Health and Emotional Health

Abstract

While the effect of antenatal stress on the offspring is well documented in the animal and human literatures, little empirical evidence is available to demonstrate the effects of antenatal stress across multiple developmental stages and multiple developmental outcomes in the same sample. Findings from the animal literature show that the offspring's stress reactivity is the primary target and the mediator of antenatal effects on physical and emotional health, findings that have not been replicated in humans. The current study examined prospectively the impact of 3rd trimester maternal distress on infant stress reactivity and developmental outcomes in toddlers, with objective behavioral measures coded from direct observation and standardized reports. The preliminary findings provided in this study suggest that (1) antenatal maternal distress predicted a) 6-month behavioral stress reactivity, b) 12- to 18-month physical health, and c) 24-month emotional health; and (2) infant stress reactivity significantly mediated the effect of antenatal maternal distress on physical and emotional health. When considerable caution is taken in interpreting the findings within the context of their potential lack of generalizability and the capitalization on chance resulted from the high predictors-to-sample size ratio, the present findings are nevertheless consistent with the growing animal and human literatures, which show that antenatal stress heightens the offspring's stress reactivity and compromises their physical and emotional health. The current findings also explored within the context of the growing human literature the transgenerational effect of antenatal adversity, where a model that links diverse findings across developmental stages is advanced and a mechanism that may underlie the antenatal effect on multiple health outcomes is proposed. These preliminary findings are

discussed in terms of the physiology of antenatal programming and its effect on the neuroendocrinological underpinnings of stress reactivity.

Introduction

Animal research shows that exposure to experimental stressors during pregnancy heightens behavioral, autonomic and neuroendocrinological responses to stress (Weinstock, 2001), compromises physical health (Seckl et al., 2005), increases behavioral disturbances (Welberg & Seckl, 2001), and decreases cognitive competence in the offspring (Vallée et al., 1999). The offspring's enhanced stress reactivity has been identified as the target and mediator of such antenatal effects (Matthews, 2002; Welberg & Seckl, 2001). In humans, it has been shown that adults who had low birth weight, an indication of adverse fetal environment, have enhanced stress reactivity (Phillips & Jones, 2006) and more metabolic and cardiovascular diseases (Barker, 1998). Because heightened stress reactivity is associated with poor health among adults who have low birth weight, it has been inferred that heightened stress reactivity mediates the health impact of antenatal adversity (Phillips et al., 2000). A growing developmental literature shows that antenatal distress predicts later stress reactivity and developmental health outcomes at all stages of human development (for a review, see Van den Bergh et al., 2005b). Missing in human research, however, is a direct empirical examination of the mediating role of the offspring's stress reactivity in the relation between antenatal stress and health outcomes. The purpose of the present study is to fill this void in the human literature.

The effect of antenatal maternal distress on the offspring's stress reactivity and developmental outcomes is evidenced in the fetal and neonatal period, infancy, toddlerhood, childhood and adolescence. For example, Ianniruberto and Tajani (1981) showed that fetuses of women who were panic-stricken during an earthquake were more

likely to exhibit hyperkinesias. Further, Van den Bergh (1990, 1992) demonstrated that women who exhibited state and trait anxiety during pregnancy were more likely to have fetuses with heightened biobehavioral responses to stress such as increased movement, increased heart rate, and reduced heart rate variability. Similarly, DiPietro, Hodgson, Costigan, Hilton, and Johnson (1996a,b) and DiPietro, Hilton, Hawkins, Costigan and Pressman (1999) reported that maternal perception of daily hassles and stress appraisal during pregnancy predicted increased fetal heart rate acceleration and reduced heart rate variability of the fetus. These studies suggest that chronic and acute antenatal maternal distress predict increased fetal stress reactivity. Antenatal maternal stress is also linked to health outcomes during the fetal period. For example, Field et al. (1985; 2003) observed that expectant mothers who were depressed or anxious had higher incidences of high-risk pregnancies and higher likelihood of fetal growth delay. Van den Bergh (1992) and DiPietro et al. (1996a) showed that increased maternal distress was also associated with reduced fetal heart rate-movement coupling, an indicator of the fetus' health status (Baser, Johnson & Paine, 1992).

Similarly, the effect of antenatal maternal stress is also evidenced during the neonatal period. For example, antenatal maternal depression and anxiety has been shown to be associated with maternal report of the neonate's behavioral reactivity to stressful/novel situations (Van den Bergh, 1990, 1992; Whiffen & Gotlib, 1989) as well as neonatal physiological indicators of stress reactivity, such as right-biased frontal EEG asymmetry and low vagal tone (Field et al., 2003). Further, expectant mothers with physiological markers of stress and anxiety (right-biased frontal EEG asymmetry, high cortisol baseline) give birth to neonates who exhibit more crying, fussing, and negative

facial emotion, more active sleep, greater number of state-changes, and higher degree of general activity (de Weerth, van Hees, & Buitelaar, 2003; Field et al., 2002). These studies indicate that maternal distress during pregnancy is linked to the offspring's enhanced behavioral and physiological reactivity to stress during the neonatal period. Research has also shown that acute and chronic mood problems, as well as moderate work-related stress are linked to indicators of compromised developmental health observed in neonates, such as low birth weight, small head circumference, low Prechtl's neurological score, low APGAR scores 1 and 5 minutes after birth, higher number of resuscitations needed at birth, abnormal sleep-wake pattern, motor immaturity, autonomic instability, difficulty in habituation to novel/adverse stimuli, behavioral dysregulation, and low cognitive competence (Brouwers, Van Baar, & Pop, 2001; Field et al., 2003; Jones, Rantakallio, Hartikainen, Isohanni, & Sipila, 1998; Lou et al., 1994; Ponirakis, Susman, & Stifter, 1998; Rieger et al., 2004; Whiffen & Gotlib, 1989).

The effect of antenatal maternal distress on the offspring's stress reactivity has also been well-documented throughout infancy and toddlerhood. A substantial body of research has shown that antenatal maternal depression and anxiety symptoms, as well as perceived daily stress during pregnancy predict laboratory-observed or maternal-reported behavioral stress reactivity among infants and toddlers (Austin, Hadzi-Pavlovic, Leader, Saint, & Parker, 2005; Davis et al., 2004; Field et al., 1985; Gutteling, de Weerth, & Buitelaar, 2005; Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2002; Martin, Noyes, Wisenbaker, & Huttunen, 1999; Pesonen, Raikkonen, Strandberg, & Jarvenpaa, 2005; Van den Bergh, 1990, 1992; Vaughn, Bradley, Joffe, Seifer, & Barglow, 1987). Infant and toddler's developmental health outcomes are also affected by antenatal

distress. General or pregnancy-related anxiety, experience of daily hassles or natural disaster during pregnancy, all predict poor health outcomes including irregularity in feeding and sleeping, poor task orientation, motor co-ordination and attention-regulation and, low infant Mental Developmental Index and Psychomotor Developmental Index (Brouwers et al., 2001; Buitelaar, Huizink, Mulder, Robles de Medina, & Visser, 2003; Huizink et al., 2002; Huizink, Robles de Medina, Mulder, Visser & Buitelaar, 2003; Laplante et al., 2004; Van den Bergh, 1990, 1992).

During childhood and adolescence the effect of antenatal maternal distress is also evidenced. Stress reactivity measured in early and late childhood in terms of the individual's neuroendocrinological responses to novel situations has been shown to be associated with maternal-reported distress during pregnancy (Gutteling, de Weerth, & Buitelaar, 2004, 2005; O'Connor et al., 2005). In terms of developmental health outcomes, maternal anxiety during pregnancy has been linked to inattention/hyperactivity and emotional problems among 3- to 4-year-old boys, emotional and conduct problems among 3- to 4-year-old girls, and persistent behavioral/emotional problems among 6- and 7-year-olds in both genders (O'Connor, Heron, & Glover, 2002a; O'Connor, Heron, Golding, Beveridge, & Glover, 2002b; O'Connor, Heron, Golding, Glover, & the ALSPAC Study Team, 2003). Further, maternal anxiety during pregnancy also predicts heightened negative emotionality in 5-year olds (Martin et al., 1999), low school grades and increased negative behavior in school among 6-year-olds (Niederhofer & Reiter, 2004), increased prevalence for Attention Deficit Hyperactivity Disorder (ADHD) symptoms and diagnoses among 7- and 8-year-olds (Rodriguez & Bohlin, 2005), increased ADHD symptoms, externalizing problems, and self-report anxiety among 8-

and 9-year-olds (Van den Bergh & Marcoen, 2004), and impulsive cognitive style and low intelligence among adolescents between age 14 to 15 (Van den Bergh et al., 2005a). Taken together, these studies indicate a link between maternal pregnancy distress and a wide range of youth mood and externalizing problems, as well as cognitive difficulties.

Consistent with non-human animal research, findings from the human literature reviewed above show that maternal distress during pregnancy is associated with heightened stress reactivity, and poor physical and emotional health in the offspring. While non-human animal research has isolated the offspring's stress reactivity as the mediator of antenatal effects on health, only indirect evidence from human birth cohort studies lend support to such a model. In addition, although evidence of antenatal effects on the human offspring can be observed at all developmental stages and across all major developmental outcomes, there appears to be no developmental study conducted to examine the process with which the effect of antenatal distress unfolds across different developmental stages and manifests across different developmental outcomes.

The Present Study

A sample of 30 mother-infant dyads was used to examine the mediating effect of the offspring's stress reactivity on the adverse health impact of antenatal distress. The study spanned from the 3rd trimester of pregnancy to 24 months. Antenatal maternal distress in the 3rd trimester, infant stress reactivity at 6 months, physical health status between 12 to 18 months and emotional difficulty at 24 months were measured.

Two questions were examined: First, does antenatal maternal distress prospectively predict the offspring's stress reactivity as well as physical health, and

emotional health? Second, does the offspring's stress reactivity mediate the effect of antenatal maternal distress on physical health and emotional health?

I hypothesized that (1) antenatal maternal distress predicts a) infant behavioral stress reactivity at 6 months, b) physical health between 12 and 18 months, and c) emotional health at 24 months; and (2) infant stress reactivity significantly mediates the effect of antenatal maternal distress on physical and emotional health.

Method

Participants

The analysis reported in the current study is based on data from a sub-sample of a larger study. In the original study, expectant mothers were recruited during the second or third trimester of pregnancy from prenatal education classes in the Greater Toronto Area for a longitudinal study extending to the second year of infants' life, for which data collection proceeded from 1996 to 1999 (Atkinson et al., 2005; Raval et al., 2001). Of the 680 mothers attending classes, 357 (52%) completed the questionnaire. Based on screening scores (i.e., preference given to those whose scores suggested non-autonomy in attachment security), 233 (65%) mothers were invited to participate. Of these, 139 (60%) agreed. Of the 139 dyads, 113 returned for the 6-month visit, 97 returned and completed the 12-month visit, 93 returned and completed the 18-month visit, and 88 returned and completed the 24-month visit. Reasons for discontinuing participation were: mother being "too busy," family moving away from the city, and illness of the baby or mother.

While all participants in the original study received the full protocol, a few additional outcome measures were also administered to two randomly selected, non-overlapping sub-samples of 30, chosen according to the match between participant

identification number and the number generated from a random table. These two independent samples of 30 are reported in the following as Studies 1 and 2. In the current study (Study 1), in addition to the original protocol, a randomly selected sample of 30 was also given the illness diary during their 12-month visit, to be returned at their 18-month visit. During their 24-month visit, this sample of 30 was given the Child Behavior Checklist (CBCL; Achenbach, 1991) in addition to the original 24-month protocol.

This sub-sample of 30 dyads consisted of 14 (47%) female infants. Most mothers were Caucasian (73%), ranging from 27 to 42 years of age (M=32.90, SD=3.64). Mothers' and fathers' schooling ranged from 11 to 23 years, averaging 15.87 for mothers and 15.57 for fathers. Over 83% of the sample earned a family income exceeding $50,000 Canadian. This sub-sample of 30 dyads was not different in terms of maternal age, mother's and father's education, and family income from either the initial sample of 139 or the subsequent samples 113, 97, 93, or 88 returning at 6, 12, 18 and 24 months respectively. Nor was this sub-sample of 30 different from either the initial or returning samples in terms of prenatal or postnatal assessment of maternal distress, or maternal reports of infant/toddler's stress reactivity.

Procedure

Data collection was conducted in one prenatal visit at the 3rd trimester of pregnancy and four postnatal visits at 6, 12, 18 and 24 months at the Hospital of Sick Children in Toronto. For the sub-sample of 30 reported in the current study, maternal distress was assessed during one prenatal visit at the 3rd trimester and 3 postnatal visits at 6, 12, and 18 months using the Beck Depression Inventory (BDI; Beck, Ward, Mendelson, Mock, & Erbaugh, 1961). Infant reactivity to stress was coded from infant

behavioral and affective responses to a series of increasingly stressful stimuli (Garcia-Coll et al., 1988) administered in the laboratory at 6 months. Information on the infant-toddler physical health was coded from the illness diaries mothers asked to keep on their infants between 12 and 18 months. The toddler's emotional/behavioral health was assessed at 24 months using the Child Behavior Checklist administered to the mothers.

Measures

Maternal Distress

Beck Depression Inventory (BDI)

A widely used measure of depression, the BDI (Beck et al., 1961), was administered in the current study to assess maternal distress during the prenatal visit in the 3rd trimester and postnatal visits at 6, 12 and 18 months. Participants were asked to rate 21 different depressive symptoms on the BDI according to 4-point scales ranging from 0 to 3. Ratings were summed to yield a single score for an overall level of self-reported depressive symptomatology, ranging from 0 to 63. Internal consistency for the BDI ranges from .73 to .92 with a mean of .86, and a split-half reliability coefficient of .93. (Beck, Steer, & Garbin, 1988). To isolate the effect of antenatal maternal distress on behavioral, physical and emotional outcomes, the effect of postnatal maternal distress was first partialled out. In cases of 12- to 18-month physical health and 24-month emotional health outcomes, the mean of 6-, 12-, and 18-month BDI scores was computed and subsequently covariated out. The mean score was used instead of individual BDI scores to better capture the postnatal environment infant experience during the first 24 months after birth, and to reduce the degrees of freedom of the regression model.

Stress Reactivity

Behavioral Response to Stimuli Presentation

Infant reactivity to stress was coded behaviorally from their responses to a presentation of 19 increasingly stressful visual, auditory, and tactile stimuli administered by an experimenter in the laboratory setting (as per Garcia-Coll et al.,1988). The entire procedure took about 15 minutes, during which time an experimenter presented each stimulus for 30 seconds to the infant who was sitting in an infant chair. The stimuli were presented in a fixed order to achieve a gradual increase of intensity and the number of sensory modalities involved: 1) the experimenter first talked to the infant several inches away with animate facial expression and vocalization, 2) the experimenter then picked up the infant and held the infant in her arms, 3) the experimenter cuddled the infant at her shoulder, 4) the experimenter silently dangled a toy composed of brightly colored blocks linked together, 5) the experimenter continued to dangle the brightly colored toy, but now accompanied with animated facial expression and vocalization, 6) the experimenter brushed the infant's hair, 7) the experimenter washed the infant's face with a soft tissue, 8) the experimenter fitted a hat on the infant's head, 9) the experimenter rang a bell for 10 seconds, paused for 20 seconds, and then again rang the bell for 5 seconds, 10) the experimenter wore a scary mask and spoke to the infant in an animated voice, 11) the experimenter wore a mask of a little girl and spoke to the infant in an animated voice, 12) the experimenter presented the jack-in-the-box and played music until it popped out, then paused for 20 seconds, and the episode was repeated for a second time, 13) the experimenter played a tape of a baby crying for 10 seconds, then paused for 20 seconds, and the episode was repeated for a second time, and 14) the experimenter showed the infant a toy robot that made buzzing noises and flashing lights while it moved. Garcia-

Coll et al., (1988) showed that this method for behavioral coding of stress reactivity was associated with infant reactivity to stress rated by maternal reports of infant behavior with respect to the dimension of fussy-difficult, unadaptability-reactivity, and activity subscales of the Infant Characteristic Questionnaire (Bates, Freeland & Lounsbury, 1979). More relevant to the current attempt to understand antenatal effects on infant behavioral outcomes, the authors showed that premature infants were coded as having a significantly higher frequency of negative responses to stimuli presentation.

Coding Behavioral Reactivity to Stress

Infant reactivity to stress is operationalized as the total frequency of negative responses in reaction to stimuli presentation, coded in terms of the frowns, negative vocalizations, or any gross motor movements accompanied by a frown or negative vocalization, in addition to turning away or pushing away from stimulus, or squirming as a response to cuddling. A high frequency count reflects more negative responses to stimuli. In the current study, the behavioral reactivity score used in the analysis was computed by subtracting the frequency of negative responses coded prior to stimuli presentation (20 seconds) from the ratio between negative responses coded during the presentation and the number of episodes presented. Interrater reliability is defined as the agreement on endorsing different types of negative behavior coded for every 5-sec epoch across all episodes. The interrater reliability of the behavioral reactivity score used in the current study was .83 (Kappa).

Physical Health

Illness Diary

Based on previous research that examined infant physical health within the context of development, mental health and temperamental reactivity (Goldberg, Gotowiec, & Simmons, 1995; Goldberg et al., 1997; Goldberg, Washington, Morris, Fischer-Fay, & Simmons, 1990; Janus & Goldberg, 1997), infant physical health in the current study was quantified as the composite of: 1) the number of days ill, 2) the number of doctor visits, 3) the number of days non-prescribed medication was taken, and 4) the number of days prescribed medication was taken based on the entry in the illness diary mothers kept between 12- to 18-month visits. The number of days the infant was ill, visited doctor, and took medication were totaled by counting the number of corresponding entries in the illness diary (see Goldberg et al., 1995; Janus & Goldberg, 1997). In the current sample, the Cronbach's alpha among the four items is .78.

Emotional Health

Child Behavior Checklist (CBCL)

The CBCL (Achenbach, 1991) has been identified as the most reliable and valid parent report measure currently available for assessing children's emotional and behavioral problems (Reitman, Hummel, Franz & Gross, 1998). Information concerning the child's behavior during the previous 6 months is obtained directly from the caregiver. The CBCL consists of 118 problem behavior items categorized as internalizing or externalizing behaviors. Internalizing behaviors include sadness, depression, and anxiety. Externalizing behaviors include opposition, aggressiveness, and hyperactivity. Parents rate each item on a 3-point scale: 0 (not true [as far as you know]), 1 (somewhat or sometimes true), and 2 (very true or often true). The CBCL consists of eight syndrome subscales (Withdrawn, Anxious/Depressed, Somatic Complaints, Social Problems,

Attention Problems, Delinquent Behavior, Thought Problems, Aggressive Behavior), which can be further collapsed into two broadband syndrome scores (Internalizing behavior and Externalizing behavior), and a total problems score. Achenbach (1991) has reported high internal consistency, test-retest reliability, construct validity, and criterion-related validity for the CBCL. Based on existing literature on the link between early stress reactivity and later internalizing symptomatology, only the internalizing subscale score were included in the following analyses.

Variable Selection

Study 1 examined the mediating effect of behavioral stress reactivity in the relation between antenatal maternal distress and infant health outcomes. The current model was tested below using the BDI total scores, laboratory observation of the change in the proportion of infant negative response in the course of the experiment (in response to the Garcia-Coll et al's protocol) from baseline, the total score of the frequency counts from each type of non-chronic poor-health indicators recorded in the illness diary, and CBCL internalization subscale.

The sample reported in Study 1 was one of the two non-overlapping samples that were drawn from a single cohort wherein multiple measures of maternal distress and infant stress reactivity were used. I specifically selected the BDI as the maternal measure in the current sample for theoretical reasons, and behavioral observation as the infant measure for methodological reason. The BDI was used in testing the current model because previous research had shown that antenatal maternal depression was associated with the offspring's physical and mental health outcomes, more so than event-based subjective stress, such as the experience of daily hassle (Van den Bergh, Mulder,

Mennes, & Glover, 2005). On the other hand, behavioral observation instead of maternal report was used in the current model, to avoid report biases since the health outcome measures in the current sample was based on maternal report.

A number of behavioral codes were generated from the laboratory observation. In the original article, Garcia-Coll et al. (1988) coded the infant response to their stimuli presentation protocol in terms of the following criteria: 1) positive response: total frequency of smiles, positive vocalization, any gross motor movements accompanied by a smile or positive vocalization, 2) negative response: total frequency of frowns, negative vocalizations, or any gross motor movements accompanied by a frown or negative vocalization, turning away from or pushing away stimulus, or squirming as a response to cuddling, 3) sociability: the total number of positive responses (as defined above) during face and voice interaction, cuddle in arms and at shoulder, 4) soothability: the intensity of intervention required to calm the crying infant [intensity rating: (1) self-quieting, (2) examiner talk, (3) pacifier, (4) pick-up, (5) mother intervenes, and (6) unconsolable] divided by the number of stimuli in response to which the child cried, and 5) activity: the total frequency of all behavioral responses regardless of the affective component (positive, neutral, or negative). Given the focus of the current study, infant behavioral stress reactivity was operationalized as the change in the frequency of negative behavior from baseline in reaction to stressor. Specifically, the behavioral stress reactivity score for each infant was calculated by subtracting the negative responses prior to stimuli presentation (20 seconds) from the ratio between negative responses coded during the presentation and the number of episodes presented. Further, because of the exploratory nature of the study, the overall frequencies of negative responses as well as other

behavioral scales were also examined in relation to maternal predictors and health outcomes. There is no theoretical or empirical basis to expect that neutral or positive response should mediate the positive relation between antenatal maternal distress and the offspring's adverse health outcomes. On the other hand, the baseline negative responses and the reactivity to and intervention-driven recovery from novel/stressful stimuli might serve as the mediators in the relation between antenatal maternal distress and adverse health outcomes. However, in order to operationalize behavioral stress reactivity in a consistent manner as the other two studies reported below, only analyses involving the difference score between post and pre-stimuli negative responses were taken as relevant and therefore reported. Because of the exploratory nature of the study, the other parameters were also tested with the minimum criteria for the mediation model in the preliminary analysis; they yielded only inconsistent results.

In terms of infant physical health, the occurrence of four different types of indicators were recorded: 1) the number of days ill, 2) the number of doctor visits, 3) the number of days non-prescribed medication was taken, and 4) the number of days prescribed medication was taken. The four indicators were summed up as a total score for the purpose of the following analysis. The composite of health indicators was selected as the outcome health measures in the model testing to represent the broad spectrum of infant health concerns.

The CBCL was administrated to the current sample as a measure of emotional health. Previous research has demonstrated the association between antenatal maternal distress and the offspring's internalizing as well as externalizing behavioral symptomatology (Van den Bergh, Mulder, Mennes, & Glover, 2005). Nevertheless, our

focus here is to examine the relation between antenatal maternal distress and the offspring's emotional health within the context of testing the mediating role of the infant's behavioral stress reactivity. To this end, the offspring's internalizing symptomatology was selected as the outcome variable in the following analyses, given its known relation with infant behavioral stress reactivity (Tubman & Windle, 1995).

Results

In the following, results from the preliminary analyses and model testing will be reported in turn. Findings reported as preliminary analyses included 1) the distribution and transformation of variables, where variables were transformed according to Tukey's (1977) "ladder of re-expression" (see also Mosteller & Tukey, 1977; Cohen, Cohen, Akins & West, 2003), 2) exploratory correlation analyses between pairs of key variables as well as their derivatives, 3) scatterplots of relations between key variables, and 4) exploratory correlation and regression analyses of the effect of different antenatal maternal distress measures on infant behavioral and health, singly or simultaneously. On the other hand, to test the overall model, I conducted a series of multiple regression analyses that examined the effect of antenatal maternal distress on infant stress reactivity, physical and behavioral health, and the mediating role of infant stress reactivity in the relation between antenatal maternal distress and the offspring's behavioral and physical health.

Preliminary Analyses

In the following, the distributions of all variables relevant to the overall model, as well as the derivatives of these variables are reported. Distributions of transformed variables are reported in addition to those of raw scores where appropriate. Secondly, the

intercorrelations among variables derived from different pairs of core constructs that constituted the overall model were examined in turn. To provide visualization for the relations between different pairs of key variables, scatterplots were also reported in the following. Finally, potential covariates were screened in terms of their confounding influences on variables selected to test the model of stability proposed in the current study.

Descriptive Statistics

Variable Distribution and Transformation

Reported in the current section are the distributions, skewness and kurtosis statistics, and the Shapiro-Wilk tests of normality on 1) antenatal and postnatal BDI total scores, 2) infant behavioral parameters coded from the Garcia-Coll protocol of novelty presentation at 6 months, 3) infant physical health indicators extracted from the illness diary kept between 12 and 18 months, and 4) subscales and total scores of the CBCL. Transformed variables are reported in addition to raw scores when the distributions of raw scores exhibited substantial skewness or kurtosis, or significant deviation from normal.

Antenatal and Postnatal Maternal Distress

3rd Trimester, 6-month, 12-month and 18-month BDI Scores. In the current study, maternal distress was assessed in the 3rd trimester, and at 6 months, 12 months and 18 months postnatally with the BDI. Figure A1.1-A1.4 report the distributions of the 3rd trimester, 6-month, 12-month and 18-month BDI scores. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality on all BDI scores are reported in Table A1.1. The results indicated that, with the exception of the 6-month BDI score, the

distributions of all BDI scores exhibited substantial skewness (the ratio between skewness statistics and the corresponding standard errors greater than 2), and the 3rd trimester and 12-month BDI scores also exhibited substantial kurtosis (the ratio between kurtosis statistics and the corresponding standard errors greater than 2). Shapiro-Wilk tests of normality indicated that the distributions of the 3rd trimester and 18-month BDI scores were significantly deviated from normal, while the distributions of the 6- and 12-month BDI scores were deviated from normal at a marginally significant level. To this end, log-transformation was performed on all BDI scores. Figures A1.5-A1.8 report the distributions of log-transformed BDI scores. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality on the log-transformed BDI scores are reported in Table A1.2. Results indicated that none of the log-transformed BDI scores exhibited substantial skewness or kurtosis. Neither was the distribution of any BDI score significantly deviated from normal. Hence, log-transformed BDI scores were used in the following analyses.

Infant Behavioral Reactivity

6-month Behavioral Reactivity Score Coded from the Garcia-Coll Protocol. In the current study, infant behavioral reactivity to stress was examined in terms of behavioral parameters coded from a laboratory procedure wherein infants were exposed to a series of increasingly stressful stimuli. Figures A1.9 to A1.12 report the distributions of the following behavioral parameters coded from the Garcia-Coll protocol: 1) the percentage of behavioral distress (coded every second) in a 20-second episode prior to the presentation of stimuli, 2) the ratio between the frequency of behavioral distress during stimuli presentation and the number of stimuli presented to the infant, 3) the ratio

between the frequency of positive vocalization during stimuli presentation and the number of stimuli with which the infant was presented, and 4) the difference between the ratio of behavioral distress in relation to the number of stimuli presented and the percentage of behavioral distress at baseline. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality on all behavioral parameters are reported in Table A1.3. The results indicated that the distribution of the percentage of distress behavior at baseline and the ratio of distress behavior during stimuli presentation were substantially skewed. The distribution of the percentage of distress behavior at baseline also exhibited substantial kurtosis. The Shapiro-Wilk tests of normality indicated that the distributions of all four behavioral parameters deviated significantly from normal. Hence, log-transformation was performed on all behavioral parameters. Figures A1.13-A1.16 report the distributions of the log-transformed behavioral parameters. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality on all log-transformed behavioral parameters scores are reported in Table A1.4. Results indicated that none of the log-transformed behavioral parameters exhibited substantial skewness or kurtosis. Nevertheless, the distributions of the log-transformed behavioral parameters remained significantly deviated from normal, with the exception of the difference score between behavioral distress during stimuli presentation and baseline. Since the difference score of behavioral distress between baseline and during stimuli presentation was the variable selected to test the proposed mediation model based on its closeness with the concept of behavioral stress reactivity, no additional transformation was conducted. Log-transformed behavioral distress difference score was used in the main analyses.

The coding of positive vocalizations during the 20 seconds prior to stimuli presentation was attempted, but was dropped eventually because of low variability: only 3 infants exhibited positive vocalizations at baseline.

In terms of the intercorrelation among different behavioral parameters, the difference score was positively and significantly correlated with behavioral distress during stimuli presentation ($r=.86$, $p<.01$). The difference score was negatively and significantly correlated with behavioral distress at baseline ($r=-.43$, $p<.05$). Behavioral distress coded at baseline was not significantly associated with behavioral distress observed during stimuli presentation. No behavioral distress parameter was significantly associated with positive vocalization during stimuli presentation.

Infant and Toddler Health Outcomes

12- to 18-month Physical Health Outcomes. Figure A1.17-A1.21 report the distributions of the following health indicators extracted from the illness diary participating mothers kept on their infants in the past 6 months: 1) the number of sick days, 2) the number of doctor visits, 3) the number of days non-prescribed medication was taken, 4) the number of days prescribed medication was taken, and 5) the composite of all four physical health indicators. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality on all four health indicators and their composite are reported in Table A1.5. The results indicated that the distributions of the number of doctor visits, the number of days prescribed and non-prescribed medication was taken, and the composite of the health indicators all exhibited substantial skewness and kurtosis. The Shapiro-Wilk tests of normality indicated that the distributions of all four health indicators and their composite significantly deviated from normal. As a consequence,

log-transformation was performed on the health indicators and their composite. Figures A1.22 to A1.26 report the distributions of the four health indicators and their composite after log transformation. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality on the log-transformed health indicators are reported in Table A1.6. The results indicated that, after log transformation, none of the health indicators, nor their composite, exhibited substantial skewness or kurtosis in their distribution. Shapiro-Wilk tests of normality revealed that even after log transformation, only the distributions of the number of days non-prescribed medication was taken and the composite of all health indicators did not significantly deviate from normality, the distribution of the number of doctor visits and the number of days prescribed medication were taken, remained significantly deviated from normal. Since the composite of health indicators was selected to test the mediation model proposed in the current study. Hence, no further transformation beyond log-transformation was performed.

24-month Behavioral Health Outcomes. Figures A1.27 to A1.29 report the distribution of the CBCL internalizing and externalizing subscale scores and the CBCL total score. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality on CBCL subscale and total scores are reported in Table A1.7. The results indicated that none of the CBCL subscale scores or its total score exhibited substantial skewness or kurtosis. The Shapiro-Wilk tests of normality revealed that, however, unlike the CBCL externalizing subscale score and total score, the CBCL internalizing subscale score significantly deviated from normal. Since the internalizing subscale is the key outcome variable selected to test the proposed mediation model, log transformation was conducted, and the distributions for the log-transformed CBCL subscale and total scores

are reported in Figures A1.30 to A1.32. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality on the log-transformed CBCL subscale and total scores are reported in Table A1.8. The result indicated that, the skewness and kurtosis of the distribution of log-transformed CBCL internalizing subscale score were no longer substantial, and the Shapiro-Wilk tests of normality was no longer significant. However, the distribution of the log-transformed CBCL externalizing score nevertheless exhibited substantial skewness and kurtosis and the Shapiro-Wilk tests of normality indicated that the distribution of the log-transformed value deviated significantly from normal. Nevertheless, no further transformation was conducted since the CBCL internalizing subscale score was the selected variable in the following model testing.

Exploratory Analyses

Reported in the current section are the basic bivariate correlation analyses that serve as the building blocks for the mediation model proposed: 1) maternal distress and infant behavioral reactivity, 2) infant behavioral reactivity and physical health, 3) infant behavioral reactivity and behavioral health, 4) maternal distress and infant physical health, and 5) maternal distress and infant behavioral health. Scatterplots are provided to describe the relations between key variables that were selected to test the mediation model proposed. In addition, since participants of the current study belonged to a larger study where maternal distress was also assessed in terms of the Impact of Event Scale (IES), correlation analyses between the IES scores and offspring's behavioral and health outcomes, as well as multiple regression analyses that examined the unique contribution of the BDI and IES on the offspring's behavioral and health outcomes are also reported in the following.

Maternal Distress and Infant Behavioral Reactivity

BDI Scores and Infant Behavioral Parameters Coded from the Garcia-Coll protocol

In the current study, maternal distress was operationalized in terms of the mother's total scores on the BDI administered at the 3rd trimester, and at 6 months, 12 months and 18 months. Infant behavioral reactivity was based on 4 different behavioral parameters coded from the Garcia-Coll experimental protocol during the 6-month visit to the laboratory: 1) the percentage of behavioral distress (coded every second) in a 20-second episode prior to the presentation of stimuli, 2) the ratio between the frequency of behavioral distress during stimuli presentation and the number of stimuli the infant was presented, 3) the ratio between the frequency of positive vocalization during stimuli presentation and the number of stimuli with which the infant was presented, and 4) the difference between the standardized ratio of behavioral distress in relation to the number of stimuli presented and the standardized percentage of behavioral distress at baseline. As noted previously, the values of all BDI scores and behavioral parameters were first log-transformed prior to any further analyses to normalize the distribution of the value. Table A1.9 reports the intercorrelations between BDI scores assessed at 3rd trimester and 6 months on one hand, and different parameters extracted from behavioral coding of the Garcia-Coll procedure at 6 months on the other. The results indicated that log-transformed antenatal maternal BDI score was significantly associated with 1) the log-transformed behavioral distress ratio during stimuli presentation ($r=.36$, $p<.05$) and 2) the log-transformed behavioral distress difference score ($r=.38$, $p<.05$). However, antenatal maternal BDI score was not significantly associated with either 1) the log-transformed percentage of behavioral distress coded at baseline, or 2) the log-transformed ratio of

positive vocalization during stimuli presentation. On the other hand, 6-month BDI was only marginally associated with 1) the ratio of behavioral distress during stimuli presentation and 2) the behavioral distress difference score. The difference score between behavioral distress in response to stimuli presentation and behavioral distress observed at baseline was selected to test the proposed mediation model. The scatterplot of the relation between antenatal BDI score and the difference score of behavioral distress are reported in Figure A1.33.

IES Scores and Infant Behavioral Parameters Coded from the Garcia-Coll protocol

As noted, the current study was based on a sub-sample of a larger study where maternal distress was also assessed using IES administered at the 3rd trimester, 6 months and 12 months. To examine the effect of antenatal maternal distress through this alternative measure of maternal distress, the relation between maternal IES score and infant behavioral parameters is also reported in the following. Table A1.10 reports the intercorrelations between IES scores assessed at the 3rd trimester and at 6 months on one hand, and different parameters extracted from behavioral coding during the Garcia-Coll procedure at 6 months on the other. The result indicated that, 3rd trimester IES score only predicted the behavioral distress difference score coded before and during stimuli presentation at a marginally significant level ($r=.32$, $p<.10$). No IES score, antenatal or postnatal, significantly associated with any other 6-month infant behavioral parameters.

Regressing Behavioral Distress Difference Scores on Antenatal BDI and IES

In addition, multiple regression analysis was conducted to examine the unique contribution of antenatal BDI and IES scores on the behavioral distress difference score. The result indicated that, when both variable were entered simultaneously, the

independent effect of BDI score on the behavioral distress difference score was significant (B=1.14, SE=.54, p<.05), while the effect of the IES score was not (B=.01, SE=.04, ns). These results indicated that antenatal BDI score contributed significant amount of unique variance to infant behavioral stress reactivity at 6 months. On the other hand, the unique contribution of antenatal IES score on 6-month behavioral reactivity did not.

Infant Behavioral Reactivity and Health Outcomes

Infant Behavioral Parameters and Infant Physical Health Indicators from the Illness Diary

As noted above, 4 behavioral parameters were coded from the 6-month laboratory procedure: 1) percentage of behavioral distress at baseline, 2) ratio of behavioral distress to total number of stimuli presented, 3) ratio of positive vocalization to the total number of stimuli presented, and 4) the difference score of behavioral distress between baseline and stimuli presentation. On the other hand, infant physical health status was based on 4 indicators extracted from the illness diary: 1) the number of sick days, 2) the number of doctor visits, 3) the number of days non-prescribed medication was taken, 4) the number of days prescribed medication was taken. The composite of all four health indicators was also computed. Table A1.11 reports the intercorrelations among different parameters coded from the 6-month laboratory observation on one hand, and different health indicators extracted from the illness diary on the other. All values are log-transformed in order to normalize the distribution. The result indicated that, none of the behavioral distress parameters, at baseline and during stimuli presentation, or the difference between the two, significantly predicted any individual health indicators. Nevertheless, the behavioral distress during stimuli presentation and the difference score of behavioral

distress between baseline and stimuli presentation were significantly associated with the health indicators composite ($r=.39$ and $r=.41$ respectively; $p<.05$). On the other hand, the ratio of positive vocalization coded during stimuli presentation was not significantly associated with the composite of health indicators, but was instead significantly associated with the number of days ill ($r=.36$, $p<.05$) and the number of days non-prescribed medicine was taken ($r=.45$, $p<.05$). In the main analyses, the composite of health indicators, rather than individual indicators, was used to test the proposed model to ensure the most accurate representation of the infant experience over a 6-month period. The scatterplot of the relation between the behavioral distress difference score and the composite of health indicators is reported in Figure A1.34.

Infant Behavioral Parameters and CBCL

As previously reported, during the 6-month laboratory observation, behavioral distress was coded at baseline and during stimuli presentation. Positive vocalization was also coded during stimuli presentation. The difference score was computed between behavioral distress coded before and during stimuli presentation. On the other hand, toddler's behavioral health was assessed using the CBCL, which includes an externalizing subscale, an internalizing subscale and their total score. Table A1.12 reports the intercorrelation among different behavioral parameters and toddler's CBCL subscale and total scores. All values are log-transformed to normalize the distributions. The results indicated that behavioral distress coded during stimuli presentation and the difference score of behavioral distress coded before and during stimuli presentation significantly predicted CBCL subscale and total scores ($r=.36$ to $.39$, $ps<.05$). Positive vocalizations coded during stimuli presentation on the other hand, predicted significantly, but

negatively the externalizing subscale score (r=-.36, p<.05). The behavioral distress at baseline was not significantly associated with either the subscale or the total score of the CBCL. The CBCL internalizing subscale score was selected to test the mediation model proposed below. The scatterplot that describes the relation between behavioral distress difference score and CBCL internalizing subscale score is reported in Figure A1.35.

Maternal Distress and Health Outcomes

Physical Health

BDI scores and Infant Physical Health Indicators from the Illness Diary.

Again, the BDI was administered at the 3rd trimester, and at 6 months, 12 months and 18 months to assess antenatal and postnatal maternal distress. Assessment of infant physical health was based on the following indicators, isolated from entries of the illness diary in the past 6 months: 1) the number of sick days, 2) the number of doctor visits, 3) the number of days non-prescribed medication was taken, 4) the number of days prescribed medication was taken, 5) the composite of all four physical health indicators. Table A1.13 reports the intercorrelations among the BDI score assessed at different time points and different health indicators extracted from the illness diary. All values are log-transformed to normalize the distribution of the variables. The result indicated that 3rd trimester BDI score was not significantly correlated with any individual health indicator. It was, however significantly associated with the composite of the four health indicators (r=.39, p<.05). On the other hand, while 6-month BDI score was significantly, though negatively associated with the number of days non-prescribed medicine was taken (r=-.39, p<.05), none of the other postnatal BDI scores significantly associated with the composite of health indicators. In the following, the composite of health indicators, rather

than any individual indicators, was used to test the proposed model. The scatterplot of the relation between 3rd trimester BDI score and the composite of health indicators is reported in Figure A1.36.

IES scores and Infant Physical Health Indicators from Illness Diary. As noted above, participants of the current study was a sub-sample of a larger study where maternal distress was also assessed using the Impact of Event Scale, administered at the 3rd trimester, and at 6 months and 12 months. To examine the effect of antenatal maternal distress via an alternative measure of maternal distress, the relation between maternal IES score at different time points and infant physical health indicators are also reported in the following. Table A1.14 reports their intercorrelation. The values of health indicators and their composite were log-transformed to normalize the distribution of the variables. The result indicated, the number of days ill was significantly associated with the 3rd trimester and 6-month IES scores (respectively, $r=.56$, $p<.01$ and $r=.38$, $p<.05$), and the number of doctor visits was significantly associated with 6- and 12-month IES scores (in both cases, $r=.39$, $ps<.05$). However, only 3rd trimester IES score was significantly associated with the composite of the four health indicators ($r=.51$, $p<.01$).

Regressing Infant Physical Health Indicators on Antenatal BDI and IES Scores. Multiple regression analysis was also conducted to examine the unique contribution of antenatal BDI and IES scores on the composite of physical health indicators. The results indicated that, when entered simultaneously, both the BDI ($B=.39$, $SE=.18$, $p<.05$) and the IES ($B=.03$, $SE=.01$, $p<.05$) scores contributed independently and significantly to the composite of the four health indicators.

Behavioral Health

BDI and CBCL Scores. BDI total scores collected at the 3rd trimester and 6, 12, and 18 months were used to measure maternal distress while CBCL subscale and total scores were used to assess the toddler's mental health. Table A1.15 reports the intercorrelation among them. All values were log-transformed to normalize the distribution of the variables. Results indicated that 3rd trimester BDI significantly predicted CBCL internalizing score ($r=.46$, $p=.01$) and total score ($r=.38$, $p<.05$). However, neither 6- nor 12-month BDI scores predicted CBCL subscale or total scores. 18-month BDI score, on the other hand, associated significantly with CBCL internalizing subscale score ($r=.41$, $p<.05$) and correlated marginally with CBCL total score ($r=.33$, $p<.10$). As noted above, the CBCL internalizing score was selected to examine the proposed mediation model. The scatterplot that describes the relation between 3rd trimester BDI score and CBCL internalizing subscale score is reported in Figure A1.37.

IES and CBCL Scores. The relation between IES score and CBCL subscale and total scores were also explored since the participants of the current study were in fact a sub-sample of a larger study wherein maternal distress was also studied using the IES, administered at the 3rd trimester, and at 6 and 12 months. The intercorrelations among different IES assessments and CBCL subscale and total scores are reported in Table A1.16. The results indicated that 3rd trimester IES score was not significantly associated with either the subscale or total scores of CBCL, and neither was 12-month IES. On the other hand, 6-month IES score significantly predicted CBCL internalizing subscale score ($r=.37$, $p<.05$).

Regressing Infant CBCL Scores on Antenatal BDI and IES Scores. When the two measures of antenatal maternal distress were entered into the model simultaneously

to examine the unique contribution of each measures on CBCL internalizing subscale score, the independent effect of 3rd trimester BDI score on CBCL internalizing score was significant (B=.40, SE=.20, p=.05) even after controlling for the effect of the 3rd trimester IES score on CBCL internalizing score. On the other hand, regression analysis indicated that the effect of 3rd trimester IES score on CBCL internalizing subscale score was not statistically significant (B=.01, SE=.02, ns). The results suggested that the contribution of the BDI on CBCL internalizing score was unique, independent of the effect of IES score.

Screening for Potential Covariates

Table 1.2 reports the descriptive statistics of 1) antenatal and postnatal BDI scores, 2) 6-month behavioral stress reactivity score, 3) 12- to 18-month physical health indicators, and 4) 24-month maternal report of CBCL internalizing subscale scores. Table 1.3 reports the correlation among outcome variables.

Insert Table 1.1 and 1.2 about here

Maternal Distress

Zero-order correlational analyses indicated that 3rd trimester BDI score was associated with postnatal BDI scores (r=.40 to r=.60, p<.05), but not with sociodemographic variables such as maternal age, years of schooling, family income, martial status or ethnics origin.

6-month Behavioral Reactivity Scores.

Zero-order correlational analyses indicated that 6-month behavioral reactivity score was not different according to the biological sex of the infant, nor was it associated with any sociodemographic variables.

12- to 18-month Physical Health

Zero-order correlational analyses indicated that indicators of physical health employed in the current study correlated with one another (r=.37 to r=.62, p<.05). The composite score generated from summing individual physical health indicators was not associated with the biological sex of the infant or any sociodemographic variables.

24-month Emotional Health

Zero-order correlational analyses indicated that CBCL internalizing scores were significantly higher among female than male 24-month-olds (t=2.18, p<.05), but had no association with any sociodemographic variables.

Hypothesis Testing

Does Antenatal Maternal Distress Predict the Offspring's Stress Reactivity, and Physical and Emotional Health?

Antenatal Maternal Distress and 6-month Stress Reactivity

Sequential multiple regression analysis was conducted to predict 6-month behavioral reactivity score from 3rd trimester BDI total score, with 6-month postnatal BDI total score first partialled out. Results indicated that 3rd trimester BDI total score predicted 6-month behavioral reactivity score, even after first controlling for the effect of 6-month postnatal BDI total score (B=.62, SE=.20, p<.01). This result suggests that the greater the maternal distress during pregnancy, the greater the infant stress reactivity, even after controlling for the effect of postnatal maternal distress.

Antenatal Maternal Distress and 12- to 18-month Physical Health

Sequential multiple regression analysis was conducted to predict the 12- to 18-month physical health composite score from BDI total score at 3rd trimester, with the mean of 6-, 12-, and 18-month postnatal BDI total scores first partialled out. Results indicated that 3rd trimester BDI total score significantly predicted 12- to 18-month physical health composite score, even after first controlling for the effect of the mean postnatal BDI total score (B=.10, SE=.03, p<.01). Hence, the results suggest that, independent of the degree of postnatal maternal distress, the greater the maternal distress during pregnancy, the poorer the offspring's physical health.

Antenatal Maternal Distress and 24-month Emotional Health

Sequential multiple regression analysis was conducted to predict 24-month CBCL Internalizing subscale scores from BDI total scores at 3rd trimester, with the mean of 6-, 12- and 18-month postnatal BDI total scores first partialled out. Results indicated that 3rd trimester BDI total score significantly predicted 24-month CBCL Internalizing subscale scores, even after first controlling for the effect of the mean postnatal BDI total score (B=8.69, SE=2.44, p<.01). Hence, the results suggest that, independent of the effect of postnatal maternal distress, the greater the maternal distress during pregnancy, the poorer the offspring's emotional health.

Does the Offspring's Stress Reactivity Mediate the Effect of Antenatal Maternal Distress on Physical and Emotional Health?

The mediating role of stress reactivity in the relations between antenatal maternal distress and 1) 12- to 18-month physical health, and 2) 24-month emotional health, was evaluated according to the regression-based guidelines proposed by Baron and Kenny

(1986; see also Judd & Kenny, 1981), including formal assessments of the mediation effect using the Goodman test (Goodman, 1960). To establish mediation, the following four minimum criteria must hold: 1) 3rd trimester BDI total score must significantly predict 6-month behavioral stress reactivity score; 2) 6-month behavioral stress reactivity score must significantly predict a) physical health composite score, and b) CBCL internalizing score; 3) 3rd trimester BDI total score must also significantly predict a) physical health composite score, and b) CBCL internalizing score; and 4) the effect of 3rd trimester BDI total score on the two developmental outcomes must be significantly reduced after controlling for the effect of 6-month behavioral stress reactivity score. To this end, Meng, Rubin and Rosenthal's (1992) adaptation of the Fisher's z transformation procedure was used (the adaptation enables the comparison between correlated correlation coefficients with overlapping variables) to examine if the drop of the regression coefficient was significant. These relations are necessary, but not sufficient, conditions for demonstrating mediation. The Goodman test (Goodman, 1960) was therefore performed to provide formal tests of the mediation hypotheses.

Criterion 1: Antenatal Maternal Distress Predicts 6-month Stress Reactivity

As reported above, antenatal maternal distress significantly predicted 6-month behavioral stress reactivity score, even after first controlling for the effect of 6-month postnatal BDI total score (B=.62, SE=.20, p<.01). The first criterion of mediation was therefore met.

Criterion 2: 6-month Stress Reactivity Predicts Developmental Outcomes

Separate simple regression analyses were conducted to predict from 6-month behavioral reactivity score: 1) the physical health composite score and 2) the CBCL

Internalizing scores. Results indicated that 6-month behavioral reactivity score significantly predicted physical health composite score (B=.05, SE=.02, p<.05) and CBCL Internalizing subscale scores (B=.51, SE=.19, p<.05). These results suggested that the greater the stress reactivity at 6-months, the poorer the physical health between 12 to 18 months and the poorer emotional health at 24 months. The second criterion of statistical mediation was therefore also met.

Criterion 3: Antenatal Maternal Distress Predicts Developmental Outcomes

As reported above, even after controlling for the mean of 6-, 12-, and 18-month postnatal BDI total score, 3rd trimester BDI total score significantly predicted health composite score (B=.10, SE=.03, p<.01) and CBCL Internalizing score (B=8.69, SE=2.44, p<.01), such that the higher the maternal distress during pregnancy, the poorer the offspring's physical and emotional health. The third criterion of statistical mediation was therefore met.

Criterion 4: Relations between Antenatal Maternal Distress and Developmental Outcomes Are Not Independent of Infant Stress Reactivity

Finally, when behavioral stress reactivity score was entered into the above multiple regression model with physical health composite score or CBCL internalizing score, the relations between antenatal distress and both physical and emotional health were no longer significant (respectively, B=.05, SE=.03, ns. and B=3.61, SE=1.94, ns.). Subsequent analyses using Meng, Rubin and Rosenthal's (1992) adaptation of the Fisher's z transformation procedure to compare correlated correlation coefficients with overlapping variables indicated that drops of the antenatal distress-health outcomes relations before and after controlling for stress reactivity were significant (z=2.20, p<.05

and z=3.38, p<.01 for physical and emotional health respectively). These results suggested that, while antenatal distress significantly predicted developmental outcomes, the relations were not independent of the effect of infant stress reactivity. The final criterion for statistical mediation was therefore met.

Assessment of the Proposed Mediation Model

Since all conditions for testing mediation were met, the first formal test of statistical mediation was conducted based on 3rd trimester BDI, 6-month behavioral reactivity score, and 12- to 18-month physical health composite score, and a second formal test of statistical mediation was conducted based on 3rd trimester BDI, 6-month behavioral reactivity score, and 24-month maternal-report CBCL Internalizing score. The Goodman test for both mediation models, with all complementary postnatal maternal effect first partialled out, were significant (t=1.99, p<.05 and t=2.07, p<.05 respectively) and consistent with the hypothesis that stress reactivity mediates the effect of antenatal distress on the offspring's physical and emotional health.

Discussion

Antenatal stress affects the offspring's stress reactivity and developmental outcomes. Non-human animal research shows that the antenatally stressed offspring exhibit heightened stress reactivity (Weinstock, 2001), poor physical health (Seckl et al., 2005), increased behavioral disturbances (Welberg & Seckl, 2001), and decreased cognitive competence (Vallée et al., 1999). In humans, epidemiological research and birth cohort studies provide retrospective evidence on the relation between antenatal adversity and 1) heightened stress reactivity (Phillips & Jones, 2006) and 2) poor physical health (Barker, 1998). In addition, a growing developmental literature has accumulated

prospective evidence on the effect of antenatal distress on the offspring's stress reactivity and emotional health across all developmental stages (Van den Bergh et al., 2005b).

However, the underlying mechanism that enables the adverse effect of antenatal stress to unfold across multiple developmental stages and to manifest across multiple developmental outcomes remains a knowledge gap in the human literature. Non-human animal literature has isolated the offspring's HPA-axis as the primary target and mediator of the adverse effect of antenatal stress (Matthews, 2002). Glucocorticoids, the end product of the HPA axis that are released in response to stress, induce long-term modification on multiple organ systems via intracellular receptors, and serve as the mechanism through which antenatal adversity programs the developing fetus (Seckl et al, 2005; Welberg & Seckl, 2001). While it is also observed in human that the effect of antenatal stress is persistent and multidimensional, no systematic investigation has been conducted to examine the underlying mechanism.

Not inconsistent with findings reported in the human and animal literatures, the preliminary results reported in this study indicated that antenatal maternal distress significantly predicts the offspring's 1) 6-month stress reactivity, 2) 12- to 18-month physical health, and 3) 24-month emotional health, even after controlling for corresponding postnatal maternal distress. Also, these preliminary findings are not inconsistent with the hypothesis that infant stress reactivity, measured behaviorally in the current study via laboratory observation, mediates the adverse effect of antenatal distress on physical and emotional health.

However, the preliminary findings reported in the current study need to be interpreted with extreme caution. According to Nunnally and Bernstein (1994),

capitalization upon chance decreases with sample size and increases with the pool of possible predictors. According to the authors, sample *R* is systematically biased upward compared to the population *R*, and one major reason for the bias is that predictors are usually selected from a larger set. Such bias is negligible when the sample size-to-predictors ratio is large, but becomes substantial when the ratio drops to 10:1, which is the level the current study is at. This biased selection takes enormous advantage of chance when it is based upon data and not theory (Nunnally & Bernstein, 1994). Although variable selection in the current study was not dictated by the data but guided by theory, the sheer number of candidate measures associated with each construct greatly increases the probability of having predictors being highly correlated with the criterion simply because of sampling error, or having spurious intercorrelation among predictors that may artificially inflate the size of *R*. In addition, even if predictors were not selected from a larger pool, the *R* obtained from a relatively small sample (with sample size-predictor ratio being around 10:1) will tend to become smaller when reapplied to a larger sample. Large samples have lower probability of producing unusually large correlation by chance because the parameter estimates are more stable (Nunnally & Bernstein, 1994).

With these cautions in mind, findings reported herein are nevertheless consistent with the animal literature, where stress reactivity (measured in terms of glucocorticoids response to novel/challenging situations) has been isolated as the primary target and mediator of antenatal effect on developmental outcomes. Similarly, when interpreted with caution, findings reported in the current study are also consistent with human findings on antenatal effects on both physical and emotional health. Notwithstanding some major flaws in the method and design of the current study, there are also a few ameliorating

factors. For example, the current study also employed multiple sources of information to reduce confounds introduced because of common methodology: maternal distress was based on maternal self-report, offspring stress reactivity was measured by means of behavioral observation in the laboratory, physical health was assessed based on frequency count of health-related incidences recorded in an illness diary, and the offspring's emotional health was measured with a standardized maternal report measure. Finally, to distinguish between antenatal and postnatal maternal influences, and to control for confounds associated with linking maternal self-report and maternal-reported infant outcomes, the current study statistically controlled for the effect of postnatal maternal distress, which was measured at multiple postnatal time-points with the same instrument with which antenatal maternal distress was measured.

Limitations

It is recognized that findings from the current study may not be generalizable due to small sample size, and the number of inferential statistics that were conducted in relation to such relatively small sample size may have in fact capitalized on chance. Nonetheless, as we shall see, the other two studies reported in this dissertation replicate, with the use of different measures, the same relation between antenatal distress and infant stress reactivity. It is also recognized that the large number of analyses conducted greatly increased the probability of type 1 error. However, given the scarcity of empirical data on the mechanism of antenatal programming in human, it would risk type 2 error if the full potential of the data set was not explored.

Further, while pregnant mothers were recruited from low risk communities and were not known to be involved in any treatment programs, no exclusion criteria existed to

screen for any inherent medical condition or congenital defects that may complicate the fetus' developmental health. Nor was there any information collected from the dyad regarding the perinatal period, including the mode of delivery, prematurity, birth complications or obstetric interventions, the existence of which would seriously confound the relations being studied.

It is also recognized that there are problems inherent in the measures being used in the current study. While data on infant physical health was extracted by tallying the number of medical-related incidents recorded in the illness diary by the mother, rather than directly from any subjective rating by the mother, and infant emotional health was assessed using a standardized diagnostic tool, the relation between maternal self-reported distress and maternal-reported infant outcomes may nonetheless be inflated because of reporter biases. Similarly, maternal self-reported distress was a poor substitution for any direct observation on maternal caregiving behavior as indicators of the quality of the offspring's postnatal environment, thus making the removal of postnatal maternal influence from the relation between antenatal maternal distress and infant outcomes incomplete. Finally, while the offspring's mental health was measured at 24 months, postnatal maternal distress was only measured up to 18 months, making the partialling out of postnatal maternal distress insufficient in controlling for postnatal maternal influence on the relation between antenatal maternal distress and the offspring's mental health at 24 months.

Future Directions

The present findings are consistent with a model that integrates existing human literature and provides a human analogue for non-human animal research on stress and

antenatal adversity. With proper caution in mind regarding the potential inflation of effects, capitalization on chance and the non-generalizability of results, findings reported in the current study are nevertheless consistent with the hypothesis that stress reactivity mediates the adverse effect of antenatal stress on physical as well as emotional health outcomes, a hypothesis that has not been examined in humans before. Unlike animal studies, the current study and most human studies assessed the offspring's stress reactivity behaviorally rather than neuroendocrinologically. Future human studies are therefore needed to demonstrate that the effects of antenatal maternal distress on the offspring's behavioral and neuroendocrinological stress reactivity are linked, and therefore bridge the animal and human literatures. To this end, two studies are reported below. Study 2 examines the hypothesis that the effects of antenatal maternal distress on the offspring's behavioral and endocrinological systems are stable, and the changes in the behavioral effect of antenatal maternal distress over time, is associated with the changes in the neuroendocrinological effect of antenatal maternal distress within the same timeframe. Study 3 examines the hypothesis that maternal distress during pregnancy programs the offspring's neuroendocrinological stress reactivity, which in turn mediates the effect of antenatal maternal distress on the offspring's behavioral reactivity. Taken together, the two studies reported below seek to provide evidences that contextualize the behavioral effect of antenatal maternal distress reported by the current study within the existing body of animal literature, which show that the offspring's HPA-axis is the mechanism of antenatal programming.

STUDY 2

Stability of the Effects of Antenatal Maternal Distress on Neuroendocrinological and Behavioral Stress Reactivity of Infants and Toddlers

Abstract

The effect of antenatal maternal distress on the offspring is stable and persistent. Non-human animal research has shown that antenatal stress induces a sustained increase in glucocorticoid levels throughout development. This in turn is responsible for the persistent behavioral hyper-reactivity observed across the lifespan from the antenatally stressed offspring. However, human evidence concerning the stability of antenatal effects on the offspring's stress reactivity is lacking, and the relation between antenatal maternal effects on neuroendocrinological and the behavioral dimensions of stress reactivity remains unexplored. The current study is a preliminary attempt to observe in human infants the stability of the effect of antenatal maternal distress on the offspring's stress reactivity over an 18-month period across neuroendocrinological and behavioral dimensions. Maternal distress was assessed at the 3rd trimester during pregnancy and in 6 and 12 months postnatal, maternal reports of infant behavioral stress reactivity were collected at 6 and 24 months, and infant salivary cortisol was sampled prior to and after a novel/challenging paradigm at 12 and 18 months. The preliminary findings reported below are consistent with the hypothesis that antenatal maternal distress is associated with 6- and 24-month behavioral stress reactivity, and that the effect is stable. However, findings from the current study do not support the hypothesis that antenatal maternal distress is associated with 12- and 18-month neuroendocrinological stress reactivity, and that the neuroendocrinological effects of antenatal maternal distress are stable, or that the changes in the behavioral and the neuroendocrinological effects of antenatal maternal distress over time are linked. When considerable caution is taken in interpreting the findings within the context of their potential lack of generalizability and the capitalization

on chance resulting from the high predictors-to-sample size ratio, the present findings are nevertheless consistent with the animal literature, and are discussed within the context of the antenatal programming hypothesis.

Introduction

Human epidemiological research has identified a fetal origin of adult diseases (Barker, 1991). "Antenatal programming" has been advanced as a mechanism that links the events at both ends of the lifespan (Barker, 1998). One central tenet of the antenatal programming hypothesis is that the effect of antenatal distress on the offspring's stress reactivity is stable, persists throughout development, and predisposes an individual to diseases in adulthood (Seckl et al, 2005). Non-human animal studies show that antenatal stress alters the expression of the glucocorticoid receptor gene along the fetal HPA-axis, causing a sustained increase in glucocorticoid levels throughout development (Welberg, Seckl, & Holme, 2000). This, in turn, heightens behavioral reactivity to stress, increases behavioral disturbances and compromises physical health across the lifespan (Welberg & Seckl, 2001). In human research, however, the effect of antenatal stress on the offspring's stress reactivity is primarily assessed behaviorally (for reviews, see Van den Bergh et al., 2005b); very few studies assessed stress reactivity neuroendocrinologically (Gutteling, de Weerth, & Buitelaar, 2005, and O'Connor et al., 2005), and none assessed antenatal maternal effects across parallel dimensions, neuroendocrinologically as well as behaviorally. Similarly, only a few studies examined the effect of antenatal maternal distress repeatedly across development (Huizink et al., 2002, 2003; O'Connor et al., 2003); one study measured antenatal effect on behavioral stress reactivity repeatedly (Huizink et al., 2002), and one directly addressed the issue of stability (O'Connor et al., 2003). The purposes of the current study were to examine 1) the stability of antenatal maternal effects on stress reactivity over time, and 2) the relation between antenatal

maternal effects across the neuroendocrinological and behavioral dimensions of stress reactivity.

The enhanced stress reactivity or poor developmental outcomes observed in *adult* antenatally stressed offspring are often cited as evidence for the long-term effect of antenatal maternal stress on the offspring (for a review, see Maccari et al., 2003). For example, numerous cross-sectional studies were conducted with the intent to demonstrate the long-term effect of antenatal maternal stress. These studies showed in adulthood the difference between antenatally stressed and control animals in terms of behavioral reactivity to novelty, defensive withdrawal, self-administration of positive reinforcing drug, and the deterioration of spatial memory and normal circadian rhythms (Alonso, Arevalo, Afonso, & Rodriguez, 1991; Alonso, Navarro, Santana & Rodriguez, 1997; Deminiére et al., 1992; Fride, Dan, Feldon, Halevy & Weinstock, 1986; Van Reeth, Koehl, Weibel, Le Moal & Maccari, 1998; Poltyrev, Keshet, Kay, & Weinstock, 1996). In addition, effects of antenatal maternal stress on the offspring are also evidenced neuroendocrinologically. Differences in the functioning and morphology of the HPA-axis between antenatally stressed and control animals have been studied in adulthood to demonstrate the long-term effect of antenatal maternal stress on the offspring (Koehl et al., 1999; Maccari et al., 1995).

Among cross-sectional studies that examined in adulthood the effect of antenatal maternal stress on the offspring, a few attempted to identify the underlying mechanism responsible for the long-term effect of antenatal maternal distress on the offspring. For example, Vallée et al. (1997) tested the differences in behavior, memory and glucocorticoid levels between antenatally stressed and control animals during adulthood.

They found that antenatally stressed adult offspring exhibited heightened behavioral reactivity to novelty, poor memory performance and enhanced glucocorticoid response compared to control animals. Vallée et al. (1997) also found that such heightened behavioral reactivity to novelty was linked to enhanced glucocorticoid response. Based on these observations in adulthood, the authors concluded that the effect of antenatal maternal stress was long-lasting, persisting even to adulthood, and the offspring's HPA-axis served as the mechanism that underlay the long-term effect of antenatal maternal stress on the offspring's behavioral and cognitive outcomes observed in adulthood.

A few animal studies repeatedly assessed stress reactivity of antenatally stressed offspring across development to isolate the mechanism that underlies the stability and persistence of antenatal effect. For example, Henry, Kabbaj, Simon, Le Moal, and Maccari (1994) found that antenatally stressed offspring exhibited persistent elevation of plasma glucocorticoid level in response to novelty when assessed repeatedly over the first 90 days of postnatal life. The authors also reported that, when assayed concurrently with the repeated assessments of the offspring's glucocorticoid reactivity, the expression of the offspring's glucocorticoid receptors at the hippocampus also exhibited stable down-regulation, indicating a long-term attenuation of the feedback mechanism of the HPA-axis. The authors concluded from the evidence that the hyper-secretion of glucocorticoids observed over the development of the antenatally stressed offspring was the long-term effect of the down-regulation of the expression of glucocorticoid receptors, induced by the experience of antenatal stress.

Such long-term functional and morphological modifications of the HPA-axis in the antenatally stressed offspring have been linked to the stable and persistent effects of

antenatal maternal stress on the offspring's developmental outcomes. For example, Vallée et al. (1999) repeatedly tested the differences in neuroendocrinological and cognitive functions between antenatally stressed and control animals throughout early adulthood, middle age, and old age, and found long-term and parallel deterioration across both dimensions among the antenatally stressed offspring. Specifically, the authors found that the antenatally stressed offspring exhibited advanced aging in both neuroendocrinological and cognitive functions, such that adult antenatally stressed offspring exhibited heightened glucocorticoid reactivity and poor cognitive performances that are characteristics of a normal middle-aged animal, and the middle-aged antenatally stressed offspring functioned at a level comparable to that of a normal animal in its old age. In conclusion, the authors argued that the advanced age-related deterioration of cognitive performance among the antenatally stressed offspring was the result of the increased reactivity of the offspring's HPA-axis induced by antenatal stress.

Similarly, Burlet et al. (2005) showed that when a pregnant animal was injected with glucocorticoids to mimic endogenous increases in glucocorticoids associated with antenatal stress, the offspring exhibited consistent delay in motor development when assessed daily during the first three weeks of postnatal life. The authors also found that the antenatally exposed offspring had lower adrenal weight compared to controls (an indication of chronic hyperactivity of the offspring's HPA-axis) and an increased expression of corticotropin releasing hormone (CRH) in relation to arginine vasopressin (AVP) mRNA (an indication of long-term increases in the HPA activity). The authors argued that antenatal glucocorticoid exposure, a condition that resembles the offspring's

experience of antenatal stress, caused long-term hyper-activation of the offspring's HPA activity, which in turn caused persistent delay throughout development.

Findings from these animal studies suggest that 1) the effects of antenatal maternal stress on the offspring's neuroendocrinological and behavioral stress reactivity and developmental outcomes may be stable across the lifespan, and 2) the stability of antenatal effects on behavioral stress reactivity and developmental outcomes is a function of the sustained hyper-reactive neuroendocrinological system of the offspring, which resulted from exposure to antenatal stress.

In humans, epidemiological research and birth cohort studies provide indirect evidence of the long-term effect of antenatal adversity based on evidence that links enhanced stress reactivity and poor health outcomes among adults who had low birth weight (Phillips & Jones, 2006). In terms of direct, prospective evidence on the long-term effect of antenatal stress, different developmental studies have independently demonstrated that antenatal maternal distress is associated with heightened stress response in fetuses, neonates, infants, and children (for review, see Van den Bergh et al., 2005b). Most studies, however, focused on the effect of antenatal maternal distress at one particular developmental stage. As an exception, Huizink et al. (2002) assessed maternal distress during pregnancy and infant behavioral stress reactivity at 3 and 8 months. The authors found that the higher the maternal distress during pregnancy, the greater the behavioral reactivity in the offspring across both time points. Subsequently, Huizink et al. (2003) also reported that antenatal maternal distress significantly and consistently predicted 3- and 8-month developmental outcomes. The authors found that the higher the maternal distress during pregnancy, the lower the cognitive performances across both

time points. While the authors made no comparison between the antenatal effects across the two time points, the results were consistent with the argument that the effect of antenatal maternal stress is persistent.

One study addressed specifically the issue of stability regarding antenatal maternal effect. O'Connor et al. (2003) not only showed that the effect of antenatal maternal distress on behavior was observable both at 41 and 85 months, indicating that the higher the maternal distress during pregnancy, the greater the externalizing symptoms at both time points, the authors also compared the magnitude of antenatal effects across both time points, and concluded from their similar numeric values that the effects were stable. While such a comparison does not constitute a direct examination of the stability of the effect, the result is consistent with the hypothesis that the effect of antenatal maternal distress is stable.

Also, only a few human studies have examined the effect of antenatal maternal distress on the offspring's neuroendocrinological, instead of behavioral, stress reactivity (Gutteling et al, 2004, 2005; O'Connor et al., 2005). These studies consistently showed that the higher the maternal distress during pregnancy, the greater the neuroendocrinological reactivity to stress. No study has examined the effect of antenatal maternal distress across multiple dimensions or their co-development across the life span.

The Present Study

The current study examined in a sample of 30 mothers and their infants the stability of antenatal maternal effects over time and across different dimensions of stress reactivity. Mothers were recruited during pregnancy and maternal distress was assessed at the 3rd trimester. Information on infant behavioral stress reactivity was collected at 6 and

24 months, using maternal report. Neuroendocrinological stress reactivity was assessed at 12 and 18 months via salivary cortisol change in response to novel/challenging paradigms.

The current study addressed the following questions: 1) Does antenatal maternal distress predict 12- and 18-month neuroendocrinological stress reactivity? Are antenatal maternal effects on neuroendocrinological stress reactivity stable over time? 2) Does antenatal maternal distress predict 6- and 24-month behavioral stress reactivity? Are antenatal maternal effects on behavioral stress reactivity stable over time? 3) Does the change in antenatal maternal effects on neuroendocrinological stress reactivity from 12 to 18 months correlate with the change in antenatal maternal effects on behavioral stress reactivity from 6 to 24 months?

I hypothesized that 1) antenatal maternal distress is positively associated with both 12- and 18-month neuroendocrinological stress reactivity and the effect is stable over time, 2) antenatal maternal distress is positively associated with both 6- and 24-month assessment of behavioral stress reactivity and the effect is stable over time, 3) the change in antenatal maternal effects on neuroendocrinological stress reactivity from 12 to 18 months is positively correlated with the change in antenatal maternal effects on behavioral stress reactivity from 6 to 24 months.

Method

Participants

The analyses reported in the current study are based on data from a sub-sample of a larger study. In the original study, expectant mothers were recruited during the second or third trimester of pregnancy from prenatal education classes in the Greater Toronto

Area for a longitudinal study extending to the second year of infants' life, for which data collection proceeded from 1996 to 1999 (Atkinson et al., 2005; Raval et al., 2001). Of the 680 mothers attending classes, 357 (52%) completed the questionnaire. Based on screening scores (i.e., preference given to those whose scores suggested non-autonomy in attachment security), 233 (65%) mothers were invited to participate. Of these, 139 (60%) agreed. Of the 139 dyads, 113 returned for the 6-month visit, 97 returned and completed the 12-month visit, 93 returned and completed the 18-month visit, and 88 returned and completed the 24-month visit. Reasons for discontinuing participation were: mother being "too busy," family moving away from the city, and illness of the baby or mother.

While all participants in the original study received the full protocol, a few additional outcome measures were also administered to two randomly selected, non-overlapping sub-samples of 30, chosen according to the match between participant identification number and the number generated from a random table. These two independent samples of 30 are reported in the following as Studies 1 and 2. In the current study (Study 2), in addition to the original protocol, salivary cortisol was collected from a randomly selected sample of 30 infants prior to, and 20 minutes after two different stressors administered to all participants in the original study during their respective visits at 12 and 18 months.

This sub-sample of 30 dyads consisted of 15 (50%) female infants. Most mothers were Caucasian (82%), ranging from 27 to 42 years of age (M=32.90, SD=3.64). Mothers' and fathers' schooling ranged from 11 to 23 years, averaging 15.87 for mothers and 15.57 for fathers. Over 74% of the sample earned a family income exceeding $50,000 Canadian. This sub-sample of 30 dyads was not different in terms of maternal

age, mother's and father's education, and family income from either the initial sample of 139 or the subsequent samples 113, 97, 93, or 88 returning at 6, 12, 18 and 24 months respectively. Nor was this sub-sample of 30 different from either the initial or returning samples in terms of prenatal or postnatal assessment of maternal distress, or maternal reports of infant/toddler's stress reactivity. Nor were the two randomly selected samples of 30 different from each other in terms of the variables mentioned above.

Procedure

Data collection was conducted in one prenatal visit at the 3rd trimester of pregnancy and four postnatal visits at 6, 12, 18 and 24 months at the Hospital of Sick Children in Toronto. For the sub-sample of 30 reported in the current study, maternal distress was assessed during one prenatal visit at the 3rd trimester and two postnatal visits at 6 and 12 months using the Impact of Events Scale (IES; Horowitz, Wilner & Alvarez, 1979; Weiss & Marmar, 1997). Infant neuroendocrinological stress reactivity was assessed during the postnatal visits at 12 and 18 months in terms of changes in cortisol levels in reaction to maternal separation and reunion at 12 months, (Ainsworth, Blehar, Waters, & Wall, 1978) and the presentation of a series of increasingly stressful stimuli at 18 months (Garcia-Coll et al., 1988). Infant behavioral stress reactivity was measured using maternal report on the Infant Characteristics Questionnaire (ICQ; Bates et al. 1979), administrated to mothers at the 6- and 24-month visits.

Measures

Maternal Distress

Impact of Events Scale The IES was administered during the prenatal, 6- and 12-month postnatal visits. The IES is a self-report measure designed to assess current

subjective distress related to a specific life event stressor (Horowitz et al., 1979; Corcoran & Fischer, 1994). Because of its sensitivity to change, it has been employed in clinical settings to monitor clients' progress in therapy (Corcoran & Fischer, 1994). In the current study, the IES was administered to assess maternal distress associated with a specific event that occurred in the past 7 days. All 15 items of the IES were anchored to a specific stressor (Briere, 1997; Horowitz et al., 1979). Respondents were asked to rate the items on a 4-point scale according to how often each had occurred in the past 7 days. The 4 points on the scale are: 0 (not at all), 1 (rarely), 3 (sometimes), and 5 (often), and all 15 items were summed to generate a total stress score. In Horowitz' original study (Horowitz et al., 1979), calculations were conducted on the data of 66 subjects. The authors found that internal consistency of IES ranged from .79 to .92, the split-half reliability was .86, and the test-retest reliability across a one-week period was .87.

Neuroendocrinological Stress Reactivity

Stressor. Two standardized procedures were used as stressors to elicit cortisol stress responses: the Strange Situation described by Ainsworth and Witting (1969), and the novel stimuli presentation protocol described by Garcia-Coll et al. (1988). The Strange Situation was conducted at the 12-month laboratory visit, and the presentation of novel stimuli took place at the 18-month visit.

The strange situation mimics naturally occurring situations and is moderately stressful for 12- to 18-month-olds. It consists of eight 3-min episodes wherein the infant is alone, with mother, with a female stranger, or with both mother and stranger, in an unfamiliar setting. These episodes are ordered to increase stress in standardized increments that are manageable for the baby. There are two separations from the mother,

one in which the infant is with the stranger and one in which the infant is first alone and then with the stranger before the mother returns. Several studies reported individual differences in cortisol stress responses to this procedure (Goldberg et al., 2003; Hertsgaard, Gunnar, Erickson & Nachmias 1995; Nachmias, Gunnar, Mangelsdorf, Parritz & Buss, 1996; Spangler & Grossmann, 1993; Spangler & Schieche, 1998). The Strange Situation was used here only as a stressor for the purposes of salivary cortisol collection. The attachment classifications generated from the Strange Situation, or any behavior or affect coded from the separation-reunion episodes were not used in the following analyses.

In the novel stimuli presentation protocol, 19 increasingly stressful visual, auditory, and tactile stimuli were presented to the infant by an experimenter in the laboratory setting. The procedure took about 15 minutes, during which time an experimenter presented each stimulus for 20 seconds to the infant who was sitting in an infant chair. The stimuli were presented in a fixed order to achieve a gradual increase of intensity and the number of sensory modalities involved. The behavioral stress reactivity observed during the presentation of novel stimuli has been linked to maternal report of infant stress reactivity (Garcia-Coll et al., 1988). For a more detailed description of Garcia-Coll et al.'s protocol, please refer to Study 1 above. The video recording of 18-month behavioral observation was not accessible to the author and was therefore not available to be coded. The ICQ was therefore the only measure of infant stress reactivity administered repeatedly within the same time frame as the infant cortisol stress reactivity.

Salivary Cortisol Collection. All 12- and 18-month laboratory visits were scheduled in the morning between 9 and 10 AM. Although afternoon sampling is typical

in adult studies, this is not the usual practice with infants (Gunnar & White, 2001). During infancy, effects of daytime stressors, meals, and naps are highly variable from child to child and from day to day. Morning collection is advised to reduce these potential sources of variation. On arrival in the laboratory, mothers read and signed the consent forms and the first (baseline) saliva collection was performed as soon as possible. Mothers also completed a brief demographic questionnaire and provided information regarding the infant's schedule that day (e.g., time of waking and last meal). The priority during the laboratory session was to be minimally disruptive of the infant's normal periods of alertness, naps, and meal schedule. At each visit, a second saliva sample was collected 20 minutes after the completion of the corresponding laboratory protocol. During the 20 minutes, the dyads could remain in the laboratory playroom or go for a walk and return at the appointed time, the only restriction being that the infant should not ingest anything other than water during these periods. The exact time of each collection was recorded.

To collect saliva, infants sucked on a 6-inch cotton dental roll. Although use of sweet powdered drink crystals to encourage sucking has been standard practice with infants, we used no stimulants because studies suggested that oral stimulants might compromise the validity of assay values (Schwartz, Granger, Susman, Gunnar, & Laird, 1998). Once wet, the tip of the dental roll was "clipped" and placed in a needleless syringe. The plunger was then depressed to squeeze saliva into a vial with a screw cap for storage. Samples were frozen until assay. Two samples were collected at the laboratory session: baseline (on arrival) and after completion of each stressor.

Cortisol Assay. Samples were assayed in duplicate using the Diagnostics Products Corporation (DPC) Coat-A-Count® Cortisol Radioimmunassay kit. The procedure standardizes assay values from batch to batch. Before shipping for analysis, identifying information (subject number, time of day) was removed and replaced with a code number so that technicians were blind to the identity of the samples. Samples were assayed in duplicate using a standard radioimmunoassay procedure (Diagnostic Products, Los Angeles, California). Correlations between duplicates typically exceeded .95 or better; however, duplicates were re-assayed if they varied by 20% or more. The assay required 50 µl of saliva for each duplicate, for a total of 100 µl.

Behavioral Stress Reactivity

Infant Characteristics Questionnaire (ICQ). In the current study, the ICQ (Bates et al., 1979) was administered to mothers during the 6- and 24-month visits to assess infant/toddler stress reactivity according to their infants/toddlers' tendency to fuss or change moods in response to novel and stressful everyday life situations. When completing the ICQ, mothers judged to what degree their infants/toddlers' behaviors corresponded to questions asked in each specific item. Items were rated on a 7-point Likert scale, where a score of 4 corresponds to an average infant response, scores of 1 are viewed as being not at all irritable, and scores of 7 are viewed as very difficult responses by the child. The ICQ consists of 4 subscales: 1) difficultness, which concerns the degree of fussiness, expressions of frustration, and soothability in daily circumstances, 2) adaptability-reactivity, which addresses the initial reactions to novel events, persons, objects and other stimuli, 3) activity level, which concerns the general responsiveness and overall activity level, and 4) unpredictability, which concerns the regularity of daily

events, or lack thereof, such as feedings, diaper changes, and sleep patterns. In the original article, Bates et al. (1979) found that internal consistency of the four subscales ranged from .79 to .39 and test-retest reliability ranged from .70 to .47. A more recent publication (Lemelin, Tarabulsy & Provost, 2002) reported item internal consistencies of the four ICQ subscales ranged from .63 (Activity) to .82 (Difficultness). Subsequent to his original study, Bates (1992) reported high levels of convergence with various subscales of other maternal reports of temperament that addressed dimensions similar to the four ICQ scales, such as the Revised Infant Temperament Questionnaire (Carey & McDevitt, 1978) and the Infant Behavior Questionnaire (Rothbart, 1986).

Variable Selection

The current study examines the impact, stability and the interrelatedness of the behavioral and neuroendocrinological effects of antenatal maternal distress. The current model was tested below using the IES total scores, 6- and 24-month ICQ total scores, and 12- and 18-month cortisol difference scores between pre- and post-stressor samples. The sample reported here was one of the two non-overlapping samples that were drawn from a single cohort wherein multiple measures of maternal distress and infant stress reactivity were used. I specifically selected the IES and ICQ as the maternal and infant measures respectively in the current study for methodological reasons. The IES was used to examine the stability and change of antenatal maternal effect because of its event-specificity and sensitivity to chance (Horowitz et al., 1979; Corcoran & Fischer, 1994). On the other hand, ICQ was the only infant reactivity measure administered repeatedly within the same time frame as infant salivary cortisol, and this enabled me to examine the

stability of antenatal effects across different, yet parallel dimensions of neuroendocrinological and behavioral stress reactivity.

The ICQ has a number of subscales: 1) Difficultness, which measures infant general non-specific fussiness in daily circumstances, 2) Inadaptability, which addresses the initial reactions to novel events, persons, objects and other stimuli, 3) Activity level, which concerns the overall level of activity, and 4) Unpredictability, which concerns the regularity of daily functioning. While each subscale of the ICQ addresses a different dimension of how the infant reacts to and regulates stress, Lemelin, Tarabulsy and Provost (2002) point out that these dimensions are complementary and that infant stress reactivity and regulation may be best captured by the ICQ total score, which represents a broader and more contextual approach to characterizing infant reaction to and the regulation of emotion. For this reason, the ICQ total score was used in the following analyses.

On the other hand, although salivary cortisol was only sampled before and after the stressors, a number of parameters had been generated, each representing a different aspect of HPA functioning. While baseline cortisol levels reflect the resting activity of the system, changes in cortisol levels in response to stressor from the baselines reflect the reactivity of the system in relation to its resting state. However, if the baseline levels were subtracted from post-challenge level to form change scores, or statistically partialled out from either the post-stressor levels or from the change scores, more direct measures of reactivity resulted. Compared to baseline measures and other measures of HPA reactivity, the difference score between pre- and post-stressor cortisol levels are more often used in the literature as an indicator of neuroendocrinological stress reactivity

(Gunnar & White, 2001), and is conceptually more compatible with 1) maternal report on infant behavioral reactivity and regulation as assessed by the ICQ in the current study, and 2) laboratory observed behavioral reactivity measures in the other two studies reported in the current dissertation.

Results

In the following, results from the preliminary analyses and model testing will be reported in turn. Findings reported as preliminary analyses included 1) the distribution and transformation of variables, where variables were transformed according to Tukey's (1977) "ladder of re-expression" (see also Mosteller & Tukey, 1977; Cohen, Cohen, Akins & West, 2003), 2) exploratory correlation analyses between pairs of key variables as well as their derivatives, 3) scatterplots of relations between key variables, and 4) exploratory correlation and regression analyses of the effect of different antenatal maternal distress measures on infant stress reactivity measures, singly or simultaneously. On the other hand, model testing begins with individual multiple regression analyses of antenatal maternal effect on infant stress reactivity, over time and across dimensions. Regression diagnosis, such as influential statistics (Mahalanobis Distances, Cook's Distances and the Centered Leverage Values) and residuals check (normality and homoscedasticity) are provided to ensure the assumptions of the regression analysis are met. Within each dimension (neuroendocrinological or behavioral), the stability of antenatal maternal effect over time is examined by 1) computing the differences between the two regression coefficients generated from the regression model of each time point, followed by testing the correlation between influential statistics generated from the regression model of the two time points. The final model being examined is the

interrelatedness between the changes of neuroendocrinological and behavioral effects of antenatal maternal distress over time, operationalized by the correlation between the changes of inferential statistics generated by regression models of each of the two dimensions.

Preliminary Analyses

In the following, the distributions of all variables relevant to the overall model, as well as the derivatives of these variables are reported. Distributions of transformed variables are reported in addition to those of raw scores where appropriate. Secondly, the intercorrelations among variables derived from different pairs of core constructs that constituted the overall model are examined in turn. To provide visualization for the relations between different pairs of key variables, scatterplots are also reported in the following. Finally, potential covariates are screened in terms of their confounding influences on the variables selected to test the model of stability proposed in the current study.

Descriptive statistics

Variable Distribution and Transformation

Reported in the current section are the distributions, skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality on 1) antenatal and postnatal IES total scores, 2) 12- and 18-month salivary cortisol parameters, and 3) 6- and 24-month ICQ subscales and total scores. Transformed variables are reported in addition to raw scores when the distributions of raw scores significantly deviated from normal.

Antenatal and Postnatal Maternal Distress

3rd trimester, 6-month and 12-month IES scores. In the current study, maternal distress was examined in terms of the mother's total scores on the IES, administered at the 3rd trimester, and at 6 months and 12 months postnatally. Figures A2.1-A2.3 report the distributions of the 3rd trimester, 6-month and 12-month IES total scores. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality on all IES scores are reported in Table A2.1. The results indicated that no IES score exhibited substantial skewness or kurtosis (the statistics were deemed to be substantial when the ratio between skewness/kurtosis statistics and their corresponding standard errors was greater than 2). The Shapiro-Wilk tests of normality also indicated that the distributions of IES scores did not significantly deviate from normal. Hence, the raw IES scores were used in the following to test the proposed model.

Infant Neuroendocrinological Stress Reactivity

12- and 18-month Salivary Cortisol Parameters. Infant neuroendocrinological stress reactivity was assessed in the following in terms of parameters generated from salivary cortisol collected from the infants at 12 and 18 months. The distribution of infant cortisol level at baseline and in reaction to challenge, the difference score between baseline and post-challenge levels, the standardized residual of the post-challenge level controlling for baseline, and the standardized residual of the difference score controlling for baseline are reported separately for 12 and 18 months in Figures A2.4-A2.8 and A2.9-A2.13 respectively. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality of each of the cortisol parameters are reported in Table A2.2. The results indicated that substantial skewness was exhibited in 12-month baseline and the difference score between baseline and the post-challenge levels, and in 18-month

baseline, post-challenge levels, and the standardized residuals of post-challenge and difference scores. Similarly, substantial kurtosis was exhibited in 12-month difference score between baseline and post-challenge levels, and in all 18-month cortisol parameters. The Shapiro-Wilk tests of normality revealed that the distributions of the 12-month cortisol baseline and difference score, and all 18-month cortisol parameters with the exception of the difference score, deviated significantly from normal. To this end, log-transformation was applied to all 12- and 18-month cortisol parameters to normalize their distributions.

Figures A2.14 to A2.23 report the distributions of log-transformed 12- and 18-month cortisol parameters. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality of each cortisol parameters are reported in Table A2.3. Results indicated that all log-transformed 12-month cortisol parameters exhibited substantial skewness and kurtosis, with the exception of the baseline value. In addition, the distributions of log-transformed 12-month cortisol parameters deviated significantly from normal. On the other hand, none of the log-transformed 18-month cortisol parameters exhibited skewness or kurtosis, nor did any of the 18-month parameter's distribution deviate significantly from normal. Hence, the log-transformed 18-month cortisol parameters were used in the proposed model. On the other hand, to further normalize the 12-month cortisol parameters, the raw values of the parameters were inversely transformed. The distributions of the inverse-transformed 12-month cortisol parameters are reported in Figures A2.24 to A2.28. Their skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality on each cortisol parameters are reported in Table A2.4. The result indicated that, among all 12-month cortisol parameters, only the baseline

and the difference score did not exhibit substantial skewness or kurtosis, and did not deviate significantly from normal after inverse transformation. Since the 12-month cortisol difference score was selected to test the overall model of stability, no further transformation was conducted. The inversed values were further reflected before any correlation or regression analyses were conducted to align the metric of the variables with the directionality of the conceptual relation.

As expected, cortisol parameters were highly correlated with one another within each time point. Correlation analyses indicated that 12-month cortisol baseline was negatively and significantly associated with the difference score between baseline and post-challenge levels ($r=-.84$, $p<.01$). 12-month post-challenge cortisol level, on the other hand, was positively and significantly associated with the magnitude of the difference score ($r=.47$, $p<.05$) and the standardized residuals of post-challenge level and difference score controlling for baselines ($rs=1.00$, $p<.01$). The difference score at 12 months was significantly correlated with the standardized residuals of post-challenge levels and difference scores controlling for baselines ($rs=.54$, $p<.01$). Similarly, 18-month cortisol baseline was significantly associated with the post-challenge level ($r=.50$, $p<.01$) and with the difference score between baseline and post-challenge levels ($r=-.65$, $p<.01$). In addition to baseline, the 18-month post-challenge level was also significantly associated with the standardized residuals ($rs=.87$, $p<.01$). As the variable selected to examine the stability of antenatal maternal effects, the reflected, inversely transformed difference score at 12 months was significantly associated with the log-transformed difference score at 18 months ($r=.36$, $p<.05$).

Infant Behavioral Stress Reactivity

6- and 24-month ICQ Subscales and Total Scores. In the current study, infant behavioral stress reactivity was measured using the Infant Characteristics Questionnaire (ICQ), administered to the participating mothers at 6 and 24 months. Figures A2.29 to A2.38 report the distributions of 6- and 24-month ICQ subscales and total scores. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality of the subscale and total scores are reported in Table A2.5. Results indicated that, with the exception of 24-Month Unpredictability subscale, the distribution of all 6- and 24-month ICQ subscale and total scores exhibited no substantial skewness, kurtosis or significant deviation from normal. Since it was the total score that was selected to examine the overall model, no transformation was performed and the raw values of the ICQ subscale and total score are reported in the following.

Exploratory Analyses

Reported in the current section are the basic correlational analyses that serve as the building blocks for the examination of the stability of antenatal maternal effect on offspring's neuroendocrinological and behavioral reactivity. In the following, the relations between maternal distress and the offspring's neuroendocrinological reactivity at 12 and 18 months are first examined, followed by the relations between maternal distress and the offspring's behavioral reactivity at 6 and 24 months. Scatterplots were provided to describe relations between key variables selected to examine the proposed model of stability. In addition, since participants of the current study belonged to a larger study where maternal distress was also assessed in terms of the Beck Depression Inventory (BDI), correlation analyses between BDI scores and offspring's neuroendocrinological and behavioral reactivity measures, as well as multiple regression

analyses that examined the unique contributions of the BDI and IES on the offspring's neuroendocrinological and behavioral reactivity measures are also reported in the following.

Maternal distress and Neuroendocrinological Stress Reactivity

IES Scores and Salivary Cortisol Parameters

In the current study, maternal distress was operationalized in terms of the mother's total scores on the IES, administered at the 3rd trimester, and at 6 months and 12 months. As previously noted, the raw values of the IES scores are reported in the following analyses. Pre- and post-challenge infant salivary cortisol was collected at 12 and 18 months. At each time point, 5 cortisol parameters were generated: baseline, post-challenge cortisol level, the difference score between baseline and post-challenge cortisol level, the standardized residual of the post-challenge level controlling for baseline, and the standardized residual of the difference score controlling for baseline. As described above, all 12-month cortisol parameters were inversely transformed while 18-month parameters were log-transformed. The inversed value was however first reflected before correlation and regression analyses were performed, such that metric of any pair of variables of interest would align with the directionality of the conceptual relation. Table A2.6 reports the intercorrelations between IES scores and all 12- and 18-month cortisol parameters. The results indicated that 3rd trimester IES score was significantly associated with 12-month cortisol difference score ($r=.36$, $p<.05$), 18-month cortisol baseline ($r=-.49$, $p<.01$) and 18-month cortisol difference score ($r=.40$, $p<.05$). Nevertheless, 6-month IES score was not associated with any cortisol parameters at either 12 or 18 months. 12-month IES score significantly predicted 12-month cortisol baseline ($r=.35$, $p=.05$) and

difference score (r=-.35, p=.05), as well as 18-month cortisol difference score (r=-.38, p<.05) and the standardized residual of cortisol difference score with the baseline value controlled for (r=-.38, p<.05).

The difference score between cortisol level at baseline and in reaction to challenges was selected to operationalize the concept of neuroendocrinological reactivity to stress in the current study. The scatterplots of the relations between antenatal IES score and the 12- and 18-month cortisol difference scores are reported in Figures A2.39 and A2.40 respectively.

BDI Scores and Salivary Cortisol Parameters

The participants of the current study belonged to a larger study where maternal distress was also assessed with the BDI, administered at the 3rd trimester of pregnancy, and at 6-, 12- and 18-months postnatal. In the following, results from correlation analyses between, on one hand, different BDI scores, and, on the other hand, all of the offspring's cortisol parameters from 12- and 18-month are reported. Results indicated that, with the exception of the 3rd trimester BDI, which predicted 18-month cortisol difference score in an unexpected direction (r=-.36, p<.05), neither antenatal nor postnatal BDI score alone significantly predicted any other 12- or 18-month cortisol parameters.

Regressing Cortisol Difference Scores on Antenatal IES and BDI Scores

To examine the unique contribution of antenatal maternal BDI and IES scores on the offspring's cortisol difference scores, multiple regression analyses were performed, with antenatal BDI and IES scores entered simultaneously as predictors. When 12-month cortisol difference score was regressed simultaneously on both 3rd trimester IES and BDI scores, the unique contribution of the IES score was significant (B=1.04, SE=.52, p=.05),

but not the contribution of the BDI score (B=6.54, SE=7.22, ns). On the other hand, when 18-month cortisol difference score was regressed simultaneously on both 3rd trimester IES and BDI scores, both 3rd trimester IES score and BDI score accounted for significant amount of unique variance from 18-month cortisol difference score (B=.03, SE=.01, $p<.05$ and B=-.44, SE=.19, $p<.05$, respectively). Nevertheless, BDI predicted the 18-month cortisol difference score in an unexpected direction.

Maternal Distress and Behavioral Stress Reactivity

IES Scores and ICQ Subscale and Total Scores

While maternal distress was measured at 3rd trimester, and at 6 months and 12 months in terms of IES total score, infant behavioral stress reactivity was assessed at 6 and 24 months using the ICQ, which consist of four different subscales: Difficultness, Inadaptability, Activity, and Unpredictability. As reported above, with the exception of 24-month ICQ Unpredictability subscale, the distribution of the raw values of both IES and ICQ did not exhibit substantial skewness or kurtosis nor did they significantly deviate from normal. Hence, the raw values of IES and ICQ were used in the following analyses. Reported in Table A2.7 are the intercorrelations between IES scores and 6- and 24-month ICQ subscale and total scores. The result indicated, 3rd trimester IES scores significantly predicted both 6- and 24-month ICQ total scores (r=.42 and .43 respectively, $ps<.05$). 3rd trimester IES scores also predicted 6-month ICQ Difficultness (r=.37, $p<.05$) and 6-month ICQ Unpredictability (r=.36, $p<.05$) subscales. 6- and 12-month IES scores predicted 6-month ICQ Difficultness subscales (r=.48 and .59 respectively, $p<.01$) and 6-month ICQ total scores (respectively, r=.33, $p<.10$ and r =.59, $p<.01$). As noted above, the ICQ total scores were selected to examine the overall model proposed in the current

study. The scatterplots of the relations between 3rd trimester IES scores and 6- and 24-month ICQ total scores are reported respectively in Figures A2.41 and A2.42.

BDI Scores and ICQ Subscale and Total Scores

As previously noted, participants of the current study belonged to a larger study where maternal distress was also assessed using the BDI, which was administered at the 3rd trimester, and at 6 months, 12 months and 18 months. 3rd trimester BDI score was not significantly associated with any of the 6- or 24-month ICQ subscales or total scores. 6-month BDI score, however, was significantly associated with 24-month Inadaptability ($r=.63$, $p<.01$) and Unpredictability ($r=.42$, $p<.05$) subscales scores as well as 24-month total score ($r=.40$, $p<.05$). 12-month BDI score, on the other hand, did not predict any 24-month ICQ subscale or total score. Nevertheless, 18-month BDI score predicted 24-month ICQ Activity subscale score ($r=.47$, $p<.01$) and total score ($r=.40$, $p<.05$) at statistically significant levels, and predicted 24-month Difficultness subscale score at a marginally significant level ($r=.34$, $p<.10$).

Regressing ICQ Adaptation-reactivity Subscales Scores on Antenatal IES and BDI scores

To examine the unique contribution of antenatal maternal BDI and IES scores on offspring's ICQ total scores, multiple regression analyses were performed, with antenatal BDI and IES scores entered simultaneously as predictors. The results indicated that 3rd trimester IES score contributed uniquely and significantly to both 6- and 24-month ICQ total scores (respectively, $B=1.14$, $SE=.46$ and $B=1.25$, $SE=.51$; $ps<.05$), while 3rd trimester BDI score accounted for no significant unique variance.

Screening for Potential Covariates

Table 2.1 reports the means and standard deviations of 1) antenatal and postnatal IES scores, 2) 12- and 18-month cortisol baselines, and 3) 6- and 24-month ICQ adaptability-reactivity subscale scores. Table 2.2 reports the correlations among outcome variables reported in the following analyses.

Insert Table 2.1 and 2.2 about here

Maternal Distress

Zero-order correlational analyses indicated that 3rd trimester IES scores were associated with postnatal IES scores (r=.39 to r=.43, p<.05) but not associated with sociodemographic variables such as maternal age, years of schooling, family income, martial status or ethnic origin.

12- to 18-month Neuroendocrinological Stress Reactivity

Zero-order correlational analyses indicated that cortisol change from baselines assessed during 12- and 18-month lab visits were correlated with each other (r=.36, p<.05), but not associated with any sociodemographic variables or the biological sex of the offspring.

6- to 24-month Behavioral Stress Reactivity

Zero-order correlational analyses indicated that ICQ total scores reported at 6- and 24-month visits were correlated with each other (r=.61, p<.01), but not associated with the offspring's biological sex or any sociodemographic variables.

Hypothesis Testing

Does Antenatal Maternal Distress Predict 12- and 18-Month Neuroendocrinological Stress Reactivity, and are Antenatal Maternal Effects on Neuroendocrinological Stress Reactivity Stable over Time?

Antenatal Effect on 12-month Neuroendocrinological Stress Reactivity

Sequential multiple regression analysis was conducted to predict 12-month cortisol change from baseline from 3rd trimester IES score, with the mean of 6- and 12-month postnatal IES scores first partialled out. Regression diagnosis revealed that, while no individual case exerted undue influence on the relation (Mahalanobis Distances, Cook's Distances and the Centered Leverage Values were all within acceptable range), the regression residuals nevertheless deviated significantly from normal distribution (Shapiro-Wilk =.91, $p<.05$) and lacked homoscedasticity (White's Test: Chi-sq[5]=12.37, $p<.05$), thus violating the assumptions of regression analyses. The violation of regression assumptions suggested that the least-square estimates could potentially be biased, and therefore the results of the current analyses would not be considered in the subsequent model testing. Hence, the result is not reported in the current section.

Antenatal Effect on 18-month Neuroendocrinological Stress Reactivity

Sequential multiple regression analysis was conducted to predict 18-month cortisol change from baseline from 3rd trimester IES score, with the mean of 6- and 12-month postnatal IES scores first being partialled out. Regression diagnosis revealed that, while no individual case exerted undue influence on the relation (Mahalanobis Distances, Cook's Distances and the Centered Leverage Values were all within acceptable range), the regression residuals nevertheless deviated significantly from normal distribution (Shapiro-Wilk = .93, $p<.05$) and lacked homoscedasticity (White's Test: Chi-sq[5] =

11.52, $p<.05$), thus violating the assumptions of regression analyses. The violation of regression assumptions suggested that the least-square estimates could potentially be biased, and therefore the result of the current analyses would not be considered in the subsequent model testing. Hence, the result is not reported in the current section.

Stability of Antenatal Effects on Neuroendocrinological Stress Reactivity over Time

The current study proposed to examine the stability of antenatal effect on neuroendocrinological stress reactivity over time by 1) comparing the two correlated and overlapping multiple regression coefficients of the IES-cortisol relations by means of Meng et al.'s (1992) adaptation of the Fisher's *z* transformation procedure and 2) examining the relation between the influential statistics of 3rd trimester IES score's effect on 12- and 18-month cortisol difference scores respectively. Unfortunately, since the regression coefficients of the relations between IES scores and 12- and 18-month cortisol difference scores were potentially biased due to the violation of regression assumptions, the stability of antenatal effect on neuroendocrinological stress reactivity over time could not be examined in the current study.

Does Antenatal Maternal Distress Predict 6- and 24-month Behavioral Stress Reactivity, and are Antenatal Maternal Effects on Behavioral Stress Reactivity Stable over Time?

Antenatal Effect on 6-month Behavioral Stress Reactivity

Sequential multiple regression analysis was conducted to predict 6-month ICQ total scores from 3rd trimester IES scores, with 6-month postnatal IES score first partialled out. Results indicated that 3rd trimester IES scores significantly predicted 6-month ICQ total scores even after first controlling for the effect of postnatal IES scores

(*B*=.97, *SE*=.48; *p*=.05). Follow-up regression diagnoses revealed that no individual case exerted undue influence on the relation (Mahalanobis Distances, Cook's Distances and the Centered Leverage Values were all within acceptable range) and the regression residuals did not deviate significantly from normality of distribution (Shapiro-Wilk = .97, ns.), nor violate the assumption of homoscedasticity (White's Test: Chi-sq[5] = 4.86, ns.). This result therefore suggested that the greater the maternal distress during pregnancy, the greater the infant behavioral stress reactivity at 6 months, even after controlling for the effect of postnatal maternal distress.

Antenatal Effect on 24-month Behavioral Stress Reactivity

Sequential multiple regression analysis was conducted to predict 24-month ICQ total score from 3rd trimester IES score, with the mean of 6- and 12-month postnatal IES scores first partialled out. Results indicated that 3rd trimester IES score significantly predicted 24-month ICQ total scores even after first controlling for the effect of postnatal IES scores (*B*=.1.24, *SE*=.55; *p*<.05). Follow-up regression diagnoses revealed that no individual case exerted undue influence on the relation (Mahalanobis Distances, Cook's Distances and the Centered Leverage Values were all within acceptable range) and the regression residuals did not deviate significantly from normal distribution (Shapiro-Wilk = .98, ns.), nor violate the assumption of homoscedasticity (White's Test: Chi-sq[5] =5.61). This result suggested that the greater the maternal distress during pregnancy, the greater the infant behavioral stress reactivity at 24 months, even after controlling for the effect of postnatal maternal distress.

Stability of Antenatal Effect on Behavioral Stress Reactivity over Time

To examine the stability of antenatal maternal effects on behavioral stress reactivity over the 6- to 24-month period, I first compared the two regression coefficients representing antenatal effects on 6- and 24-month stress reactivity. While, the null hypothesis cannot be proven, the lack of statistical difference over time is consistent with the hypothesis that antenatal effects are stable. I therefore examined the stability of antenatal effects in terms of the correlation between the individual dyad's contributions to the antenatal IES-cortisol relations measured at 6 and 24 months.

To compare the two correlated and overlapping multiple regression coefficients, Meng et al.'s (1992) adaptation of the Fisher's *z* transformation procedure was employed. Analyses indicated that there was no statistically significant difference between the two coefficients (z=.04, ns.). Although the null hypothesis cannot be proven, the lack of statistical difference between the coefficients is consistent with the hypothesis that the effects of antenatal distress on the offspring's behavioral stress reactivity were stable between 6 and 24 months.

In addition, to examine the correlation of antenatal maternal effects on the offspring's stress reactivity between 6 and 24 months, each individual dyad's contribution to the relation between antenatal IES and the offspring's ICQ at each time point was first quantified in terms of the dyad's influential statistic in the antenatal IES-ICQ relation at that particular time point. The influential statistics for each dyad was the magnitude of standardized change in the regression coefficient of the antenatal IES-ICQ relation resulting when the particular dyad was removed from the regression model. Subsequently, zero-order correlational analysis was conducted to examine the association between the 6- and 24-month influential statistics. Results indicated that the dyad's

influential statistic in the antenatal IES-ICQ relation at 6 months was significantly correlated with the dyad's influential statistic at 24 months (r=.61, p<.01), suggesting that the effect of antenatal maternal distress on the offspring's behavioral stress reactivity was stable between 6 and 24 months.

Does the Change in Antenatal Maternal Effects on Neuroendocrinological Stress Reactivity from 12 to 18 Months Correlate with the Change in Antenatal Maternal Effects on Behavioral Stress Reactivity from 6 to 24 Months?

In the final model which I proposed to examine in the current study, the change in antenatal maternal effects on stress reactivity over time was calculated for each infant based on each individual dyad's contribution to the relation between antenatal maternal distress and the offspring's stress reactivity, quantified in terms of the individual's influential statistics and the antenatal distress-stress reactivity relation. The influential statistics for each dyad is the magnitude of change (standardized) in the regression coefficient when the dyad was removed from the regression model. The difference scores between each dyad's influential statistics across the two time points would then be computed for each dimension of stress reactivity to represent changes in either the neuroendocrinological or the behavioral effect of antenatal distress. Finally, Zero-order analysis of the two difference scores was employed to examine whether changes in antenatal effect on neuroendocrinological stress reactivity over time were associated with antenatal maternal effect on behavioral stress reactivity over time. Unfortunately, since the regression coefficients of the relations between IES scores and 12- and 18-month cortisol difference scores might be potentially biased due to the violation of regression assumptions, the stability of antenatal effect on neuroendocrinological stress reactivity

over time could not be examined, and thus the links between neuroendocrinological and behavioral effect of antenatal maternal distress over time could not be tested in the current study.

Discussion

The high prevalence of cardiovascular and metabolic diseases among adults who had low birth weight has led epidemiologists to conclude that there may be a fetal origin of adult diseases (Barker, 1991), wherein the experience of antenatal adversity permanently alters the set point of normal fetal growth, predisposing individuals to diseases across the life-span (Barker, 1998). Birth cohort studies show that enhanced neuroendocrinological stress reactivity was linked to poor health among adults who had low birth weight, and it was therefore hypothesized that antenatal adversity induces long-term change in the offspring's HPA system, which is in turn responsible for the effect of antenatal adversity observed in adulthood (Phillips et al, 2000). Non-human animal studies have shown that 1) the effects of antenatal stress on offspring's neuroendocrinological and behavioral stress reactivity are stable across the life span (Vallée et al., 1999) and 2) the stability of behavioral stress reactivity is a function of the sustained hyper-reactive neuroendocrinological system, the result of exposure to antenatal stress (Henry et al., 1994). It remains to be demonstrated prospectively in humans that the effect of antenatal stress is stable, and that the stability in behavioral effect of antenatal stress is associated with the programming of the HPA axis. In this preliminary study I hypothesized that 1) antenatal maternal distress is associated with 12- and 18-month infant neuroendocrinological stress reactivity, and the effect is stable, 2) antenatal maternal distress is associated with 6- and 24-month behavioral stress

reactivity, and the effect is stable, 3) the change in antenatal maternal effect on neuroendocrinological stress reactivity from 12 to 18 months is correlated with the change in antenatal maternal effect on behavioral stress reactivity from 6 to 24 months.

Unfortunately, the current study is not able to provide conclusive evidence to support the hypothesis that antenatal maternal distress is associated with either 12- or 18-month infant neuroendocrinological stress reactivity, and for this reason the current study is not in a position to examine whether the effect of antenatal maternal distress on neuroendocrinological stress reactivity is stable over time, or whether changes in neuroendocrinological effect of antenatal maternal distress over time is correlated with changes in behavioral effect of antenatal maternal distress within the same time frame. Nevertheless, findings from this preliminary study are not inconsistent with the hypothesis that antenatal maternal distress is associated with 6- and 24-month behavioral stress reactivity as reported by the mothers, and that the behavioral effect of antenatal maternal distress is stable over time.

However, the preliminary findings reported in the current study need to be interpreted with extreme caution. According to Nunnally and Bernstein (1994), capitalization upon chance decreases with sample size and increases with the pool of possible predictors and outcomes. In their view, sample *R* is systematically biased upward compared to the population *R*, and one major reason for the bias is that predictors are usually selected from a larger set. Such bias is negligible when the sample size-to-predictors ratio is large, but becomes substantial when the ratio drops to 10:1, which is the level where the current study is at. This biased selection takes enormous advantage of chance when it is based upon data and not theory (Nunnally & Bernstein, 1994).

Although variable selection in the current study was not dictated by the data but guided by theory, the sheer number of candidate measures associated with each construct greatly increases the probability of having predictors being highly correlated with the criterion simply because of sampling error, or having spurious intercorrelations among predictors that may artificially inflate the size of *R*. In addition, even if predictors were not selected from a larger pool, the *R* obtained from a relatively small sample (with sample size-predictor ratio being around 10:1) will tend to become smaller when reapplied to a larger sample. Large samples have lower probability of producing unusually large correlation by chance because the parameter estimates are more stable (Nunnally & Bernstein, 1994).

One major factor that contributed to the inability of the current study to provide valid evidence consistent with the hypotheses that involve cortisol parameters as outcomes (for example, predicting 12- and 18-month infant neuroendocrinological stress reactivity from antenatal maternal distress, the stability of the neuroendocrinological effect of antenatal maternal distress, and the covariation between behavioral and neuroendocrinological effect of antenatal maternal distress over time) is the lack of normality in the regression residuals of models conducted to test these hypotheses. Despite the non-normal distribution of regression residuals, individual cortisol parameters in the current study have in fact been transformed according to Tukey's graphical diagnostic procedure to offset the parameters' non-normality based on the "ladder of re-expression," which indirectly normalizes the distribution of regression residuals through the normalization of individual variables. Unfortunately, for all cortisol parameters examined in the current study, the transformation performed on individual variables was not sufficient in normalizing the distribution of regression residuals. A more direct

analytical method is nevertheless available, which estimates the exact power transformation on the variables that minimize the regression residuals' deviation from normal (Box & Cox, 1964). Further, least-square multiple regression analysis is very sensitive to outlier and influential points (Stevens, 1984), which may result in the non-normally distributed regression residuals observed in the current study. While regression diagnostics indicate that influence and leverage of all cases fall within acceptable range, as scatterplots in Figures A2.39 and A2.40 indicate, some cases are more influential on the relationship than others. It has been proposed that robust regression analysis that utilizes maximum likelihood estimator rather than least square estimator may circumvent the potential impact of individual influential points on the distribution of regression residuals (Huber, 1981). Finally, the fact that the distributions of cortisol parameters in most samples contain outliers and are not normally distributed (Gunnar & White, 2001; Goldberg et al., 2003), may reflect the possibility that they were drawn from a non-normal population distribution. In combination with small sample size, the least-square estimation of the current study may be seriously biased. As such, Fox (2002) proposed that bootstrapping regression model may potentially be a solution to regression analysis involving variables drawn from non-normal population distribution and with small sample size.

The preliminary findings reported in this study are consistent with the animal literature and limited human literature available. However, considerable caution has to be taken to interpret them within the context of their potential lack of generalizability, the potential capitalization on chance resulting from the high predictors-to-sample size ratio, and their inability to replicate the core relationships across dimensions due to the

violation of modeling assumptions. Current animal and human literatures indicate that antenatal maternal distress is associated with heightened stress reactivity observed repeatedly over the lifespan of the offspring. In the current study, stability was operationalized as 1) the lack of statistically significant difference between repeated assessments of stress reactivity over time and 2) the significant correlation between the magnitude of individual's contribution to the effect of antenatal maternal distress at time one and two. While the preliminary findings reported in the current study are consistent with the hypothesis, the findings are far from conclusive: 1) the null hypothesis cannot be proven, making the lack of statistical difference between parameters a poor proxy for stability and 2) individual contributions to the least-square estimate as the parameter of stability may capitalize on the spuriousness of parameter estimation associated with a small sample.

Limitations

One of the limitations of the current study is its small sample size, which undermines the generalizability of its findings. The number of inferential statistics conducted in the current study in relation to its relatively small sample size may increase Type I error rate. Nonetheless, as reported in Study 1 above and Study 3 below, the relation between antenatal maternal distress and infant stress reactivity was not replicated across dimensions (significant at the behavioral, but not endocrinological dimension), making the positive preliminary findings reported here possibly attributable to chance. While I recognize that the large number of exploratory analyses conducted greatly increased the probability of type 1 error, given the scarcity of empirical data on the

mechanism of antenatal programming in human, it would risk type 2 error if the full potential of the data set was not explored.

In addition, there were no exclusion criteria to screen participants for any physical or mental health conditions during pregnancy, or any congenital defects that may complicate pregnancy or fetal health. Nor was there any information collected from the dyads regarding the perinatal period, such as mode of delivery, prematurity, birth complications or obstetric intervention. Nevertheless, pregnant mothers were recruited from a low risk community, and had no known involvement with any treatment program.

Further, there exist limitations inherent in the design of Study 2. The IES assesses maternal distress and the ICQ reports infant behavioral stress reactivity, and both rely on maternal report which possibly inflates the relation due to reporter bias. I am not able to replicate the effect of antenatal maternal distress on infant stress reactivity with neuroendocrinological stress reactivity in the current study. Nevertheless, the relation between antenatal maternal distress and infant stress reactivity has been replicated with behavioral observation reported in Study 1 above and Study 3 below. Another limitation is the timing of the stress reactivity assessment, which was not symmetrical across the behavioral and neuroendocrinological dimensions, where cortisol samples and maternal reports were not collected at the same time, and the interval between the repeated collections of stress reactivity was not equivalent across dimensions.

While the preliminary findings from Study 2 are not inconsistent with the hypothesis that the effect of antenatal maternal distress on the offspring's stress reactivity are stable over time, this relation could also be explained in terms of the persistent effect

of genetic inheritance. Only if the dyad's genetic information was collected could we isolate the differential influence from antenatal, postnatal and genetic effects.

Future Directions

The preliminary findings from the current study are consistent with the hypothesis that the effect of antenatal maternal distress on behavioral stress reactivity is stable over time. They are also consistent with the findings of the animal literature, where the effect of antenatal maternal distress on behavior persists over the life span. The animal literature also suggests that such a persistent behavioral outcome is the result of the antenatal programming of the offspring's HPA axis. The current study did not demonstrate that the effect of antenatal maternal distress on the offspring's neuroendocrinological stress reactivity is significant and stable, and the stable behavioral effect of antenatal maternal distress is linked to the long-term modification of offspring HPA axis during the fetal period. To this end, Study 3 is reported below to provide preliminary findings that are nevertheless consistent with the animal literature which suggests that the fetal HPA system mediates the effect of antenatal maternal distress on the offspring's future behavioral stress reactivity.

The preliminary findings reported here suggest that further investigation on the subject of stability of the effect of antenatal maternal distress may be warranted. Future studies are needed which will include a larger sample sizes than reported herein and repeated measurements of both behavior and cortisol over time at multiple age points, to ensure the reliability and generalizability of the present findings. Future study should also utilize a more direct method in linearizing and normalizing regression residuals than the graphical procedure employed in the current study. Future study should also estimate the

neuroendocrinological effect of antenatal maternal effect by means of robust statistics, given the extreme cases that are frequently observed in different samples of cortisol parameters. Finally, future investigation may want to consider using bootstrapping regression model to examine the antenatal effect on cortisol parameters in general, which may be drawn from non-normally distributed population.

STUDY 3

Antenatal Programming of Offspring's Perinatal and Postnatal HPA Functioning: Mediation and Moderation of Antenatal Effects on Stress Reactivity in Healthy Human Infants

Abstract

Non-human animal studies show that antenatal effects on the offspring's stress reactivity are evidenced behaviorally, neuroendocrinologically, and simultaneously across both dimensions; and that the parallel between the behavioral and neuroendocrinological effects of antenatal programming reflects the mediating role of offspring's neuroendocrinological function in the relation between antenatal maternal distress and the offspring's behavioral stress reactivity. While evidence from human studies confirms the adverse effect of antenatal distress on the offspring's behavioral or neuroendorinological stress reactivity, there is no evidence linking the behavioral and neuroendocrinological effects of antenatal programming, nor is there any evidence supporting the hypothesis that the offspring's neuroendocrinological function mediates the antenatal programming of behavioral reactivity.

The current study examined the impact of maternal distress during pregnancy on the offspring's 24-hour and 10-month stress reactivity. Maternal distress during pregnancy was reported retrospectively 24 hours after labour. Postnatal maternal distress was assessed at 10 months. The offspring's neuroendocrinological stress reactivity was measured 24 hours after birth and again at 10 months in terms of changes in salivary cortisol levels in response to a heel stick and a toy removal procedure respectively. Behavioral reactivity at 10 months was coded from direct observation during the same toy removal task.

The preliminary findings reported in the current study showed that 1) distress during pregnancy predicted the offspring's perinatal neuroendocrinological stress reactivity and infant behavioral stress reactivity at 10 months, 2) the effect of antenatal

distress on the offspring behavioral stress reactivity at 10 months was mediated by the offspring's perinatal neuroendocrinological stress reactivity, independent of the effects of postnatal maternal distress measured concurrently, and 3) postnatal maternal distress at 10 months moderated the effects of antenatal distress on infant behavioral stress reactivity measured at 10 months. However, the current study was not able to provide valid evidence supporting the hypothesis that postnatal maternal distress moderates the effect of antenatal distress on infant postnatal neuroendocrinological stress reactivity.

The preliminary findings reported below are interpreted keeping in mind their potential lack of generalizability, and the potential capitalization on chance resulting from the high predictors-to-sample size ratio. Nevertheless, these findings are consistent with the nonhuman animal literature and the growing human literature on antenatal programming, providing evidence in support of the argument that the offspring's neuroendocrinological system is the target and the mediator of antenatal effects on the offspring's behavior. Findings are discussed in terms of their implications in understanding fetal programming of the HPA system in early socio-emotional development in normal human infants.

Introduction

Recent nonhuman animal studies have isolated the fetal hypothalamic-pituitary-adrenal (HPA) system, with its central regulators – the amygdala and the hippocampus – as the primary target and mediating mechanism of antenatal effects on the offspring's behavioral and health outcomes (see Matthews, 2002, for a review). These studies have also demonstrated that the window of development for the HPA system extends beyond the fetal period, making it susceptible to postnatal influences, such that at least some HPA-mediated antenatal effects can be reversed by postnatal means (see Weinstock, 2002, for a review). In humans, notwithstanding the growing literature on the physical, emotional, behavioral and neuroendocrinological effects of antenatal maternal distress on the offspring, there appears to have, however, no direct empirical evidence to demonstrate that the HPA system is the primary target and mediating mechanism of antenatal effect, nor is there any evidence to isolate antenatal and postnatal effects on HPA-mediated outcomes. The purpose of the present study was to fill this void in the human literature by examining the relation between antenatal influences and stress reactivity in healthy human infants.

The influence of antenatal stress on postnatal behavioral reactivity to novel/challenging situations is well-documented in non-human animals. Prenatally stressed adult rats exhibit a higher degree of behavioral inhibition in reaction to stressful situations than controls (Fride et al., 1986; Plotsky & Meaney, 1993). For example, when exposed to an open field or elevated open-arm maze, prenatally stressed rats typically show active and/or passive avoidance behavior, inhibition of locomotion, and increased defecation (Pellow & File, 1986; Poltyrev et al., 1996; Vallée et al., 1997; Wakshlak &

Weinstock, 1990; Zimmerberg & Blaskey, 1998). Prenatally stressed rats also exhibit enhanced prepulse inhibition of the startle response, reduced propensity to play (Takahashi, Haglin, & Kalin, 1992a), increased tendency to prolonged freezing after foot shock (Takahashi, Turner, & Kalin, 1992b), reduced movement in an activity wheel (Lambert et al., 1995), increased ultrasonic vocalizations in an open field (Williams, Hennessy, & Davis, 1998), and increased ultrasonic vocalizations to tail shock and isolation (Takahashi et al., 1992a). When given the choice of remaining in the defensive withdrawal chamber or exploring a well-lit, novel environment, prenatally stressed rats show more and longer lasting retreats into the withdrawal chamber than controls (Ward et al., 2000). Prenatally stressed rats also inhibit food-seeking activities in novel or stressful environments even when they had been, in fact, food-deprived (Fride, Dan, Gavish, & Weinstock, 1985; Fride et al., 1986).

Such stress-induced inhibition of behavior among prenatally stressed offspring was equally evident within the context of the relationship. When compared to controls, 14-day-old prenatally stressed rat pups had a lower rate of ultrasonic vocalizations when separated from their mothers (Morgan, Thayer, & Frye, 1999; Takahashi et al., 1992b). In adulthood, prenatally stressed rats were more likely to inhibit pup-retrieval activities under adverse conditions when they were separated from their offspring (Fride et al., 1985; Fride et al., 1986). Furthermore, Weinstock (2001) reported that, when compared to controls, prenatally stressed female rats were significantly less likely to initiate contact with their partners, although they spent significantly more time following their partner around after initial contact. Similarly, juvenile male prenatally stressed rats have shown a reduced propensity for social interaction and rough and tumble play characteristic of

normal young male behaviors (Ohkawa, 1987; Ward & Stehm, 1991). These findings indicate that antenatally stressed rats are more likely to exhibit heightened behavioral reactivity to novel or stressful situations, even in situations when such behaviors are at odds with the animal's instinctual needs for food and mate, and for providing protection to their offspring.

In humans, there is also a growing developmental literature documenting the effect of antenatal maternal distress on the offspring's behavioral reactivity to stress across different stages of development, spanning from the fetal period to adolescence (for a review, see Van den Bergh et al., 2005b; see also Study 1 and Study 2 above).

Animal research has accumulated a substantial body of evidence on the dysregulation of the HPA axis among the adult offspring of mothers who were stressed during gestation. There are animal studies showing that prenatally stressed rats have higher basal levels of corticosterone compared to controls (Fride et al., 1986; Peters, 1982; Ward et al., 2000). Several studies also reported significant differences in the activation of the HPA axis on exposure to stress between adult prenatally stressed offspring and controls. For example, when exposed to stressors such as a novel environment, tail shocks, restraint, or saline injections, there was a greater and more prolonged elevation of the plasma adrenocorticotrophic hormone (ACTH) and/or cortiscosterone (Barbazanges, Piazza, Le Moal, & Maccari, 1996; Dugovic, Maccari, Weibel, Turek, & Reeth, 1999; Henry et al., 1994; McCormick, Smythe, Sharma, & Meaney, 1995; Peters, 1982; Vallée et al.,1997; Weinstock, Davidson, Rosin, & Schnieden, 1982). Others have found that prenatally stressed rats have adrenal hypertrophy, indicating chronic over-stimulation of the adrenal gland by ACTH (Ward et

al., 2000). The HPA system of prenatally stressed rats is also known to be compromised due to chronic stress. Fride et al. (1986) found that, unlike control rats that stopped releasing corticosterone after repeated exposure to the same open field, prenatally stressed rats continued to release high amounts of corticosterone even after prolonged exposure. And when they were subjected only to a single 5 min exposure to a novel intimidating situation such as the open field or the plus maze, prenatally stressed rats exhibited higher morning basal corticosterone three weeks later compared to controls (Weinstock, 1998). As well, unlike control rats that were stressed repeatedly, the prenatally stressed rats did not compensate for these increased levels by reducing plasma corticosterone in the afternoon and evening (Akana & Dallman, 1992). Prenatally stressed rats also accelerate the age-related HPA-axis dysfunction. In rodents, the period of hyporesponsiveness was abolished in prenatally stressed newborns (Henry et al., 1994), and circulating glucocorticoids levels of middle-aged prenatally stressed rats were similar to those found in older controls (Vallée et al., 1999). Taken together, these findings show that antenatally stressed animals exhibit heightened neuroendocrinological reactivity and advanced age-related deterioration of HPA function and dysregulation of HPA reactivity.

In humans, evidence for the antenatal effect on the offspring's neuroendocrinological stress reactivity is sparse. Epidemiological and birth cohort studies have found that adults with low birth weight, an indication of the experience of antenatal adversity, have enhanced neuroendocrinological stress responses (Phillips & Jones, 2006). Though indirect and retrospective, results from these studies are consistent with the antenatal programming hypothesis of the stress system. Only a few human

developmental studies have examined the effects of maternal distress during pregnancy and subsequent neuroendocrinological reactivity during mid- to late-childhood (Gutteling et al., 2004, 2005; O'Connor et al., 2005), nonetheless these results are consistent with the antenatal programming hypothesis, showing that antenatal maternal distress is associated with heightened neuroendcrinological stress reactivity in the offspring.

Several animal studies have shown that the antenatal effects on both behavioral and neuroendocrinological stress reactivity are long-lasting, spanning across development in dimensions parallel to one another. Burlet et al. (2005) observed that antenatal exposure of the offspring to glucocorticoids, which mimics the intrauterine environment associated with antenatal stress, significantly affected the offspring's behavior across time when repeatedly assessed throughout the juvenile period. Henry et al. (1994) and later Kitraki, Karandrea, and Kittas (1999), after having repeatedly examined the levels of glucocorticoids, levels of glucocorticoids receptors, and levels of glucocorticoids receptor-gene expressions in rodents from the juvenile period to maturity, showed that antenatal stress produced consistent differences in the physiology of the HPA system across the lifespan. Comparisons between antenatally stressed and control animals also parallel the development of behavioral and neuroendocrinological reactivity. For example, Vallée et al. (1999) showed that antenatally stressed rats exhibited parallel advanced age-related deterioration in behavior and circulating glucocorticoids levels. This evidence suggests that antenatal effects have long-term implications on the offspring's behavioral and neuroendocrinological reactivity to stress, which appear coupled in development among antenatally stressed as well as control animals.

Only a few human studies have directly addressed the issue of stability when the effect of antenatal programming is concerned. Huizink et al. (2002, 2003) found that antenatal maternal distress significantly predicted the offspring's behavioral stress reactivity and cognitive performances at both 3 and 8 months. O'Connor et al. (2003) not only showed that the effect of antenatal maternal distress on the offspring's behavior observed at 41 months was again observed at 85 months, the authors also compared the magnitude of antenatal effects across both time points, and concluded from their similar numeric values that the effects were stable (see also Study 2 reported above for evidence on the stability of the antenatal effect on the offspring's behavioral reactivity). However, there is only indirect evidence from birth cohort studies, where heightened HPA functioning remained apparent even among adults who had low birth weight, suggesting that the effect of the offspring's neuroendocrinological reactivity is stable (for an exception, see Study 2).

In addition to demonstrating the parallel between behavioral and neuroendocrinological effect of antenatal programming, animal research has also shown that the offspring's neuroendocrinological system is in fact the mediator of antenatal effects on offspring's behavioral stress reactivity (Welberg et al., 2001). For example, research has shown that antenatally stressed animals up-regulate the expression of corticotropin-releasing hormone (CRH) and CRH receptors in the amygdala (Cratty et al., 1995), which initiates, in part, fear-related and defensive withdrawn behaviors (Davis, 1992; LeDoux, 1996; Rosen & Schulkin, 1998; Schulkin, Morgan, & Rosen, 2005), behaviors that are often observed among antenatally stressed animals (for review, see Welberg & Seckl, 2001). More direct evidence of the HPA system's mediating role in the

relation between antenatal stress and postnatal behavior has been provided by Ward et al. (2000). They demonstrated that when a CRH antagonist was administered, the behavioral differences between antenatally stressed and control animals were abolished, indicating that CRH receptors were in part the mediator of the behavioral abnormalities of prenatally stressed rats. These findings have shown directly and indirectly that the offspring's HPA system mediates the antenatal effect on behavior.However, no human study has examined the mediating role of the offspring's neuroendocrinological system in the antenatal programming of the offspring's behavioral stress reactivity.

The offspring's neuroendocrinological system has also been identified as the primary target and mediator of postnatal maternal effect on pups' behavior. By artificially enhancing (Caldji, Diorio, & Meaney, 2000a; Levine, 1957, 1962; Meaney, Aitken, Viau, Sharma, & Sarrieau, 1989; Plotsky & Meaney, 1993;) or depriving (Caldji, Francis, Sharma, Plotsky, & Meaney, 2000b; Ladd, Owens, & Nemeroff, 1996; Liu, Caldji, Sharma, Plotsky, & Meaney, 2000; Plotsky & Meaney, 1993) early mother-infant interaction, or cross-fostering the offspring between dams whose maternal behaviors lie at the two extremes of natural variation in rodents (Anisman, Lacosta, Kent, McIntyre, & Merali, 1998; Francis, Diorio, Liu, & Meaney, 1999; Moore & Lux, 1998; Zaharia, Kulczycki, Shanks, Meaney, & Anisman, 1996), researchers observed that the central and peripheral sites of glucocorticoid activities mediated the effects of postnatal maternal care on the offspring's behavioral and physiological reactivity to stress (Meaney et al., 1989).

Since antenatal and postnatal maternal influences share a common programming target and mediator of their effects on behavior (Seckl et al., 2005; Seckl & Meaney, 2004), a number of animal studies have examined the moderating effect of postnatal

maternal environment on the effects of antenatal stress on the offspring's neuroendocrinological and behavioral reactivity to stress. Smythe, McCormick, and Meaney (1996) showed that neonatal handling, a postnatal manipulation procedure that enhanced maternal behavior, decreased the CRH levels among prenatally stressed animals to a level that was comparable to that of the non-stressed controls. Furthermore, Smythe and his colleagues also showed that, while neonatal handling increased the glucocorticoids receptor-mediated feedback efficacy of the HPA system, such postnatal manipulation did not result in a significant improvement in the feedback mechanism among the antenatally stressed offspring (Smythe et al., 1996). Similarly, Maccari et al. (1995) found that adoption by foster dams with enhanced maternal behavior reversed the effect of antenatal stress on the offspring's neuroendocrinological reactivity. However, only early adoption was able to reverse the effect of antenatal stress on neuroendocrinological stress reactivity (Barbazanges et al., 1996). Hence, the evidence cited above indicates that the effects of postnatal environmental influence significantly moderate the effect of antenatal stress on the functioning of the offspring's HPA system. In humans, however, there is no evidence available to support the hypothesis that postnatal maternal influence moderates the effect of antenatal maternal distress on the offspring stress reactivity.

The Present Study

A sample of 33 healthy mothers and their healthy infants was recruited and followed across the first 10 months of postnatal life. Antenatal and postnatal assessments of maternal distress, the offspring's perinatal and postnatal salivary cortisol in response to an age-appropriate stressor, and the direct observation of infant behavioral reactivity at

10 months were collected. Perinatal cortisol, as an indicator of the offspring's HPA function during the antenatal period, was collected from newborns 24 hours after birth in response to a heel stick, and postnatal cortisol was collected at 10 months of age in response to a toy removal task. Behavioral reactivity measures were also collected in response to toy removal at 10 months.

Three questions were addressed: First, is the effect of antenatal distress on neuroendocrinological reactivity evident at multiple developmental stages and stable over time in healthy human infants? Second, is the effect of antenatal distress on postnatal behavioral reactivity mediated by perinatal neuroendocrinological stress reactivity independent of the effects of postnatal maternal distress and neuroendocrinological reactivity in healthy human infants? Third, does postnatal maternal distress moderate the effect of antenatal distress on behavioral and neuroendocrinological stress reactivity in healthy human infants?

This study predicted (1) antenatal effects on neuroendocrinological reactivity would be apparent at multiple developmental stages and the effect would be stable across time; 2) the offspring's neuroendocrinological stress reactivity during the perinatal period would mediate the effect of antenatal distress on the offspring's behavior at 10 months; and 3) antenatal effects on neuroendocrinological and behavioral stress reactivity would be moderated by the postnatal environment.

Method

Participants

The target population was defined as all healthy full-term infants born between June 11 and December 18, 2000, to mothers of a geographically defined region in

southern Ontario who presented for delivery at McMaster University Medical Centre, Hamilton, Canada.

Of the 231 newly delivered mothers eligible for participation, 121 (52.4%) initially consented to the study. Reasons for non-participation included: mother exhausted, recovering from labor and delivery (L/D) and/or Cesarean section (C/S); overwhelmed with visitors; father of the baby refused participation; uncomfortable with saliva sampling; inconvenient time; and health concerns about her newborns such as feeding difficulty. Of the 121 mothers who initially consented to participate, 84 (46%) mothers and their neonates satisfied all inclusion and exclusion criteria and were recruited into the sample. Inclusion criteria constrained participation to: 1) gestation age not less than 37 completed weeks, 2) birth weight $\geq$ 2500g, 3) 1- and 5-minute Apgar Score $\geq$ 7, 4) maternal age > 18 years, and 5) consent from the physician or midwife, whoever was the primary care provider for the mother. Exclusion criteria included: 1) the newborn spending more than the first 24 postpartum hours in the Level II nursery, 2) chromosomal anomalies and/or major fetal anomalies, 3) the newborn to be adopted or Children's Aid Society involvement, 4) antepartum exposure to illicit drugs and/or alcohol, 5) mother not proficient in spoken and written English, and 6) mothers received prenatal synthetic glucocorticoids (e.g., dexamethasone) treatment. The sample of 84 comprised of all Caucasian mothers, 48 (57%) of whom gave birth to female infants. Maternal age ranged from 18 to 41 years (M = 30.26), and 74 (88%) of the mothers were married. Mothers' and fathers' schooling ranged from grade 12 to postgraduate/professional degree, with the mean level of education for both mother and father at university levels.

Of the sample of 84, perinatal cortisol samples were successfully collected in 33 (39%) newborns, prior to and 20 min after the medical examination (including heel stick) conducted 24 hours after birth. Reasons for missing data included: the newborn was either being fed during or an hour prior to the time scheduled for cortisol collection, saliva collected from the newborn was insufficient for assaying, the newborn was too distressed to complete the cortisol collection procedure, the newborn was discharged before the completion of either the cortisol collection procedure or the questionnaires, the newborn was transferred to the Level II nursery, and/or the nursing staff was attending the newborn during the time scheduled for saliva collection. The sample of 33 comprised of 18 (55%) female infants, and was not significantly different from the sample of 84 in terms of maternal age, maternal marital status, parental education and occupation, antenatal or perinatal obstetric risks and complications, newborn Apgar scores, and birth weight; nor was there any difference between the two groups on the measure of antenatal maternal distress. Despite the gestational age of all newborns in the sample being at least 37 weeks, there was a significant difference in gestational age between newborns who were available for cortisol sampling (median = 40 weeks) and those who were not (median = 39 weeks; $t = 2.30, p < .05$).

Of the 33 newborns and their mothers, 26 (79%) consented to participate in the 10-month lab visit. The sample of 26 consisted of 13 (50%) female infants, and was not different significantly from either the initial sample of 84 or the sample of 33 in terms of maternal age, maternal marital status, parental education and occupation, antenatal or perinatal obstetric risks and complications, newborn Apgar scores, birthweight, or gestational age; nor was there any significant difference in terms of 24-hour cortisol level

and measures on antenatal and postnatal maternal distress. In the following, only information collected from the 26 dyads who have completed data are presented.

Antenatal and Perinatal Health and Demographic Background Information

Information pertaining to the presence of social complication during pregnancy, obstetric complication, peripartum intervention, delivery complication, delivery intervention, birth complication, and the newborn's health status and characteristics was collected from the medical charts in the maternity ward to screen for potential confounding influences in the attempt to isolate the relations among antenatal maternal stress, antenatal programming of the newborn's HPA function, and infant stress reactivity. Descriptive statistics of antenatal and perinatal demographic background information are summarized in Table 3.1. In addition, relations between the offspring's perinatal cortisol reactivity and a number of factors that may affect maternal attitude and behavior toward the newborn during the first 24 hours after birth were also examined. Descriptive statistics of factors that potentially influenced newborn's cortisol reactivity during the first 24 hours of postnatal life are summarized in Table 3.2.

Insert Tables 3.1 and 3.2 about here

Procedure

Data were collected in the hospital maternity ward 24 hours after birth and during a lab visit at 10 months. Maternal distress during pregnancy was assessed 24 hours after giving birth using the Abbreviated Psychosocial Scale (APS; Goldenberg et al., 1997; Neggers, Goldenberg, Cliver, & Hauth, 2006). Perinatal cortisol baseline and post-

stressor level were sampled before and 20 min after medical examination that took place 24 hours after birth.

During the 10-month laboratory visit, the APS was administered again to the mothers to assess postnatal maternal distress, and their infants participated in a toy removal procedure (Stifter & Braungart, 1995). Saliva was sampled both before and 20 min after the toy removal procedure at 10 months of age. Saliva samples were collected 20 min after the stressor because it takes at least 15 min to observe increases in salivary cortisol in response to a stressor (see Stansbury & Gunnar, 1994). All procedures were approved by the McMaster Faculty of Health Science Research Ethics Board.

Measures

Maternal Distress

The Abbreviated Psychosocial Scale (APS) was derived from five previously validated tools that measure trait anxiety (Spielberger Trait Anxiety Scale; Spielberger, Gorsuch, & Lushene, 1970), self-esteem (Rosenberg Self Esteem Scale; Rosenberg, 1965), mastery (Pearlin Mastery Scale; Pearlin, Lieberman, Menaghan, & Mullan, 1981), depression (Centre for Epidemiologic Studies Depression Scale; Radloff, 1977), and stress (Schar Subjective Stress Scale; Schar, Reeder, & Dirken, 1973). Items in the abbreviated scale were selected on the basis of their loadings on 6 different orthogonal factors extracted out of items pooled from the five full scales. In the original article, Goldenberg et al. (1997) reported that the overall scores on the 28-item abbreviated scale were highly correlated with the overall score of items pooled from the five full scales (r = .97). The APS was used in the Preterm Prediction Study of the NIH Maternal Fetal Medicine Units Network (Copper et al., 1996), which showed that high psychosocial

distress, as defined by this scale, was associated with spontaneous preterm birth and low birth weight even after adjustment for maternal demographic and behavioral characteristics. Goldenberg et al. (1997) showed that the abbreviated scale and the composite of the five full scales were comparable in predicting pregnancy risks and the newborn's health outcomes. The abbreviated scale is divided in to five subscales, reflecting the five full instruments where the abbreviated scale draws its items from. The abbreviated scale consists of 28 items, each with 5 possible answers, ranging from 0 (not true) to 5 (always true). Given the focus on stress and reactivity in the current study, only the Stress subscale of the APS was used in the following analyses.

Neuroendocrinological Stress Reactivity Procedure and Measures

Perinatal Salivary Cortisol Collection

The current study measured the effect of antenatal programming on fetal HPA activity 24 hours after birth. Perinatal saliva was collected from the newborn prior to, and 20 min after, neonatal medical examination, typically conducted 24 hours after birth. Blood was drawn from the newborn's heel during the neonatal medical examination, which began with lancing the heel, followed by repeatedly squeezing the heel to generate enough blood to screen for metabolic disorder and elevated bilirubin. The average duration of the heel stick was 5 min. Research has shown that the heel stick elicits behavioral as well as neuroendocrinological responses to stress (Gunnar, 1992; Keenan, Grace, & Gunthorpe, 2003; Ramsay & Lewis, 1995). In the current study, salivary cortisol was collected by having the newborns suck on a small cotton swab (Kendall Curex™ 8 Ply 5cm x 5cm) wrapped around the mother's index finger. After saliva collection, the cotton swab was put into a 20 cc syringe, and 0.5 - 2.0 ml (500 - 2000 μg)

of the sample were syringed into a cryogenic vial and stored at -20°C until assayed.

Postnatal Salivary Cortisol Collection

Stimulated saliva samples at 10 months of age were collected between 0900 and 1130 hours, and at least one hour after the last feeding. The infant's mouth was gently rinsed before each saliva collection. The time of collection and the state of the infant were recorded for both saliva samples per infant. Baseline salivary cortisol was collected on the infant's arrival to the laboratory, and a reactive (post-stressor) sample was collected 20 min after completion of the toy removal procedure. Saliva was collected from the infant's mouth by having the infant suck on a small cotton swab (Kendall Curex™ 8 Ply 5cm x 5cm) wrapped around the investigator's index finger. After the absorption phase, the cotton swab was put into a 20 cc syringe and 2.0 ml (2000 μg) of the collected sample were syringed into a cryogenic vial and stored at -20°C until assayed. A difference score subtracting the baseline salivary cortisol level from the post-stressor salivary level was used to quantify HPA reactivity to stress in response to the toy removal procedure at 10 months of age.

Salivary Cortisol Assaying

Samples were assayed at St. Joseph's Hospital & Healthcare Endocrinology Laboratory (Hamilton, ON) in duplicate using the Diagnostics Products Corporation (DPC) Coat-A-Count® Cortisol Radioimmunassay kit. The lower detection limit for this assaying method was 1nmol/L (0.036 μg/dL), and the upper limit was 145 nmol/L (5.1 μg/dL). The average between-assay variance was 5.7% and 4.4% for high and low concentrations respectively, and the average within-assay variance was 6.2% and 4.1%. Salivary cortisol is expressed in ug/dl.

Parameterization

Five different neuroendocrinological parameters are generated from the 2 samples of salivary cortisol collected prior to and after medical examination during the perinatal period and the toy removal paradigm at 10 months. Cortisol levels at baseline and after challenge indicate the offspring's HPA system at rest and in an active state respectively. On the other hand, the difference between post-challenge and baseline values, or the standardized residuals where the baseline values were partialled out from either the post-challenge value or from the difference score, represent the reactivity of the offspring's HPA system in response to challenge (Gunnar & White, 2001).

Behavioral Stress Reactivity Procedures and Measures

Toy Removal Procedure

Stifter and Braungart's (1995; Stifter & Jain, 1996) toy removal procedure was conducted at the 10-month laboratory visit to frustrate the infant in order to observe stress reactivity at the behavioral level. Infants were placed in an infant high chair, and mothers were seated toward the side of the high chair but in front of the infant. For 90 sec mothers and infants played with an attractive toy (Little Smart® Nursery Rhyme Land, manufactured by VTECH®) that consisted of several moving and music generating parts. When given a cue from the experimenter, mothers removed the toy from their infants and held it out of reach but within the infant's sight. Mothers were also instructed to assume a neutral expression and to refrain from interacting with their infants during this time. The toy removal lasted for a maximum of 120 sec, but could be terminated at any point after 20 sec of hard crying. Mothers were then cued to return the toy to their infants but to

remain non-interactive. After 60 sec, mothers resumed interacting with their infants. All 26 infants were able to complete the entire 120 sec toy removal episode.

Behavioral Coding of Reactivity

According to Stifter and Braungart (1995) two types of behaviors could be coded from the toy removal paradigm: reactivity, deduced from negative vocalization elicited by the toy removal challenge, and regulatory behaviors such as visual-behavioral disengagement (for example, orienting away from the toy/mother or struggle to escape) and self-comforting behaviors (for example, thumb or toe sucking). Negative vocalization is rated every 10 second based on the following scale: 0 (no negative vocalization), 1 (intermittent fussing), 2 (escalated fussing with at least one sob), 3 (steady crying), and 4 (escalated crying with at least one shriek). On the other hand, the number of seconds the infant engaged in regulatory behaviors within each 10-second epoch was noted.

A primary coder coded all 26 infants, and a secondary coder coded a subset of 20% of the tapes. Interrater reliability is defined as the agreement on endorsing one of the four possible ratings on negative vocalization coded for every 10-sec epoch. The mean interrater reliability of negative vocalization was .81 and the mean reliability for regulatory behavior was .76.

Parameterization

Following Stifter and collegues' (Fish, Stifter & Belsky, 1991; Stifter & Braungart, 1995; Stifter & Jain, 1996) approach to parameterization of behavioral codes generated by experimental challenge, the current study captures the event-related increase or decrease of behavioral stress response by 1) subtracting the sum of behavioral

rating/count at baseline from the post-challenge sum, and 2) averaging the differences between consecutive coding of behaviors over the duration of the post-challenge episode.

Variable Selection

Study 3 examined the mediating role of perinatal neuroendocrinological stress reactivity in the relation between antenatal maternal distress and the offspring's behavioral stress reactivity. The current model was tested below using maternal APS Stress subscale score, 24-hour cortisol change (the difference between log-transformed pre- and post-stressors cortisol levels), and 10-month laboratory observation of behavioral stress reactivity in the Toy Removal paradigm (the log-transformed mean differences in the intensity of negative vocalization between every two consecutive epochs over the duration of the post-challenge episode).

The APS includes five subscales: Anxiety, Depression, Self-esteem, Mastery, and Subjective Stress. While previous research has demonstrated that antenatal maternal anxiety, depression and subjective stress were all significant contributors of poor developmental outcomes in the offspring (Van den Bergh et al., 2005b), the relation between offspring's development outcomes and maternal anxiety and depression is likely to be undermined in this highly selective middle class sample, where the range of participants' response on the two clinical scales are likely to be restricted given the stringent exclusion criteria imposed. On the other hand, previous research has demonstrated in a similar middle class sample the sensitivity of the offspring's developmental outcomes to the subjective experience of stress during pregnancy such as daily hassle (Van den Bergh et al., 2005b). Self-esteem and mastery, while they contribute to the risk and coping of distress the mother experiences during pregnancy, are

not direct measures of maternal distress the mother experienced during the antenatal period, and is therefore outside the scope of the current study. Hence, the Subjective Stress subscale of the antenatal maternal APS was selected as the variable to use in the following analyses.

Among the five neuroendocrinological parameters generated from the two samples of salivary cortisol collected before and after the stressors, the baseline and the post-challenge levels reflect respectively the status of the offspring's HPA system at rest and in active state, neither of which could be considered a reactivity measure of the offspring's neuroendocrinological function, and is therefore not a valid operationalization of the offspring's neuroendocrinological stress reactivity, the focus of the current study. While the difference score and the two standardized residuals all represent the change in the offspring's HPA system in reaction to the challenge, methodologically speaking, given the size of the current sample and the commonly non-normality of cortisol levels' distribution (Gunnar & White, 2001), using standardized residuals as the variable may compound the error in estimation inherited in running inferential statistics in a small sample like the current one. Hence, the difference score between cortisol post-challenge and baseline levels are selected as the variable to test the mediation model below.

According to Stifter and colleagues (Stifter & Braungart, 1995; Stifter & Jain, 1996), infant behavior during the toy removal paradigm can be coded in terms of behavioral reactivity or regulatory behavior. Behavioral reactivity was coded via negative vocalization in response to the removal of the toy (rated as one of the four levels, from no negative vocalization to escalated crying with at least one shriek). On the other hand, regulatory behaviors were coded in terms of the presence and duration of the following

behavior: visual-behavioral disengagement, self-comforting, and communication attempts. Given the focus of the current study, only behavioral reactivity in the form of changes in the intensity of negative vocalization was most appropriate in operationalizing the construct of behavioral reactivity. In terms of data reduction, previous research favored the mean of the differences between consecutive coding-windows following experimental challenges (Stifter, C.A., & Braungart, J.M. (1995).). Hence, the mean differences in the intensity of negative vocalization between consecutive 10-second epochs during the 120 seconds following toy removal is selected as the variable to examine the mediation model proposed below.

Results

In the following, results from the preliminary analyses and model testing will be reported in turn. Findings reported as preliminary analyses included 1) the distribution and transformation of variables, where variables were transformed according to Tukey's (1977) "ladder of re-expression" (see also Mosteller & Tukey, 1977; Cohen, Cohen, Akins & West, 2003), 2) exploratory correlation analyses between pairs of key variables as well as their derivatives, and 3) scatterplots of relations between key variables employed in the testing of the mediation model proposed in the current study. On the other hand, individual regression models that examine the mediating effect of perinatal neuroendocrinological stress reactivity in the relation between antenatal maternal distress and 10-month infant behavioral reactivity, as well as the formal testing of the mediation model, will be presented in turn. Finally, the moderating influence of postnatal maternal distress on the relation between antenatal maternal distress and infant behavioral and neuroendocrinological stress reactivity will be examined. For each regression analysis

reported below, regression diagnosis, such as influential statistics (Mahalanobis Distances, Cook's Distances and the Centered Leverage Values) and residuals check (normality and homoscedasticity) are provided to ensure the assumptions of the regression analysis are met.

Preliminary Analyses

In the current section, the distributions of all variables relevant to the overall model, as well as the derivatives of these variables are reported. Distributions of transformed variables are reported in addition to those of raw scores where appropriate. Secondly, the intercorrelations among variables derived from different pairs of core constructs that constituted the overall model were examined in turn. To provide visualization for the relations between different pairs of key variables, scatterplots are also reported in the following. Finally, potential covariates were screened in terms of their confounding influences on variables selected to test the model of stability proposed in the current study.

Descriptive statistics

Variable Distribution and Transformation

Reported in the current section are the distributions, skewness and kurtosis statistics, and the Shapiro-Wilk tests of normality on 1) antenatal and postnatal maternal APS subscales and total scores, 2) perinatal and postnatal infant salivary cortisol parameters of the offspring, and 3) infant behavioral parameters coded from the toy-removal procedure at 10 months. Transformed variables are reported in addition to raw scores when the distributions of raw scores exhibited substantial skewness and/or kurtosis, or significant deviation from normal.

Antenatal and Postnatal Maternal Distress

Antenatal APS Subscale and Total Scores In the current study, antenatal maternal distress was assessed during the perinatal period with the Abbreviated Psychosocial Scale (APS). The APS consists of 5 subscales: Anxiety, Self-esteem, Mastery, Depression and Subjective Stress. Figures A3.1 to A3.6 report the distributions of the raw values of the antenatal APS subscale and total scores. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality for each of the subscale scores and total score are reported in Table A3.1. The results indicated that, with the exception of the Self-Esteem subscale score and the total scores, all antenatal APS subscale scores did not exhibit substantial skewness or kurtosis (the statistics were deemed to be substantial when the ratio between skewness/kurtosis statistics and their corresponding standard errors was greater than 2). On the other hand, the Shapiro-Wilk tests of normality indicated that the distributions of the Depression, Self-esteem subscale scores and the Total scores significantly deviated from normal. However, since the distribution of the target variable, the antenatal Subjective Stress subscale score did not exhibit substantial skewness or kurtosis, nor did it significantly deviate from normal, the raw APS subscale and total scores were used in the following exploratory analyses and modeling testing.

Table A3.2 reports the intercorrelations among different subscale and total scores of antenatal APS. The results showed that most subscales correlated significantly with one another and with the total score except the Mastery subscale, which was not significantly correlated with either the Depression or the Self-esteem subscale scores. All

significant correlations coefficients among different subscale scores and between subscale scores and the total score ranged between .41 and .85 (ps<.05).

Postnatal APS Subscale and Total Scores. Similarly, postnatal maternal distress was assessed at 10 months with APS. Figures A3.7 to A3.12 report the distributions of the subscale and total scores of the postnatal APS. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality of each of the postnatal APS subscale and total scores are reported in Table A3.3. The results indicated that, with the exception of the Self-Esteem subscale score, all the other subscale scores and the total score were not substantially skewed, and none of the subscale or total scores exhibited substantial kurtosis. On the other hand, the Shapiro-Wilk tests of normality indicated that, with the exception of Depression and the Subjective Stress subscale score, distributions of the other subscale scores and the total score significantly deviated from normal. However, since the distribution of the target variable, the postnatal Stress subscale score exhibited no substantial skewness, kurtosis, or significant deviation from normal, the raw values of the APS subscale and total scores are reported in the following exploratory analyses and model testing. Table A3.4 reports the intercorrelations among different postnatal APS subscale scores. The results showed that the intercorrelations among postnatal APS subscales and between the subscale scores and the total scores ranged between .21 and .82, with most correlations being significant except the relations between Subjective Stress and Anxiety subscale scores (r=.21), between Subjective Stress and Self-Esteem subscale scores (r=.22), and between Anxiety and Mastery subscale scores (r=.35).

Perinatal and Postnatal Neuroendocrinological Stress Reactivity

In the current study, offsprings' neuroendocrinological stress reactivity was measured 24 hours after birth and again at 10 months. Salivary cortisol samples were collected prior to and after the medical examination 24 hours after birth and toy-removal procedure at 10 months. Based on the pre- and post- salivary cortisol sample, additional parameters were computed: one difference score and two standardized residuals, where the baseline was controlled from either the post-challenge level or from the difference score.

Prenatal Salivary Cortisol Parameters Figures A3.13 to A3.17 report the distributions of perinatal cortisol level at baseline and at post-challenge levels, the difference score between baseline and post-challenge levels, the standardized residual of the post-challenge level controlling for baseline, and the standardized residual of the difference score controlling for baseline. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality for each of the cortisol parameters are reported in Table A3.5. The results indicated that perinatal cortisol level at baseline exhibited substantial skewness and kurtosis, and the distributions of the baseline value and the difference score between baseline and the post-challenge level deviated significantly from normal according to the Shapiro-Wilk tests of normality. Since the difference score between baseline and post-challenge cortisol levels was selected as the parameter to test the mediation model, all perinatal cortisol parameters were log-transformed to normalize distributions. The distributions of the log-transformed perinatal cortisol parameters are reported in Figures A3.18 to A3.22. Their skewness and kurtosis statistics, as well as their Shapiro-Wilk tests of normality are reported in Table A3.6. Results indicated that, with the exception of the baseline value, none of the log-transformed perinatal cortisol

parameters exhibited substantial skewness or kurtosis, or significant deviation from normality according to the Shapiro-Wilk tests of normality. Hence perinatal cortisol parameters reported in the current study were log-transformed prior to further analyses.

Table A3.7 reports the intercorrelations among different log-transformed perinatal cortisol parameters. The results showed that cortisol level at baseline was significantly correlated with post-challenge cortisol level ($r=.56$, $p<.01$). Baseline cortisol level, however, was significantly but negatively correlated with the difference score ($r=-.68$, $p<.01$), and was not significantly correlated with the standardized residuals of either the post-challenge level or the difference score with the baseline value controlled for. The post-challenge value, on the other hand, was not significantly correlated with the difference score but was significantly correlated with both standardized residuals, where the baseline value was partialled out of the post-challenge value or the difference score ($rs=.83$, $p<.01$). The correlations between the difference score and the standardized residual scores were also significant ($rs=.74$, $p<.01$).

Postnatal Salivary Cortisol Parameters Figures A3.23 to A3.27 report the distribution of postnatal cortisol levels at baseline and post-challenge, the difference score between baseline and post-challenge levels, the standardized residual of the post-challenge level controlling for baseline, and the standardized residual of the difference score controlling for baseline. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality for all postnatal cortisol parameters are reported in Table A3.8. The results indicated that all postnatal cortisol parameters exhibited substantial skewness and, with the exception of 10-month post-challenge cortisol level, postnatal cortisol parameters also exhibited substantial kurtosis. The Shapiro-Wilk test of

normality also revealed that the distributions of all 10-month cortisol parameters significantly deviated from normality. Because of the extreme skewness and kurtosis of postnatal cortisol parameters in the current sample, both log- and inverse-transformation were performed and are reported below. The distributions of the log- and inverse-transformed cortisol parameters are reported in Figures A3.28 to A3.32 and A3.33 to A3.37 respectively. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality for log- and inverse-transformed cortisol parameters are reported in Table A3.9 and Table A3.10 respectively. Results indicated that even after transformations, all postnatal cortisol parameters continued to exhibit substantial skewness and kurtosis, as well as significant deviation from normal. Nevertheless, by comparing the skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality between log- and inverse-transformation, log-transformation appeared to be a more normalizing transformation in offsetting the non-normal distribution of the raw cortisol parameters, though the differences were not statistically significant. Since perinatal cortisol values were log-transformed in the following analyses, log-transformed postnatal cortisol levels provided the added value of consistency.

Table A3.11 reports the intercorrelations among different log-transformed postnatal cortisol parameters. The results showed that cortisol level at baseline was significantly correlated with the post-challenge cortisol level ($r=.56$, $p<.01$) and the difference score between baseline and post-challenge values ($r=-.68$, $p<.01$).However, baseline cortisol value was not significantly correlated with the standardized residual of the post-challenge level where the baseline was first partialled out, nor with the standardized residuals of the difference score where the baseline was first partialled out.

The post-challenge cortisol level, on the other hand, was significantly correlated with the standardized residuals of both the post-challenge level and the difference score ($rs=.83$, $p<.01$). In addition, the difference score between baseline and post-challenge levels was significantly correlated with the standardized residuals of both the post-challenge level and the difference score ($r=.74$, $p<.01$).

Infant Behavioral Stress Reactivity

Figures A3.38 to A3.44 report the distribution of different infant behavioral parameters coded at 10 months, which includes 1) the mean differences in the intensity of negative vocalization between consecutive 10-second epochs during the 120 seconds following toy removal, 2) the mean differences in the duration of visual-behavioral disengagement between consecutive 10-second epochs during the 120 seconds following toy removal, 3) the mean difference in the duration of self-comforting behaviors between consecutive 10-second epochs during the 120 seconds following toy removal, 4) the sum of the mean differences in negative vocalizations intensity, duration of visual-behavioral disengagement and duration of self-comforting behaviors between consecutive 10-second epochs during the 120 seconds following toy removal, 5) the difference between the total intensity of negative vocalization prior to and after toy removal, 6) the difference between the total number of times visual-behavioral disengagment observed prior to and after toy removal, 7) the difference between the total number of self-comforting behaviors observed prior to and after toy removal, and 8) the sum of the differences in negative vocalization, visual-behavioral disengagement and self-comforting behaviors observed prior to and after toy removal. The skewness and kurtosis statistics, as well as the Shapiro-Wilk tests of normality for all infant behavioral parameters are reported in

Table A3.12. The results indicated that the mean differences in the intensity of negative vocalization between consecutive 10-second epochs during the 120 seconds following toy removal exhibited neither skewness nor kurtosis. Similarly, the difference score between negative vocalization rated at baseline and after toy removal exhibited no substantial kurtosis, but was nevertheless skewed. All other behavioral parameters exhibited severe skewness and kurtosis. Furthermore, the distributions of all infant behavioral parameter significantly deviated from normality.

To normalize the distributions, log-transformation was applied to the raw values of all behavioral parameters. The distributions of log-transformed infant behavioral parameters are reported in Figures A3.45 to A3.51. The skewness and kurtosis statistics, as well as the results of Shapiro-Wilk tests of normality are reported in Table A3.13. Results indicated that the mean differences in the intensity of negative vocalization between consecutive 10-second epochs during the 120 seconds following toy removal exhibited neither skewness nor kurtosis. Similarly, the difference between the total intensity of negative vocalization prior to and after toy removal exhibited no substantial kurtosis, but was nevertheless skewed. All other behavioral parameters exhibited severe skewness and kurtosis. In terms of normality of distribution, only the log-transformed mean differences in the intensity of negative vocalization between consecutive 10-second epochs during the 120 seconds following toy removal did not significantly deviate from normality. Since the mean differences in the intensity of negative vocalization between consecutive 10-second epochs during the 120 seconds following toy removal was selected as the parameter to examine the mediation model below, no further transformation was conducted.

Table A3.15 reports the inter-correlations among different log-transformed infant behavioral parameters. The results indicated that the parameters of visual-behavioral disengagement and self-comforting behaviors are significantly correlated, whether presented as the mean differences in the duration of visual-behavioral disengagement between consecutive 10-second epochs following toy removal ($r=.76$, $p<.01$), or in terms of the difference between the total number of times behaviors were observed prior to and after toy removal ($r=.79$, $p<.01$), and were in turn significantly correlated with their corresponding total scores. All mean difference scores between consecutive epochs were significantly correlated with their corresponding difference scores between episode prior to and after toy removal. Negative vocalization, however, was not significantly associated with either the visual-behavioral disengagement or self-comforting behaviors regardless of the data reduction methodology.

Exploratory Analyses

Reported in the current section are the basic bivariate correlation analyses that were the building blocks for the mediation model proposed: 1) maternal distress and perinatal neuroendocrinological stress reactivity, 2) perinatal neuroendocrinological stress reactivity and infant behavioral stress reactivity, and 3) maternal distress and infant behavioral stress reactivity. Scatterplots were provided to help visualize relations between each pair of key variables selected to examine the mediation model proposed.

Antenatal Maternal Distress and Perinatal Neuroendocrinological Stress Reactivity

Antenatal APS and Perinatal Salivary Cortisol Parameters

Antenatal maternal distress was measured in the current study using the 5 different subscales of the APS: Anxiety, Self-esteem, Mastery, Depression and

Subjective Stress. On the other hand, perinatal HPA function was assessed in terms of the 5 different cortisol parameters extracted from the salivary cortisol collected prior to and after medical examination 24 hour after birth, these parameters include: cortisol baseline, post-challenge cortisol level, the difference score between baseline and post-challenge levels, and standardized residuals controlling baseline value from the post-challenge level and from the difference score. Based on the above-noted distributions of individual variables, the raw values of APS subscale and total scores and the log-transformed cortisol parameters are used in the following analyses. Reported in Table A3.16 is the intercorrelation between different antenatal APS subscale scores and log-transformed perinatal cortisol parameters. The results indicated that the difference score between log-transformed baseline and post-challenge levels was significantly associated with antenatal maternal APS Subjective Stress subscale score ($r=.44$, $p<.05$) and the total score ($r=.44$, $p<.05$). While significantly correlated with the difference scores, the two standardized residuals were only significantly associated with the antenatal APS subjective stress subscale score ($rs=.41$, $p<.05$). On the other hand, the perinatal cortisol value at baseline was significantly and negatively associated with the antenatal APS Subjective Stress subscale score ($r=-.60$, $p<.01$). The relation between antenatal maternal APS Subjective Stress subscale score and the offspring's perinatal cortisol difference score was one of the building blocks for the mediation model tested below. Figure A3.52 reports the scatterplot of their relation.

Perinatal Neuroendocrinological Stress Reactivity and Infant Behavioral Stress Reactivity

Perinatal Salivary Cortisol Parameters and Infant Behavioral Parameters

As previously noted, 5 neuroendocrinological parameters were extracted from saliva samples collected from the offspring prior to and after medical examination 24-hour after birth. On the other hand, 8 behavioral parameters were extracted from incidences of negative vocalization, visual-behavioral disengagement and self-comforting behaviors the infant exhibited prior to and/or after the toy-removal procedure at 10 months. Table A3.17 reports the intercorrelations between these two sets of parameters. To normalize the distributions of individual variables, all neuroendocrinological and behavioral parameters were log-transformed. The results indicated, perinatal cortisol levels obtained after perinatal examination, and the change of cortisol levels from baseline in reaction to perinatal examination, were significantly associated with the mean differences in the intensity of negative vocalization between consecutive 10-second epochs during the 120 seconds following toy removal at 10 months experimental paradigm ($r=.71$ and $r=.57$ respectively, $ps<.01$), and with the difference in negative vocalization intensity between episodes prior to and after toy removal ($r=.47$ and $r=.38$, $p=.05$). The standardized residuals of the post-challenge cortisol level and the difference score were also significantly associated with the mean difference in the intensity of negative vocalization between consecutive 10-second epochs during the 120 seconds following toy removal ($rs=.47$, $p<.05$). In addition, the post-challenge cortisol level and the cortisol difference score were significantly and negatively associated with self-comforting behavior in response to the toy removal ($r=-.46$, $p<.05$ and $r=-.38$, $p=.05$). In the following, the log-transformed cortisol difference score and the log-transformed mean difference in the intensity of negative vocalization between consecutive epochs were

selected to examine the mediation model proposed. Figure A3.53 reports the scatterplot of their relation.

Antenatal Maternal Distress and Infant Behavioral Stress Reactivity

Antenatal APS and Infant Behavioral Parameters

Antenatal maternal distress was assessed in the current study using the 5 different subscales of the APS: Anxiety, Self-esteem, Mastery, Depression and Subjective Stress. Infant behavioral reactivity measures, on the other hand, were extracted from behavioral parameters derived from negative vocalization and behaviors of visual-behavioral disengagement and self-comfort observed prior to and/or after the toy-removal procedure. Reported in Table A3.18 are the intercorrelations between log-transformed behavioral parameters and the raw values of APS subscale and total scores. The results indicated, the only statistical significant relation was between antenatal maternal APS Subjective Stress subscale scores and infant mean differences in the intensity of negative vocalization between consecutive 10-second epochs during the 120 seconds following toy removal ($r=.41$, $p<.05$). The scatterplot of the relation between these two key variables is reported in Figure A3.54.

Screening for Potential Covariates

Table 3.3 reports the descriptive statistics of 1) antenatal and postnatal APS scores, 2) perinatal and infant cortisol baseline and reactivity levels, and 3) infant behavioral stress reactivity scores at 10 months.

Insert Table 3.3 about here

Maternal Distress

Zero-order correlational analyses indicated that the antenatal maternal APS Stress score was 1) stable across the 10-month period (r=.46, p<.05); 2) not related to any antepartum and peripartum complications or health risks; 3) not related to the newborn's gestation age, birth weight and health status; 4) not associated with socioeconomic indicators. The 10-month APS Stress subscale score was not associated with the concurrent sociodemographics of the mother.

Perinatal and Postnatal Neuroendocrinological Stress Reactivity

Zero-order correlational analyses indicated that perinatal cortisol baseline, post-stressor level, and change from baseline were not associated with 1) the number of hours between cortisol collection and birth or feeding; 2) known antepartum or peripartum complications or health risk; 3) newborn's biological sex, gestation age, health indicators, and sociodemographics; 4) whether and how soon the baby was cuddled and placed on the mother's breast, and 5) maternal attitude toward labor and birth, the newborn, and motherhood. Similarly, 10-month cortisol levels were not related to the number of hours the infant was fed prior to cortisol collection, nor was it significantly related to infant biological sex or concurrent sociodemographic factors at 10 months.

10-month Behavioral Stress Reactivity

Zero-order correlational analyses indicated that the behavioral reactivity score at 10 months was not associated with any infant characteristics such as gender or age; nor was this measure significantly associated with any maternal sociodemographic variables.

Hypothesis Testing

Is the Effect of Antenatal Distress on Neuroendocrinological Stress Reactivity Evidenced in Multiple Developmental Stages and Stable Over Time?

Stability of Cortisol Reactivity from Perinatal to Postnatal Period

In the current section, a series of simple regression analyses were conducted to examine the stability of the effect of antenatal maternal distress on the offspring's neuroendocrinological stress reactivity over a 10-month period. 10-month cortisol difference score was regressed on perinatal cortisol difference score, and again on antenatal maternal Subjective Stress score. Perinatal cortisol difference score was similarly regressed on antenatal maternal Subjective Stress score. Should the result be consistent with the hypothesis that the effect of antenatal maternal distress on offspring's neuroenocrinological stress reactivity are stable, we would expect that the relation between perinatal and postnatal cortisol difference scores to be significant, and the effects of antenatal Subjective Stress score on offspring's perinatal and postnatal cortisol difference scores to be both significant and containing comparable regression coefficients. However, regression diagnosis revealed that when log-transformed 10-month cortisol difference score was regressed on either perinatal cortisol difference score or antenatal maternal Subjective Stress score, a number of cases in both occasions exerted undue influences on the relations (Mahalanobis Distances > 10, Cook's Distances > 1 and the Centered Leverage Values > .40), and the residuals resulted from both models deviated significantly from normality, violating the assumption of regression analyses. The violation of regression assumption suggested that the least-square estimator could potentially be biased, and therefore the result of the current analyses would not be

considered in the subsequent model testing. Hence, the result is not reported in the current section.

Is the Effect of Antenatal Distress on Postnatal Behavioral Reactivity Mediated by Perinatal Neuroendocrinological Stress Reactivity Independent of the Effects of Postnatal Maternal Distress and Infant Neuroendocrinological Reactivity?

The mediating role of perinatal cortisol change in the relation between the antenatal maternal APS Stress score and infant behavioral reactivity score at 10 months was first evaluated according to the regression-based guidelines proposed by Baron and Kenny (1986; see also Judd & Kenny, 1981), including a formal assessment of the mediation effect using the Goodman test (Goodman, 1960). To establish mediation, the following four minimum criteria must hold: 1) the antenatal maternal APS Stress score must significantly predict perinatal cortisol change, 2) perinatal cortisol change must significantly predict the 10-month behavioral reactivity score, 3) the antenatal maternal APS Stress score must significantly predict the 10-month behavioral reactivity score, and 4) the effect of antenatal maternal APS Stress score on the behavioral reactivity score must be reduced after controlling for the effect of perinatal cortisol change. To demonstrate that the mediating effect of perinatal cortisol reactivity was independent of postnatal influences, effects of postnatal maternal distress and infant cortisol reactivity on infant behavioral reactivity, where appropriate, were first statistically controlled for in the following tests of the minimum criteria for statistical mediation. These relations are necessary, but not sufficient, conditions for demonstrating mediation. The Goodman test (Goodman, 1960) was therefore performed to provide a formal test for the mediation hypothesis.

Criterion 1: Antenatal Maternal Distress Predicts Perinatal Neuroendocrinological Stress Reactivity.

A regression analysis was conducted to predict perinatal cortisol change from antenatal maternal APS Stress score. Analyses indicated that the antenatal APS Stress score significantly predicted perinatal cortisol change (B=.07, SE=.03, p<.05). Regression diagnoses revealed that no individual case exerted undue influence on the relation (Mahalanobis Distances, Cook's Distances and the Centered Leverage Values were all within acceptable range) and the regression residuals did not deviate significantly from normality of distribution (Shapiro-Wilk = .95, ns.), nor violate the assumption of homoscedasticity (White's Test: Chi-sq[2] = 2.21, ns.). The result suggest that the greater the distress during pregnancy, the greater the perinatal neuroendocrinological stress reactivity to the medical examination 24 hours after birth. Therefore, the first criterion of statistical mediation was met.

Criterion 2: Perinatal Neuroendocrinological Stress Reactivity Predicts Infant Behavioral Stress Reactivity.

A regression analysis was then conducted to predict the 10-month behavioral reactivity score from perinatal cortisol change. The result was significant (B=.35, SE=.10, p<.01). Follow-up regression diagnoses revealed that no individual case exerted undue influence on the relation (Mahalanobis Distances, Cook's Distances and the Centered Leverage Values were all within acceptable range) and the regression residuals did not deviate significantly from normality of distribution (Shapiro-Wilk = .99, ns.), nor violate the assumption of homoscedasticity (White's Test: Chi-sq[2] = 2.60, ns.). The result suggests that the greater the change in 24 hour perinatal neuroendocrinological stress

reactivity, the greater the 10-month behavioral stress reactivity. Therefore, the second criterion of statistical mediation was met.

Criterion 3: Antenatal Maternal Distress Predicts Infant Behavioral Stress Reactivity.

A sequential multiple regression analysis was conducted to predict 10-month behavioral stress reactivity score from the antenatal maternal APS Subjective Stress score, with the effect of postnatal maternal Subjective Stress score first partialled out. The analysis indicated that antenatal maternal Subjective Stress score significantly predicted 10-month behavioral stress reactivity score even after the effect of the postnatal APS Stress score was first controlled for (B=.05, SE=.02, p<.05). Regression diagnoses revealed that no individual case exerted undue influence on the relation (Mahalanobis Distances, Cook's Distances and the Centered Leverage Values were all within acceptable range) and the regression residuals did not deviate significantly from normality of distribution (Shapiro-Wilk = .97, ns.), nor violate the assumption of homoscedasticity (White's Test: Chi-sq[5] = 2.57, ns.). The results suggested that the greater the maternal distress during pregnancy, the greater the behavioral reactivity observed at 10 months, and the effect of maternal distress during pregnancy on infant behavioral stress reactivity was independent of the effect of postnatal maternal distress measured concurrently with infant behavioral stress reactivity. Therefore, the third criterion of statistical mediation was met.

Criterion 4: Relation between Antenatal Maternal Distress and Infant Behavioral Reactivity is Not Independent of Perinatal Neuroendocrinological Reactivity.

Finally, when perinatal cortisol difference score was entered into the above multiple regression model, the relation between the antenatal APS Stress score and the

10-month behavioral stress reactivity score dropped to non-significant levels (B=.03, SE=.02, *ns.*). Regression diagnoses of this multiple regression model indicated that no individual case exerted any undue influence on the relation studied (Mahalanobis Distances, Cook's Distances and the Centered Leverage Values were all within acceptable range) and the regression residuals did not deviate significantly from normality of distribution (Shapiro-Wilk = .96, ns.), nor did it violate the assumption of homoscedasticity (White's Test: Chi-sq[9] = 9.41, ns.). These results suggested that, although antenatal distress significantly predicted 10-month behavioral reactivity, such that the greater the distress during pregnancy, the greater the infant behavioral reactivity to stress, the relation was not independent of the effect of perinatal neuroendocrinological reactivity. Therefore, the final criterion for statistical mediation was met.

Assessment of the Proposed Mediation Model

Since all conditions for testing mediation were met, the Goodman Test, a formal test of statistical mediation, was conducted based on the antenatal maternal Subjective Stress score, perinatal cortisol change, and 10-month behavioral reactivity, with maternal and infant postnatal influences controlled for in their respective regression model. The Goodman test was significant (t=2.03, p<.05), which was consistent with the hypothesis that perinatal neuroendocrinological stress reactivity mediates the effect of antenatal distress on behavioral stress reactivity.

Does Postnatal Maternal Distress Moderate the Effect of Antenatal Distress on Infant Behavioral and Neuroendocrinological Stress Reactivity?

To test the moderating effect of the postnatal environment on the relation between antenatal distress and postnatal stress reactivity, multiple regression analyses were

conducted to predict the 10-month behavioral stress reactivity score and 10-month cortisol change from the main effects and the interaction of antenatal and postnatal maternal Subjective Stress scores. Regression-based moderation analyses are highly sensitive to multivariate non-normality, which could potentially inflate the effect of the interaction term. To this end, the multivariate normality was first examined in each regression-based moderation model. Results indicated that the assumption of multivariate normality was valid only for the regression model where 10-month behavioral stress reactivity score was regressed on the main effects and the interaction of antenatal and postnatal maternal Subjective Stress scores. The analysis showed that the interaction between the antenatal and postnatal Stress score significantly predicted 10-month behavioral reactivity (B=.84, SE=.31; p<.05) even after the main effects were first partialled out. Regression diagnoses of this multiple regression model indicated that no individual case exerted any undue influence on the relation studied (Mahalanobis Distances, Cook's Distances and the Centered Leverage Values were all within acceptable range) and the regression residuals did not deviate significantly from normality of distribution (Shapiro-Wilk = .96, ns.), nor did it violate the assumption of homoscedasticity (White's Test: Chi-sq[8] =2 .29, ns.).

On the other hand, the assumption of multivariate normality was violated when 10-month cortisol difference score was regressed on the main effects and the interaction of antenatal and postnatal maternal Subjective Stress scores. Such violation puts into question the validity of the estimate, and the result is therefore not reported in the current section.

Discussion

The effect of antenatal stress on the offspring's stress reactivity is known to manifest across multiple dimensions in nonhuman animal studies. These studies have shown that (a) antenatal stress predicts the offspring's behavioral as well as neuroendocrinological stress reactivity (Weinstock, 2001), (b) the effects of antenatal stress on behavioral and neuroendocrinological reactivity are long-lasting, spanning from the juvenile period to maturity in parallel dimensions (Henry et al., 1994; Vallée et al., 1999), (c) the HPA system mediates in part the effect of antenatal stress, through the process of fetal programming (Welberg & Seckl, 2001), and (d) the effects of antenatal stress on the HPA functioning and HPA-mediated functions are moderated by the postnatal environment (Maccari et al., 1995; Smythe et al., 1996). However, parallel human empirical evidence on the stability and the mediation of the effect of antenatal programming is scant. In this preliminary study, I hypothesized that 1) the effect of antenatal maternal distress on offspring's neuroendocrinological stress reactivity is stable between perinatal and postnatal period, 2) the effect of antenatal maternal distress on the offspring's behavioral stress reactivity at 10 months is mediated by the offspring's perinatal neuroendocrinological stress reactivity, independent of the effect of postnatal maternal distress measured at 10 months, 3) postnatal maternal distress moderate the effect of antenatal maternal distress on infant neuroendocrinological and behavioral stress reactivity.

The preliminary findings from the current study are consistent with the hypothesis that (1) the effect of antenatal maternal distress on the offspring's behavioral stress reactivity at 10 months is mediated by the offspring's perinatal neuroendocrinological stress reactivity, but is independent of the effect of postnatal maternal distress measured

at 10 months; and (2) the effect of antenatal maternal distress on the offspring's behavioral stress reactivity at 10 months is moderated by concurrent postnatal maternal distress. However, these findings need to be interpreted with extreme caution. According to Nunnally and Bernstein (1994), capitalization upon chance decreases with sample size and increases with the pool of possible predictors and outcomes. In their view, sample *R* is systematically biased upward compared to the population *R*, and one major reason for the bias is that predictors are usually selected from a larger set. Such bias is negligible when the sample size-to-predictors ratio is large, but becomes substantial when the ratio drops to 10:1, which is the level where the current study is at. This biased selection takes enormous advantage of chance when it is based upon data and not theory (Nunnally & Bernstein, 1994). Although variables selection in the current study was not dictated by the data but guided by theory, the sheer number of candidate measures associated with each construct greatly increases the probability of having predictors being highly correlated with the criterion simply because of sampling error, or having spurious intercorrelation among predictors that may artificially inflate the size of *R*. In addition, even if predictors were not selected from a larger pool, the *R* obtained from a relatively small sample (with sample size-predictor ratio being around 10:1) will tend to become smaller when reapplied to a larger sample. Large samples have lower probability of producing unusually large correlation by chance because the parameter estimates are more stable (Nunnally & Bernstein, 1994).

On the other hand, the current study was not able to provide valid evidence to support the hypothesis that the effect of antenatal maternal distress on the offspring's HPA function is stable between the perinatal and the postnatal period. Similarly, the

current study was unable to provide valid evidence to support the hypothesis that postnatal maternal distress moderates the effect of antenatal maternal distress on infant neuroendocrinological stress reactivity.

One major factor that resulted in the inability of the current study to provide valid evidence consistent with the hypotheses involving the model that examines post-natal cortisol difference score (for example, stability of antenatal maternal effect on offspring's neuroendocrinological function between perinatal and postnatal period, and the moderation of the relation between antenatal maternal distress and infant neuroendocrinological stress reactivity by postnatal maternal distress), could be attributable to the non-normality of 10-month cortisol parameter as well as the non-normality of residuals produced by regression model involving postnatal cortisol parameters. While individual cortisol parameters in the current study have in fact been transformed according to Tukey's graphical diagnostic procedure to offset the parameters' non-normality based on the "ladder of re-expression," distributions of 10-month cortisol parameters remained significantly deviated from normality, which contributed to the non-normality of regression residuals when cortisol difference score was included as one of the variables.

Unfortunately, for all postnatal cortisol parameters examined in the current study, the transformation performed on individual variables was not sufficient in normalizing the distribution of regression residuals. A more direct analytical method is nevertheless available, which estimates the exact power of transformation on the variables that minimize the regression residuals' deviation from normal (Box & Cox, 1964). Further, least-square multiple regression analysis is very sensitive to outlier and influential points

(Stevens, 1984), which may result in the non-normally distributed regression residuals observed in the current study. As regression diagnosis suggests, some cases have significantly more influence and leverage on relations involving infant cortisol difference score. It has been proposed that robust regression analysis that utilizes maximum likelihood estimator rather than least-square estimator may circumvent the potential impact of individual influential points on the distribution of regression residuals (Huber, 1981). Finally, the fact that the distributions of cortisol parameters in most samples contain outlier and are not normally distributed (Gunnar & White, 2001; Goldberg et al., 2003), may reflect the possibility that they were actually drawn from a non-normal population distribution. In combination with small sample size, the least-square estimation of the current study may be seriously biased. As such, Fox (2002) proposed that bootstrapping regression model may potentially be a solution to regression analysis involving variables drawn from non-normal population distribution and with small sample size.

. The preliminary findings reported in this study are consistent with the growing nonhuman animal literature, which has shown that the effect of antenatal maternal distress on the offspring's behavior is mediated by the offspring's neuroendocrinological system (Welberg & Seckl, 2001), and moderated (exacerbated) by postnatal maternal distress (Maccari et al.,1995; Smythe et al., 1996). However, considerable caution has to be taken to interpret them within the context of their potential lack of generalizability, and the capitalization on chance. The findings reported here are also consistent with recent findings involving humans. For example, O'Connor et al. (2005) and Gutteling et al. (2005) also found that antenatal maternal distress was associated with enhanced HPA

functions in the offspring. Different from these two studies wherein cortisol samples were collected during childhood and pre-adolescence, the current study collected cortisol samples from the offspring during the perinatal period as a gross representation of the effect of antenatal programming of the fetal HPA axis. If salivary cortisol measured 24 hours after birth is not an entirely invalid representation of fetal HPA function, the preliminary finding reported in the present study is consistent with the hypothesis that adverse behavioral outcomes in the offspring is associated with antenatal programming of the *fetal* HPA axis. To ensure that perinatal HPA function approximates fetal HPA function, the current study pre-screened for any potential contextual influences, including the nature and quality of the birth experience, the quality and quantity of maternal contact, as well as other medical related events within the first 24 hours of postnatal life.

Recent research has also demonstrated that early contextual influences play an important role in affecting stress-reactivity measures in humans. For example, Fox and his colleagues (Hane & Fox, 2006) have noted that variations in parenting in early infancy affect stress reactivity measures (i.e., behavioral and frontal EEG asymmetry) in children. Recent unpublished findings from our own laboratory have also shown that maternal characteristics such as maternal behavior, attention regulation, and cognitive strategy of approach and withdraw predicted infant neuroendocrinological reactivity (Atkinson et al., in preparation). Taken together, the recent findings from the extant literature, along with the preliminary findings reported in the current study, suggest the importance of early contextual influences in sculpting different levels of the stress reactivity system in humans.

Limitations

Sample attrition may have undermined the representativeness and the generalizability of the findings reported in the current study. This limitation was exacerbated by the number of inferential statistics conducted in the study, which may have increased Type I error rate. Nevertheless, preliminary findings reported in Studies 1 and 2 above replicated the relation between antenatal maternal distress and the offspring's stress reactivity identified in the current study. While a large number of analyses conducted greatly increased the probability of type 1 error, however, given the scarcity of empirical data on the mechanism of antenatal programming in human, it would risk type 2 error if the full potential of the data set was not explored.

Unlike Studies 1 and 2 reported above, the current study had very stringent exclusion criteria, in combination with thorough perinatal information collection on the mother and her offspring. Such vigorous selection protocol may have biased the sample, making it neither representative nor generalizable. Nonetheless, the more community-based recruitment strategy reported in Study 1 and 2 resulted in findings that, albeit preliminary, replicated findings reported in the current study.

There also exists a further limitation inherent in the design of Study 3. In the current study, antenatal maternal distress and the offspring's antenatally programmed HPA system were both assessed 24 hours *after* birth rather than during pregnancy, which potentially introduced confounds to the nature of influence during pregnancy. Recognizing the potential problem of measuring the antenatal relation between maternal stress and infant HPA-function postnatally, steps were taken to isolate the antenatal effect from postnatal ones by partialling out perinatal influence on maternal mood and attitude toward the newborn in the first 24 hours. I acknowledge that statistically partialling out

postnatal maternal influence to isolate the antenatal influence is insufficient at best; future study needs to assess the functioning of fetal HPA system directly through the assessment of fetal cortisol. Similarly, it is recognized that measuring antenatal maternal distress during the perinatal period poses confounds. Nevertheless, the relation between antenatal maternal distress and the offspring's behavioral stress reactivity was replicated by the preliminary findings reported in Studies 1 and 2, wherein antenatal maternal distress and the offspring's stress reactivity were measured prospectively.

Future Directions

The preliminary findings reported in the current study are consistent with findings from the nonhuman animal stress literature. In a rudimentary way, the current findings show in a very small sample of human infants that antenatal distress predicts behavioral stress reactivity during infancy and such an effect is mediated by the offspring's perinatal neuroendocrinological stress reactivity, and moderated by postnatal maternal distress. Future studies are needed which include a larger sample size than reported herein, repeated measurements of behavior and salivary cortisol over time at multiple age points, and a measure of fetal neuroendocrinological activity to ensure the reliability and generalizibility of the present findings. The preliminary findings from Study 1 above replicates at the behavioral level the neuroendocrinologically mediated effect of maternal distress on offspring's behavioral outcomes. Study 2 further explores this parallel between the animal and human models in terms of the parallel between neuroendocrinological and behavioral effects of antenatal maternal distress on the offspring. The findings reported in the current study, in a very rudimentary way, explore

the role of the offspring's neuroendocrinological stress reactivity as the potential mechanism for the effect of antenatal maternal distress on infant behaviors.

Future work should also consider the influence of antenatal programming on developmental outcomes in clinical populations such as survivors of low birth weight and examine if the influences of antenatal program on behavioral and neuroendocrinological reactivity in humans can be modified or reversed.

GENERAL DISCUSSION

The high prevalence of cardiovascular and metabolic diseases among adults who had low birth weight has sparked the search for a fetal origin of adult diseases (Barker, 1991, 1998). The "antenatal programming" hypothesis has been advanced, not only to explain the increased morbidity of individuals who have low birth weight, but also the poor developmental and health outcomes among the offspring who experience antenatal adversity, of which low birth weight is an indicator (Phillips & Jones, 2006). Non-human animal studies have accumulated a substantial body of prospective evidence in support of the antenatal programming hypothesis (Seckl, 2005; Weinstock, 2001, 2002; Welberg & Seckl, 2001), which suggested that: 1) antenatal stress predicts the offspring's behavioral and health outcomes, 2) the effects of antenatal stress are stable, and 3) the effects of antenatal stress on the offspring's behavioral and health outcomes are mediated by the offspring's stress reactivity. Findings available from human developmental research are consistent with the animal literature in showing that antenatal stress predicts the offspring's behavioral and emotional health outcomes (Van den Bergh et al., 2005b), and that the offspring's neuroendocrinological stress reactivity is a target of the antenatal maternal effects (Gutteling et al., 2005; O'Connor et al., 2005). Nevertheless, human evidence that addresses the stability of, and the linkage between, the behavioral and neuroendocrinological effects of antenatal maternal distress is lacking. Also missing is evidence that replicate findings from the animal literature and shows that the offspring's HPA system mediates the antenatal programming of the offspring's behavior.

The three studies reported above were attempts to explore this knowledge gap. Animal studies have demonstrated that the offspring's neuroendocrinological stress reactivity is the primary target and mediator of antenatal programming. On the other

hand, the majority of human literature examines the effects of antenatal maternal distress on the offspring's behavioral rather neuroendocrinological stress reactivity. To this end, Study 1 was conducted to examine if the offspring's stress reactivity, when measured behaviorally rather than neuroendocrinologically, indeed serves as the target and the mediator of antenatal maternal effect. The results of Study 1 showed, in a very rudimentary way that antenatal maternal distress predicted laboratory-observed behavioral stress reactivity at 6 months, physical health status generated from an illness diary between 12 to 18 months, and standardized maternal report of internalizing behavioral difficulty at 24 months. Study 1 also showed that stress reactivity, measured behaviorally here, statistically mediates the effect of antenatal maternal distress on physical and emotional health in a very small sample.

Analyses reported in Study 2 and 3 were reported to bridge the gap between, on one hand, the model reported in Study 1 where behaviorally measured stress reactivity mediates the antenatal effect, and on the other hand, a model documented in the animal literature where neuroendocrinologically measured stress reactivity mediates the antenatal effect. Unfortunately, Study 2 offers no valid evidence to demonstrate the antenatal maternal effect on the offspring neuroendocrinological function, nor does it show the relation between the effect of antenatal maternal distress on offspring's neuroendocrinological and behavioral stress reactivity. Nevertheless, preliminary findings of Study 2 are consistent with the hypothesis that antenatal maternal distress predicts behavioral stress reactivity, and the effect of antenatal maternal distress on behavioral stress reactivity is stable over time. Study 3 further explores the nature of the relation between the behavioral and the neuroendocrinological effects of antenatal

maternal distress. Findings of Study 3 showed, rudimentarily in a small sample, that the offspring's perinatal neuroendocrinological stress reactivity (as a gross representation of fetal HPA function) statistically mediated the impact of antenatal maternal distress on infant behavioral stress reactivity.

However, the preliminary findings reported in these three study need to be interpreted with extreme caution. According to Nunnally and Bernstein (1994), capitalization upon chance decreases with sample size and increases with the pool of possible predictors and outcomes. This biased selection takes enormous advantage of chance when it is based upon data and not theory (Nunnally & Bernstein, 1994). Although variable selection in the current study was not dictated by the data but guided by theory, the sheer number of candidate measures associated with each construct greatly increases the probability of having predictors being highly correlated with the criterion simply because of sampling error, or having spurious intercorrelation among predictors that may artificially inflate the effect size. In addition, small samples have greater probability of producing unusually large correlation by chance because the parameter estimates are much more unstable (Nunnally & Bernstein, 1994), and are particularly susceptible to the influence of individual variable in the optimization process of the least-square estimation, and therefore artificially bias the result. For this reason, least-square regression model in a small sample is particularly sensitive to biases, resulted from the violation of one or more modeling assumptions. Two out of three studies reported above violated one or more assumptions of regression analyses, and hence proper care should be taken in the interpretation of the preliminary findings reported above.

Nevertheless, when considerable caution has been taken to interpret the findings within the context of their potential lack of generalizability, their potential capitalization on chance and the potential violation of modeling assumption, the findings of these 3 preliminary studies, as a group, are nevertheless consistent with the antenatal programming hypothesis, where 1) antenatal stress predicts the offspring's behavioral and health outcomes, 2) the effects of antenatal stress are stable, and 3) the offspring's HPA system is the primary target and mediator of antenatal effect.

1. *Antenatal Stress Predicts the Offspring's Behavioral and Health Outcomes*

Consistent with both the animal and human literature, all three studies reported above have shown in a rudimentary way that antenatal maternal distress significantly predicted the offspring's behavioral stress reactivity. The nature of maternal distress being measured was different across studies, ranging from depressive symptomatology to the impact of everyday-life stressful events, yet all predicted the offspring's behavioral stress reactivity in similar magnitudes. These relations were consistent with previous human literature, where maternal distress that ranges from depressive or anxiety symptoms to perceived stress from daily hassles, all predict poor outcomes in the offspring (Van den Bergh et al., 2005b). Such equifinality of the effect of maternal distress on the offspring is consistent with the animal literature, which shows that maternal glucocorticoids, the end product of the neuroendocrinological stress system, is the agent of antenatal programming (Matthew, 2000, 2002).

The three studies reported above also assessed the offspring's behavioral stress reactivity with multiple but converging measures, including maternal report (Study 2) as well as laboratory observations (Study 1 and Study 3). Nevertheless, the subscale selected

from the maternal report and the behavioral coding scheme shared by the two laboratory paradigms were selected on a theoretical ground to capture specifically the offspring's reactivity to stress under novel situations, such that construct validity is preserved. While no published study has included both maternal report and laboratory observation in examining the effect of antenatal maternal distress on the offspring's behavioral stress reactivity, the preliminary findings reported in this dissertation are consistent with the general findings in the literature, which show that antenatal maternal distress predicts the offspring's behavioral stress reactivity, independent of how it was measured (Van den Bergh et al., 2005b).

Also in a rudimentary way, Study 1 has shown that antenatal maternal distress significantly predicted both physical and emotional health of the offspring. Such preliminary findings are consistent with the animal literature, where the antenatally stressed offspring are more likely to suffer from physical illnesses and exhibit heightened emotionality (Weinstock, 2001). Such preliminary findings are also consistent with the human literature, where epidemiological research has demonstrated that the offspring's poor physical health is associated with antenatal adversity (Barker, 1998) and developmental research has shown that the offspring's poor emotional health is linked to antenatal maternal distress (Van den Bergh et al., 2005b). In this regard, the preliminary findings reported in Study 1 are consistent with the findings of the animal literature, where the effect of antenatal maternal distress is observed across both physical and emotional health outcomes among the offspring. While the effects on both health outcomes are linked physiologically through the HPA system (Seckl et al., 2005), the two

outcomes themselves are not significantly correlated, giving weight to the preliminary findings of such multiple effects.

In predicting behavioral and health outcomes from antenatal distress, all three studies reported above have first statistically controlled for the effect of the corresponding postnatal maternal distress to isolate the antenatal from the postnatal effect on the offspring. It is, however not a standard practice in the human literature on antenatal programming (for exceptions, see Huizink et al., 2003 and O'Connor et al., 2002, 2003). Hence, the significant association between the antenatal maternal distress and behavioral and health outcomes reported above reflects the unique portion of variance of behavioral and health outcomes accounted for by antenatal maternal distress.

2. *The Effects of Antenatal Stress are Stable*

Consistent with animal literature and the limited findings available from human literature, Study 2 showed, in a rudimentary way that the effects of antenatal maternal distress on behavioral stress reactivity (as reported by the mother) were stable over an 18-month span, from infancy to toddlerhood. However, such findings were not replicated across different dimensions.

3. *The Offspring's HPA system is the Primary Target and Mediator of Antenatal Effect*

Notwithstanding that the preliminary findings reported in the current dissertation lack generalizability, capitalize on chance, and violate assumption of regression model, these findings, when interpreted with appropriate caution, are nonetheless consistent with the animal literature, which indicates that the offspring's HPA system is the target and mediator of antenatal maternal effect. In a rudimentary way, Study 3 shows that antenatal maternal distress affects offspring's perinatal neuroendocrinological stress reactivity

(arguably a gross presentation of fetal HPA function), which in turn mediates the effect of antenatal maternal distress on the offspring's behavioral reactivity at 10 months.

Limitations

Limitations: Sample size

Because of small sample size, either by attrition or by design, any single study has only limited generalizablility. Such limitation was exacerbated by the number of inferential statistics conducted in the study, which might have increased Type I error rate. In balancing Type 1 and Type 2 error, studies reported here tended toward minimizing Type 2 error given the scarcity of human empirical data. As a result, Type 1 error might have been inflated.

Limitation: Design

The current dissertation reports secondary analyses of data collected to answer very different research questions; for this reason, the timing of data collection may not completely capture the developmental process set out to study. For example, the studies lacked symmetry in the assessment of parallel developmental dimensions (Study 1: physical and emotional health; Study 2: different time frame of cortisol vs. behavioral assessments) and were unable to track precise timing of antenatal development (antenatal maternal distress was assessed only at the 3rd trimester in Study 1 & 2, and was measured retrospectively in Study 3).

Limitation: Recruitment

Again, because the cohorts reported in the current dissertation were not recruited specifically for the purpose of examining the antenatal programming hypothesis, their inclusion and exclusion criteria could have introduced biases and confounds. For

example, the Toronto cohort (Study 1 & 2) was drawn from the community sample recruited from prenatal classes without first screening for any pregnancy risks or complications, which might co-occur with antenatal maternal distress. On the other hand, the Hamilton cohort had very stringent selection criteria, possibly undermining the representativeness of the sample with respect to the Ontario population. The Hamilton cohort was also recruited from the hospital after labour, which ran the risk of self-selection bias.

Limitation: Antenatal maternal measures

Antenatal distress was only measured using self-report, limiting our understanding of the nature of the antenatal environment a fetus experienced. Given that many outcome measures were also based on maternal reports, relying on maternal self-report as the only source of information on antenatal distress might have introduced reporter biases.

Limitation: Postnatal maternal measures

To isolate the unique contribution of antenatal maternal distress from that of postnatal maternal distress on the offspring's behavioral and health outcomes, postnatal maternal distress was statistically controlled from the relation between antenatal maternal distress and child developmental outcome. However, postnatal maternal influence on the infant is complex and multifactorial, which may not be best captured by maternal self-report alone. In addition, antenatal and postnatal environment are generally stable, making such incomplete control of postnatal contribution a confounding factor.

Limitation: Infant Behavioral and Health Outcomes

Although precaution was taken in the dissertation as a whole to measure the offspring's behavioral and health outcomes with convergent evidences collected from different number of sources, Study 1 and Study 2 relied heavily on maternal report as the source of information for behavioral reactivity (ICQ), as well as behavioral (CBCL) and physical health (illness diary) outcomes. Given that maternal self-report was the only source from which we gather information on maternal distress, reporter-biases might have introduced confounds to the prediction.

Limitation: Alternative explanations

The linkage between antenatal maternal distress and infant stress reactivity can be explained alternatively by the stability of genetic trait from one generation to the next, as well as the stability of the larger social context surrounding the dyad. Currently the 3 studies reported in the dissertation are not able to isolate their relative contributions. Future studies that examine the mechanism with which antenatal maternal distress influences offspring's developmental outcomes may be benefited from collecting data pertaining to the dyad's genetical inheritance as well as social economic circumstances.

Implications

Findings reported above may serve as reference for clinical practice and social policy. As has been shown previously, antenatal maternal distress, either in terms of the impact of everyday-life stress or depressive/anxiety symptomatology, affects future physical and emotional health of the offspring. Standardized screening processes could be instituted in gynecologist offices and women's health clinics to identify families at risk, facilitating the reception of immediate clinical and social support. Further, low birth weight and prematurity are of much higher prevalence among low SES populations

(Kramer, Yaffe, Lengenfelder, & Delis, 2003), which is an indication of high antenatal maternal adversity. Given the long-term medical and emotional cost antenatal adversity has on the society, there is a preventive need that could be met by increasing social and family services available to pregnant mothers. The findings reported above also indicate that the effect of antenatal maternal distress can be buffered by the postnatal caregiving milieu, underscoring the importance of postnatal clinical and social support, including home visitation programs and high quality universal daycare.

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TABLES

Table 1.1

Descriptive Statistics of Maternal Distress, Behavioral Stress Reactivity Score, Physical Health Indicators, and CBCL Internalizing Score

	M	SD
MATERNAL DISTRESS		
3rd trimester BDI	7.97	4.74
6-month BDI	6.06	4.68
12-month BDI	7.38	5.75
18-month BDI	6.68	5.75
STRESS REACTIVITY		
6-month behavioral stress reactivity	.89	.42
PHYSICAL HEALTH INDICATORS		
no. of days ill	16.85	12.75
no. of doctor visits	1.21	1.21
no. of days non-prescribed medication was taken	.76	1.06
no. of days prescribed medication was taken	2.07	1.81
EMOTIONAL HEALTH		
CBCL Internalizing Score	14.33	6.95

Table 1.2

Intercorrelation Amongst Outcome Variables

	no. of doctor visits	no. of days non-prescribed medication taken	no. of days prescribed medication taken	CBCL Internalizing Score
no. of days ill	.42	.52	.33	.52
no. of doctor visits		.53	.62	.45
no. of days taken non-prescribed medication			.43	.50
no. of days taken prescribed medication				.16

Table 2.1

Descriptive Statistics of Maternal Distress, Cortisol Levels and Behavioral Reactivity Scores

	M	SD
MATERNAL DISTRESS		
3rd trimester IES	29.26	9.52
6-month IES	33.41	7.13
12-month IES	31.99	7.88
STRESS REACTIVITY		
12-month cortisol baseline	.21	.16
12-month cortisol reactivity	1.10	2.85
18-month cortisol baseline	.26	.16
18-month cortisol reactivity	-.09	.58
STRESS REACTIVITY		
6-month ICQ total scores	81.46	19.49

24-month ICQ total scores	106.38	19.77

Table 2.2

Intercorrelation Amongst Outcome Variables

	18-month cortisol reactivity	6-month behavioral reactivity	24-month behavioral reactivity
12-month cortisol reactivity	.36	.46	.33
18-month cortisol reactivity		.38	.43
6-month behavioral reactivity			.61

Table 3.1

Descriptive Statistics of Antenatal and Perinatal Demographics and Background Information

	Descriptives Statistics	
Maternal Variables		
Mother's age	M = 30.24 years	SD = 5.16
Marital Status (perinatal)	96% married	
Marital Status (at 10 months)	91% married	
Education	72% were at least high school graduated	
Antenatal medication	36% had been medicated during pregnancy	
Smoking during pregnancy	14% smoked during pregnancy	
Alcohol use during pregnancy	1% used alcohol during pregnancy	
Illicit drug use during pregnancy	1% used illicit drugs	
Obstetric complication	45% had at least one type of obstetric complication	
Peripartum intervention	24% had at least one form of peripartum intervention	
Delivery complications	45% had at least some types of non-threatening	

	complication
Birth Intervention	57% received at least one form of birth intervention
Mode of delivery	25% C-section

Table 3.1 (continued)

Infant Variables		
Newborn's biological sex	51% female	
Birth order	41% first born, 50% second or third born	
Apgar 1 minute	M=8.15	SD=1.40
Apgar 5 minute	M=8.94	SD=.66
Birth weight	M=3559.54g	SD=447.90
Gestational Age	M = 39.34	SD=1.19

Table 3.2

Descriptive Statistics of Potential Confounding Factors on Maternal Attitude and Behavior within the First 24 Hours of Postnatal Life

	Descriptives Statistics
Allowed to cuddle baby first hour after birth	84% yes
Newborn was placed on mom's breast	58% immediately
Attitude toward labour and birth	57% between somewhat positive to positive
Attitude toward baby at birth	89% between somewhat positive to positive
Attitude toward motherhood	65% between somewhat positive to positive

Table 3.3

Means and Standard Deviation of 1) Antenatal and 10-month Maternal Distress, 2) Offspring's Perinatal and 10-month Cortisol and 3) 10-month Behavioral Stress Reactivity

	M	SD
Maternal distress 24 hours after birth	7.11	1.45
Maternal distress at 10 months	6.93	1.36
Cortisol baseline 24 hours after birth	0.44	0.46
Cortisol change in reaction to 24-hours medical examination	-0.11	0.2
Cortisol baseline at 10 months	0.53	0.93
cortisone change in reaction to 10-month toy-removal procedure	-0.05	0.1
10-month baseline infant distress	0.23	0.51
10-month infant stress reactivity	0.9	4.02

Appendices

Table A1.1

Distribution Statistics of 3rd Trimester, 6-month, 12-month and 18-month Beck Depression Scores

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
3rd Trimester	1.92 (.43)	4.12 (.83)	.79**
6-Month	.45 (.44)	-.93 (.87)	.93^{+}
12-Month	1.16 (.50)	2.46 (.97)	.91^{+}
18-Month	1.22 (.43)	.59 (.85)	.84**

Table A1.2

Distribution Statistics of 3rd Trimester, 6-month, 12-month and 18-month Log-transformed Beck Depression Scores

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
3rd Trimester	.40 (.43)	.65 (.83)	.95
6-Month	-.50 (.45)	-.55 (.87)	.94
12-Month	-.61 (.50)	-.01 (.97)	.94
18-Month	-.22 (.43)	-.64 (.85)	.97

Table A1.3

Distribution Statistics of Infant Behavioral Reactivity Score

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Baseline Distress	2.27 (.47)	4.99 (.92)	.64**
Distress/Stimuli	1.20 (.44)	.51 (.87)	.81**
Positive Vocalization/Stimuli	-.11 (.44)	-1.13 (.86)	.90**
Distress Difference Score	.52 (.49)	.11 (.95)	.90*

Table A1.4

Distribution Statistics of Log-transformed Infant Behavioral Reactivity Score

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Baseline Distress	.74 (.47)	-.76 (.92)	.84**
Distress/Stimuli	.70 (.45)	-.71 (.87)	.85**
Positive Vocalization/Stimuli	-.30 (.44)	-1.24 (.86)	.87**
Distress Difference Score	-.01 (.43)	-.47 (.83)	.98

Table A1.5

Distribution Statistics of Physical Health Indicators and their Composites

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Sick Days	.51 (.44)	-1.17 (.87)	.90*
Doctor Visits	1.40 (.43)	2.21 (.85)	.80**
Non-prescribed medication	1.56 (.43)	3.21 (.85)	.85**
Prescribed medication	1.69 (.43)	2.60 (.85)	.72**
Composite	2.51 (.45)	8.05 (.87)	.74**

Table A1.6

Distribution Statistics of Log-transformed Physical Health Indicators and their Composites

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Sick Days	-.35 (.44)	-1.13 (87)	.92*
Doctor Visits	.23 (.43)	-.51 (.85)	.86**
Non-prescribed medication	-.02 (.43)	-.18 (.85)	.93
Prescribed medication	.81 (.43)	-.36 (.85)	.77**
Composite	.49 (.45)	-.09 (.87)	.97

Table A1.7

Distribution Statistics of CBCL Internalizing and Externalizing Subscale Scores and CBCL total Scores

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Internalizing Scores	.38 (.43)	-1.11 (.83)	.93*
Externalizing Scores	.15 (.43)	-.45 (.83)	.99
Total Scores	.16 (.43)	-84 (.83)	.98

Table A1.8

Distribution Statistics of Log-transformed CBCL Internalizing and Externalizing Subscale Scores and CBCL total Scores

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Internalizing Scores	-.51 (.43)	.00 (.83)	.95
Externalizing Scores	-1.18 (.43)	1.79 (.83)	.92*
Total Scores	-.81 (.43)	.66 (.83)	.95

Table A1.9

Intercorrelations between Log-transformed BDI Scores and Log-transformed Behavioral Reactivity Scores

	Baseline Distress	Distress/stimuli	Positive Vocalization /stimuli	Distress Difference Scores
3rd Trimester BDI Scores	-.14	.36*	-.26	.38*
6-Month BDI Scores	.03	.32+	-.19	.32+

**p≤.01, *p≤.05, $^{+}$p≤.10

Table A1. 10

Intercorrelations between Log-transformed IES Scores and Log-transformed Behavioral Reactivity Scores

	Baseline Distress	Distress/Stimuli	Positive Vocalization/Stimuli	Distress Difference Scores
3rd Trimester IES Scores	-.04	.24	-.05	$.32^{+}$
6-month IES Scores	-.16	.09	-.09	.11

**p≤.01, *p≤.05, $^{+}$p≤.10

Table A1.11

Intercorrelation between Log-transformed Health Indicators and Log-transformed Behavioral Reactivity Scores

	Distress Difference Scores	Distress/Stimuli	Positive Vocalization/Stimuli	Baseline Distress
Sick Days	.12	.16	0.36*	.02
Doctor Visits	-.06	-.05	.37	-.00
Prescribed Medication	-.17	-.16	.08	-.02
Nonprescribed Medication	-.26	-.26	.45*	.18
Composite	39*	.41*	-.08	-.16

**p≤.01, *p≤.05, $^{+}$p≤.10

Table A1.12

Intercorrelations between Log-transformed CBCL Subscale and Total Scores and Log-transformed Behavioral Reactivity Scores

	Distress Difference Score	Distress/Stimuli	Positive Vocalization/Stimuli	Baseline Distress
CBCL Internalizing Scores	.36*	.39*	-.15	.02
CBCL Externalizing Scores	.37*	.37*	-.36*	.06
CBCL Total Scores	.37*	.38*	-.23	.05

**p≤.01, *p≤.05, $^{+}$p≤.10

Table A1.13

Intercorrelations between log-transformed BDI Scores and Log-transformed Health Indicators

	Sick Days	Doctor Visits	Prescribed Medication	Nonprescribed Medication	Composite
3rd Trimester BDI Scores	.23	.26	.18	-.08	.39*
6-month BDI Scores	-.00	-.10	-.26	-.39*	.24
12-month BDI Scores	.07	.26	-.07	-.29	.11
18-month BDI Scores	.22	.26	.14	.01	.15

**p≤.01, *p≤.05, $^{+}$p≤.10

Table A1.14

Intercorrelations between IES Scores and Log-transformed Health Indicators

	Sick Days	Doctor Visits	Prescribed Medication	Nonprescribed Medication	Composite
3rd Trimester IES Scores	.56*	.27	-.02	.03	.51 **
6-month IES Scores	.37*	.39*	.15	.11	.28
12-month IES Scores	.24	.39*	.11	.16'	.04

**p≤.01, *p≤.05, $^{+}$p≤.10

Table A1.15

Intercorrelations betweenLog-transformed BDI Scores and Log-transformed CBCL Subscale and Total Scores

	CBCL Internalizing Scores	CBCL Externalizing Scores	CBCL Total Scores
3rd Trimester BDI Scores	.46*	.24	.38*
6-month BDI Scores	-.01	.04	.04
12-month BDI Scores	.09	.10	.10
18-month BDI Scores	.41*	.27	.33^{+}

**p≤.01, *p≤.05, $^{+}$p≤.10

Table A1.16

Intercorrelations between IES Scores and Log-transformed CBCL Subscale and Total Scores

	CBCL Internalizing Scores	CBCL Externalizing Scores	CBCL Total Scores
3rd Trimester IES Scores	.17	.22	.26
6-month IES Scores	.37*	.19	.23
12-month IES Scores	.22	.08	.12

**p≤.01, *p≤.05, $^{+}$p≤.10

Figure A1.1. Histogram of Third Trimester Beck Depression Scores (Raw Scores)

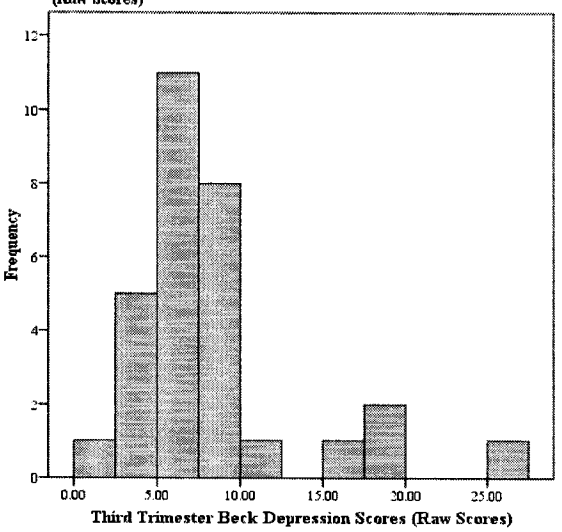


Figure A1.2. Histogram of 6-Month Beck Depression Scores (Raw Scores)

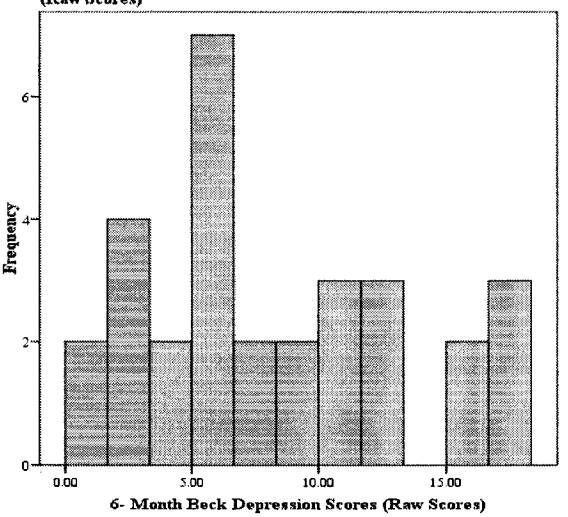


Figure A1.3. Histogram of 12-Month Beck Depression Scores (Raw Scores)

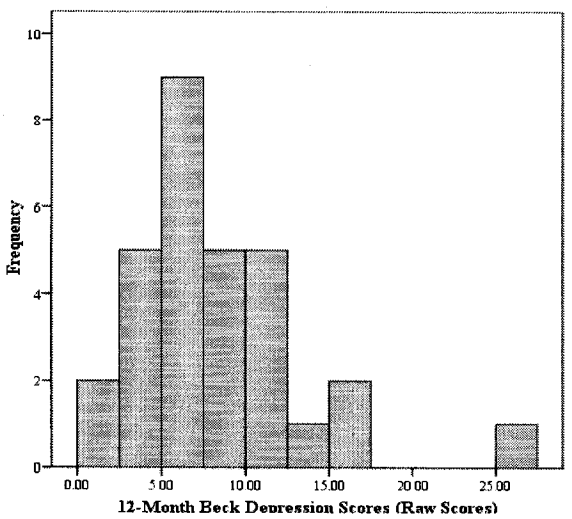


Figure A1.4. Histogram of 18-Month Beck Depression Scores (Raw Scores)

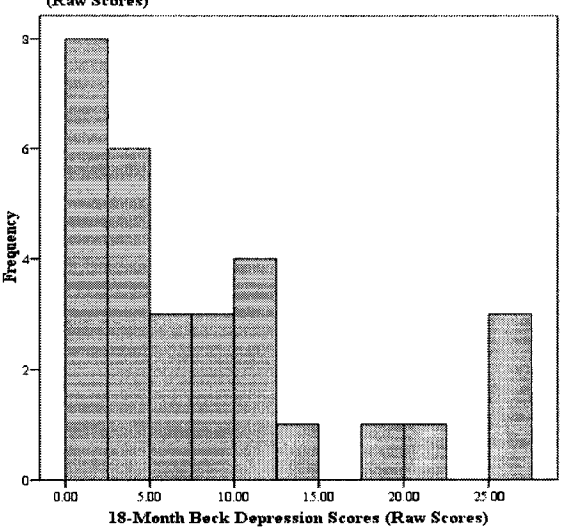


Figure A1.5. Histogram of Trimester Beck Depression Scores (Log Scores)

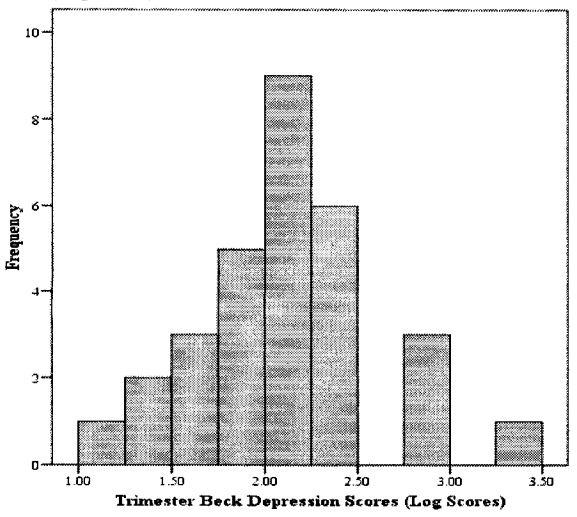


Figure A1.6. Histogram of 6-Month Beck Depression Scores (Log Scores)

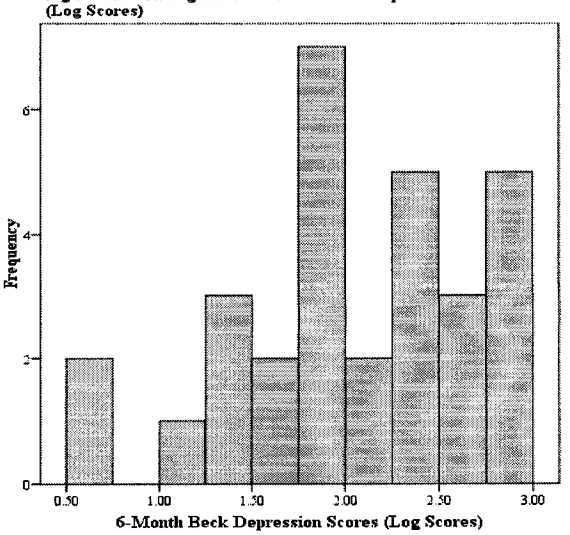


Figure A1.7. Histogram of 12-Month Depression Scores (Log Scores)

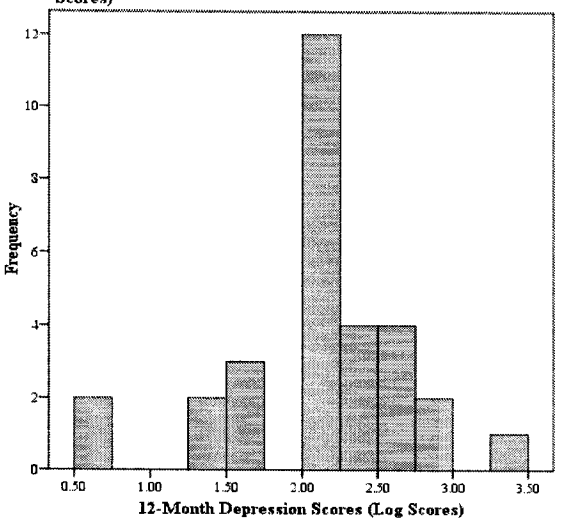


Figure A1.8. Histogram of 18-Month Beck Depression Scores (Log Scores)

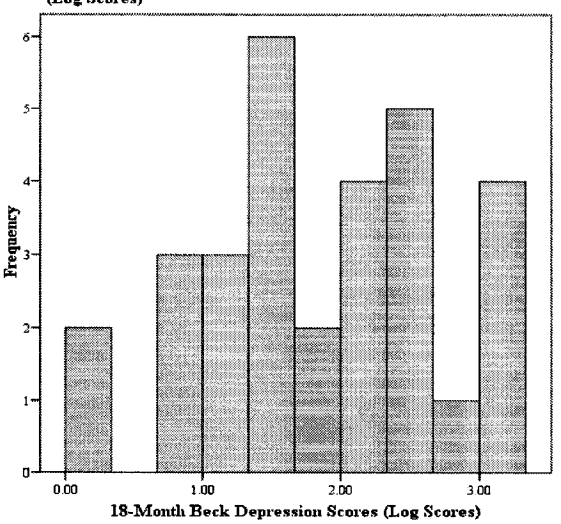


Figure A1.9. Histogram of Behavioral Distress (%) Prior to Stimuli Presentation (Raw Scores)

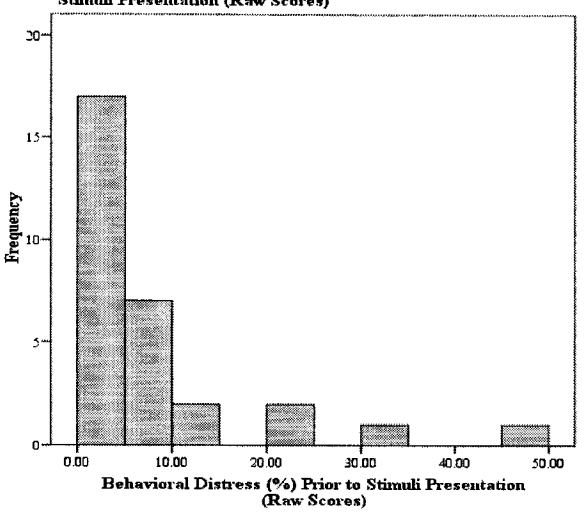


Figure A1.10. Histogram of Behavioral Distress in Relation to the Number of Stimuli Presented (Ratio) (Raw Scores)

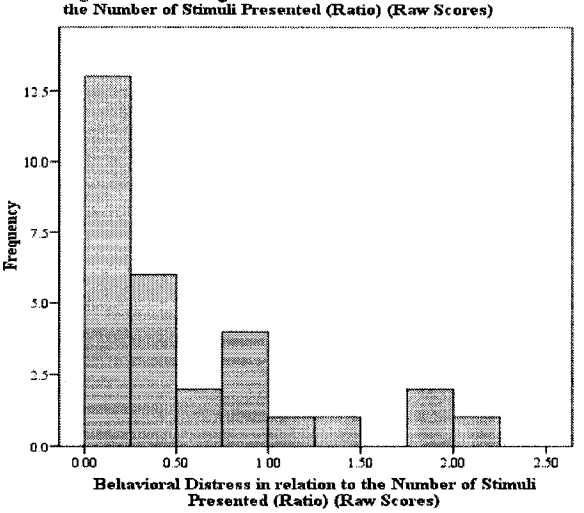


Figure A1.11. Histogram of Positive Vocalization in Relation to the number of Stimuli Presented (Ratio) (Raw Scores)

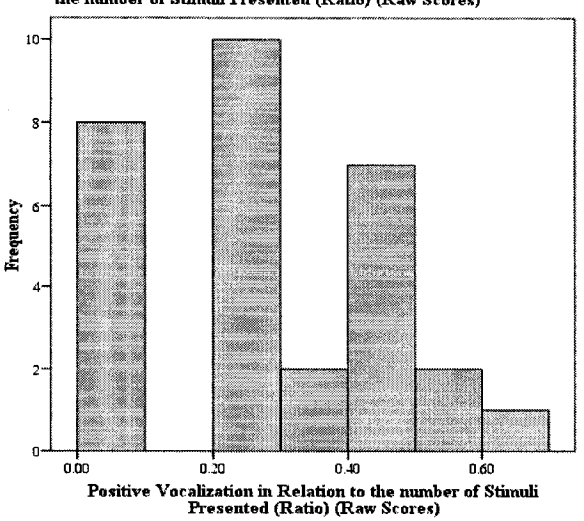


Figure A1.12. Histogram of Difference Scores between Behavioral Distress at Baseline and during Stimuli Presentation (Raw Scores)

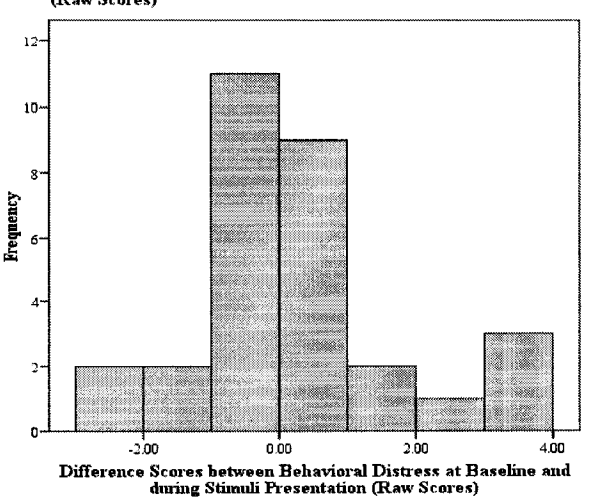


Figure A1.13. Histogram of Behavioral Distress (%) prior to Stimuli Presentation (Log Scores)

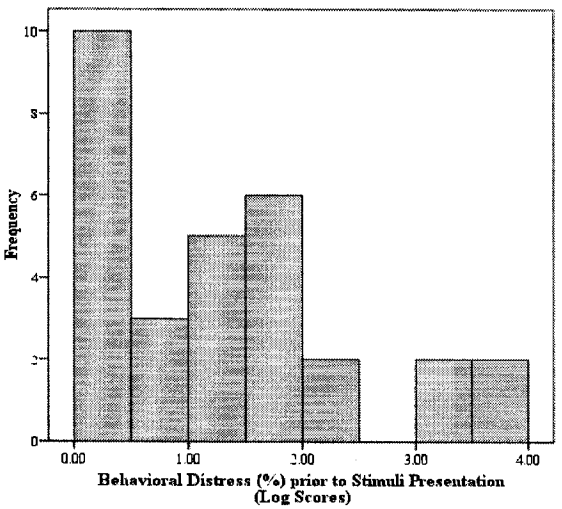


Figure A1.14. Histogram of Behavioral Distress in Relation to the Number of Stimuli Presented (Ratio) (Log Scores)

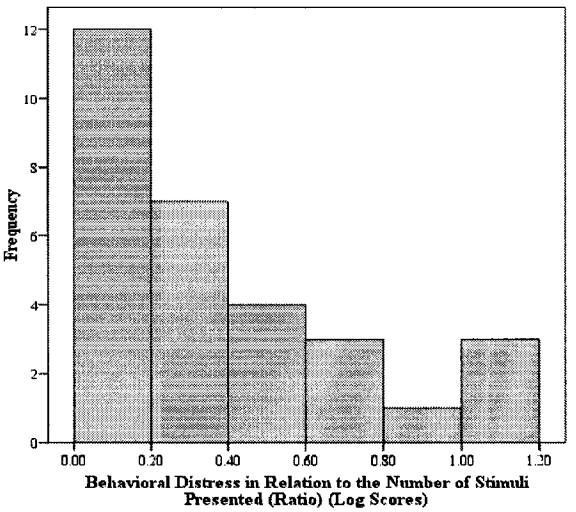


Figure A1.15. Histogram of Positive Vocalization in Relation to the Number of Stimuli Presented (Ratio) (Log Scores)

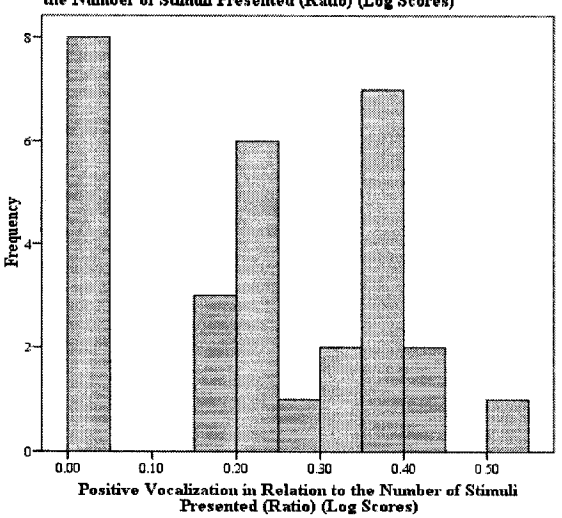


Figure A1.16. Histogram of Difference Scores between Behavioral Distress at Baseline and during Stimuli Presentation (Log Scores)

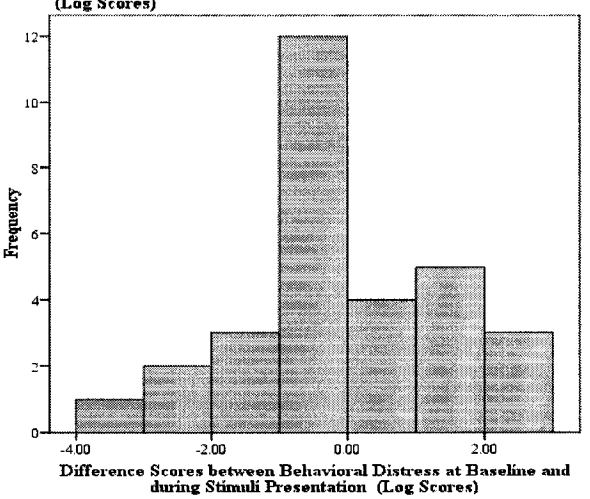
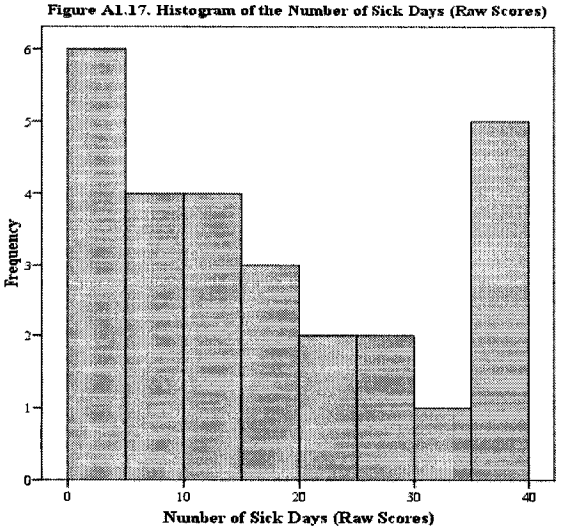


Figure A1.17. Histogram of the Number of Sick Days (Raw Scores)



FigureA1.18. Histogram of the Number of Doctor Visits (Raw Scores)

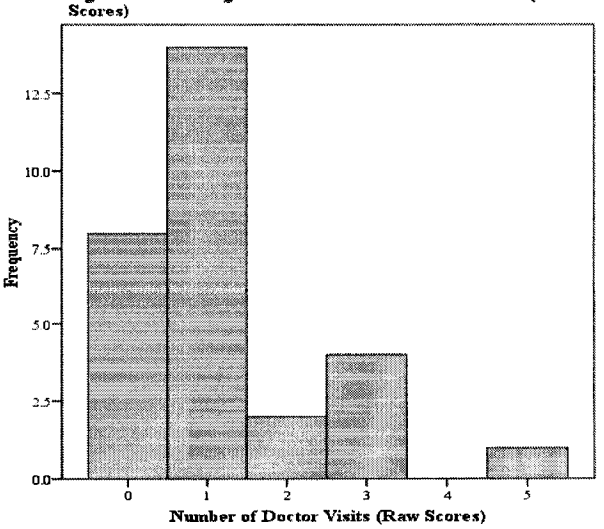


Figure A1.19. Histogram of the Number of Days Non-prescribed Medication Taken (Raw Scores)

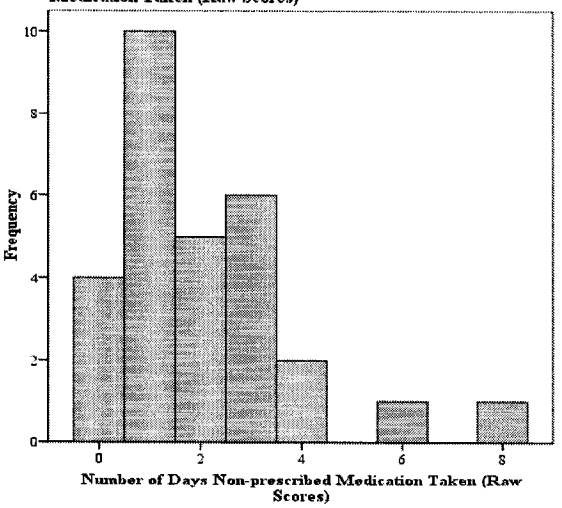


Figure A1.20. Histogram of the Number of Days Prescribed Medication Taken (Raw Scores)

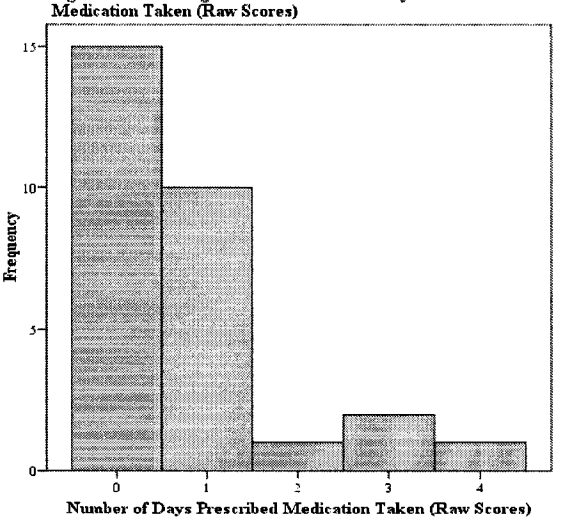


Figure A1.21. Histogram of the Composite of All Four Health Indicators (Raw Scores)

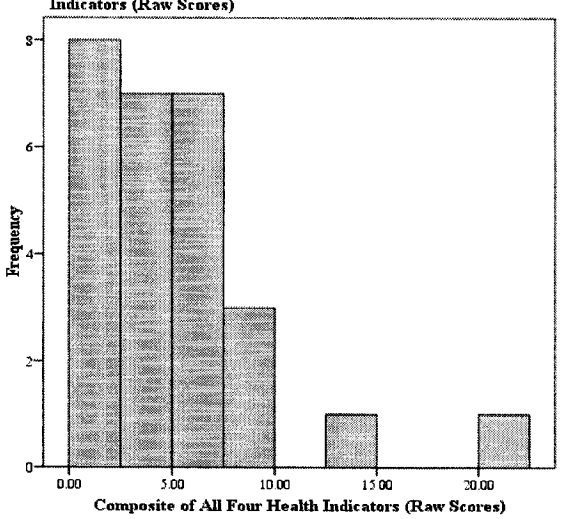


Figure A1.22. Histogram of the Number of Sick Days (Log Scores)

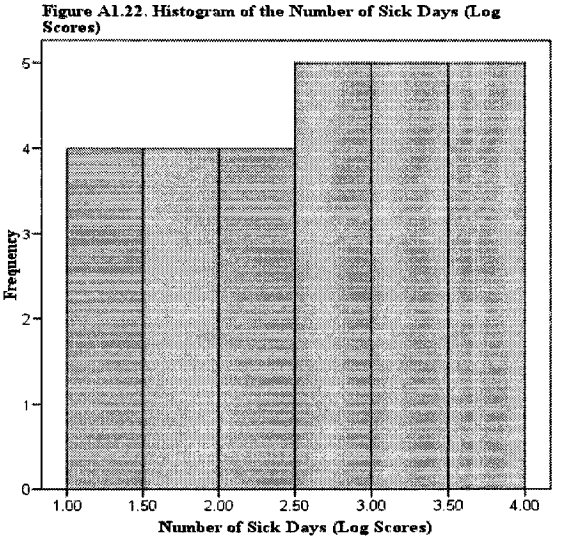


Figure A1.23. Histogram of the Number of Doctor Visits (Log Scores)

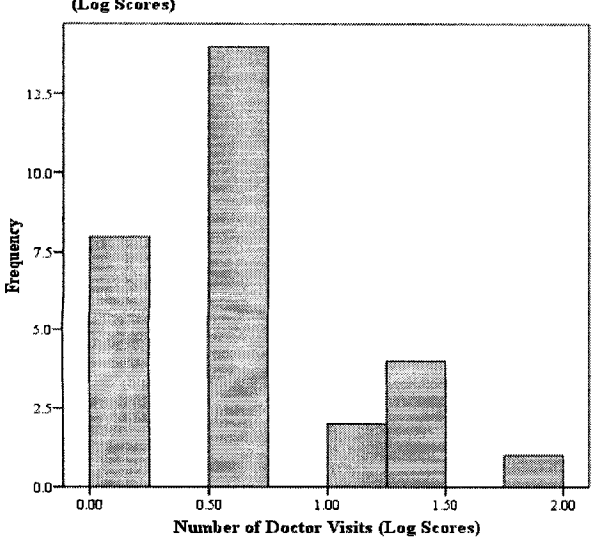


Figure A1.24. Histogram of the Number of Days Non-prescribed Medication Taken (Log Scores)

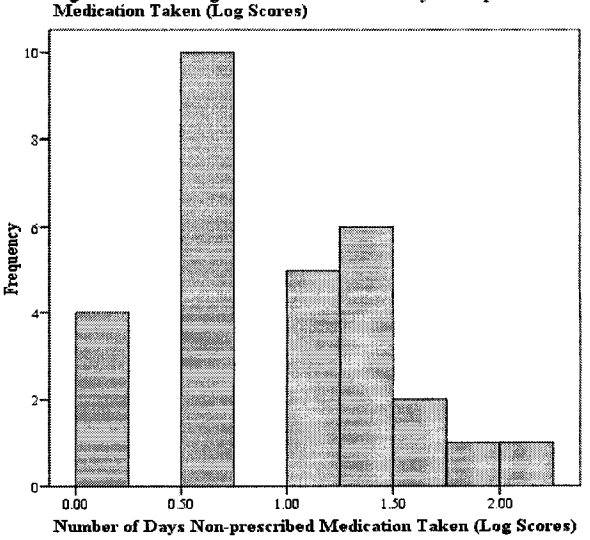


Figure A1.25. Histogram of the Number of Days Prescribed Medication Taken (Log Scores)

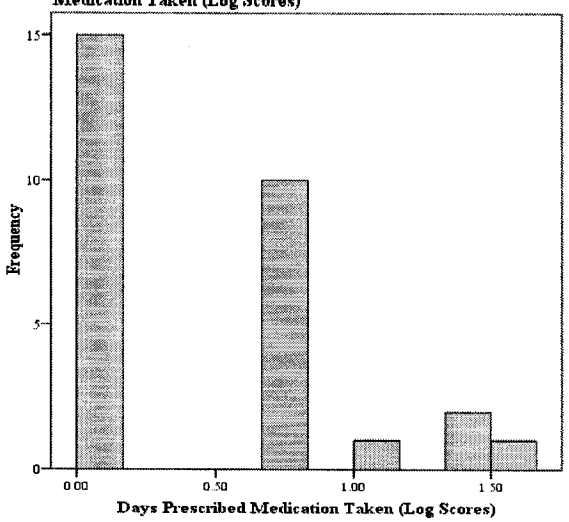


Figure A1.26. Histogram of the Composite of All Four Health Indicators (Log Scores)

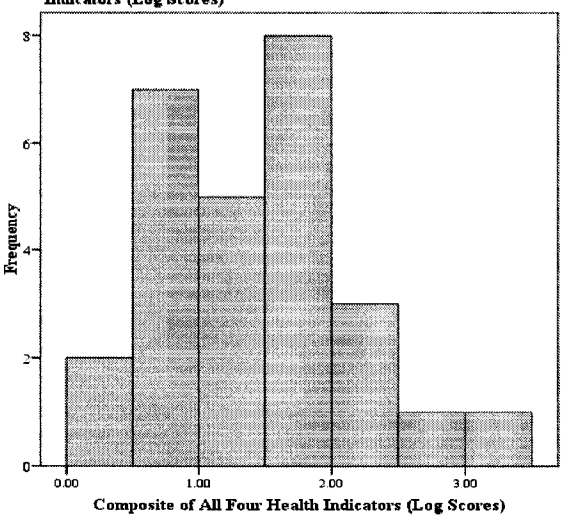


Figure A1.27. Histogram of Child Behavior Checklist (CBCL) Internalizing Scores (Raw Scores)

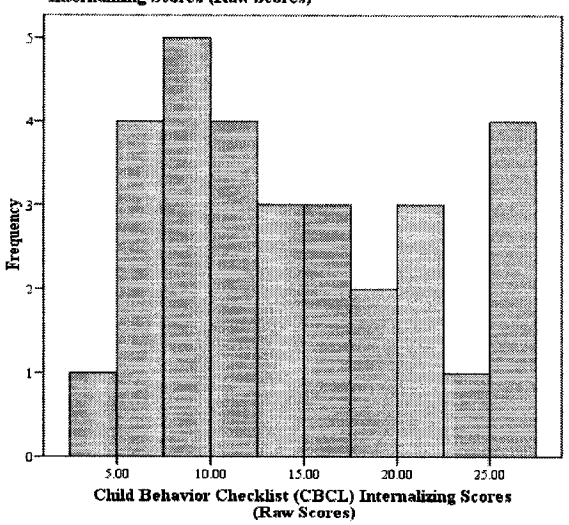


Figure A1.28. Histogram of CBCL Externalizing Scores (Raw Scores)

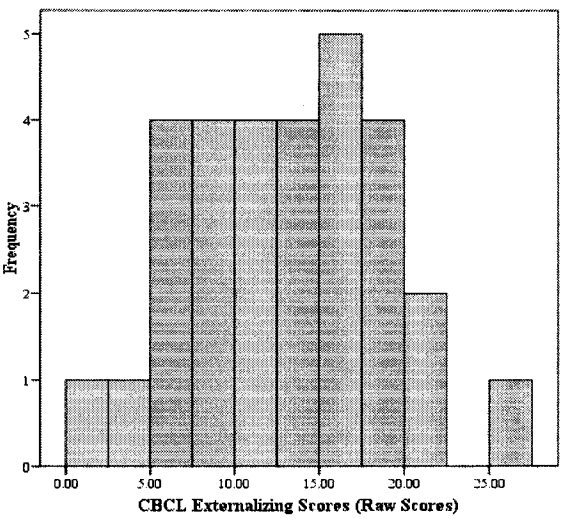


Figure A1.29. Histogram of CBCL Total Scores (Raw Scores)

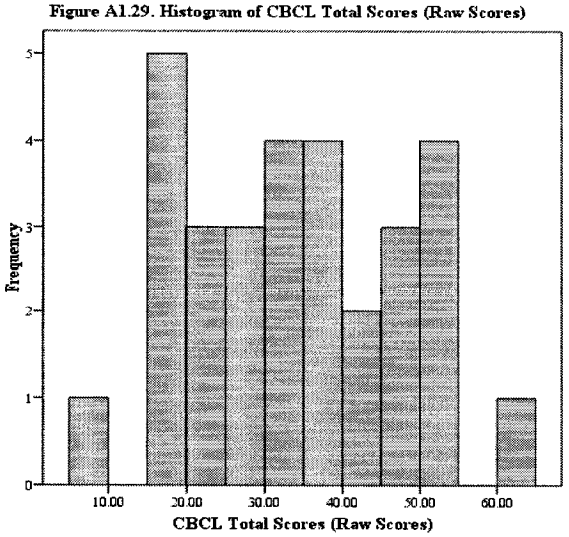


Figure A.1.30. Histogram of CBCL Internalizing Scores (Log Scores)

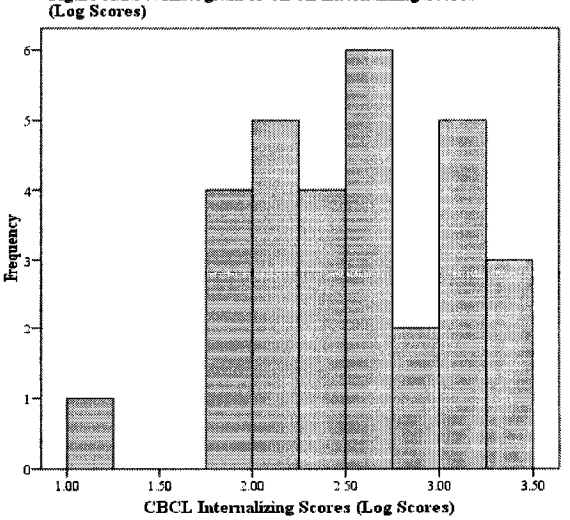


Figure A1.31. Histogram of CBCL Externalizing Scores (Log Scores)

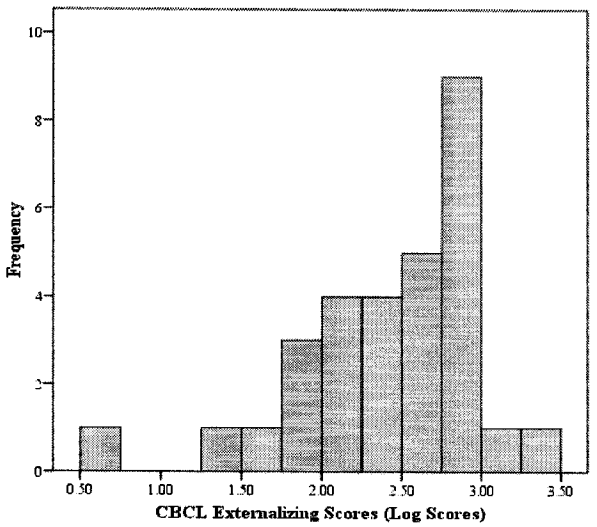


Figure A1.32. Histogram of CBCL Total Scores (Log Scores)

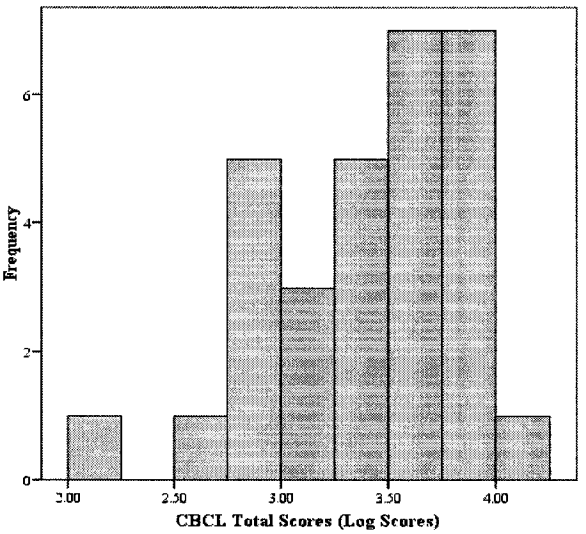


Figure A1.33. Scatterplot of the Relation between Thrid Trimester BDI Scores (Log-transformed) and 6-month Behavioral Distress Difference Scores (Log-transformed)

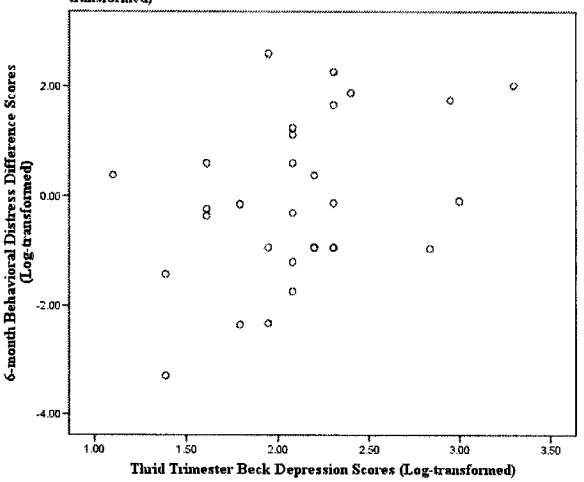


Figure A.1.34. Scatterplot of the Relation between 6-month Behavioral Distress Difference Scores (Log-transformed) and Composite of Health Indicators (Log-transformed)

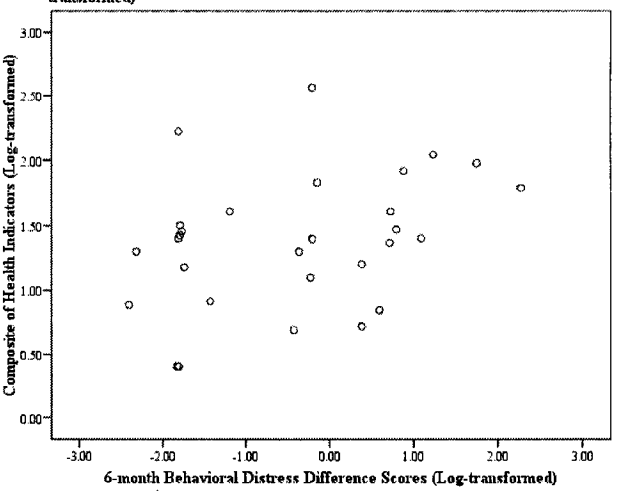


Figure A1.35. Scatterplot of the Relation between 6-month Behavioral Distress Difference Scores (Log-transformed) and CBCL Internalizing Subscale Scores (Log-transformed)

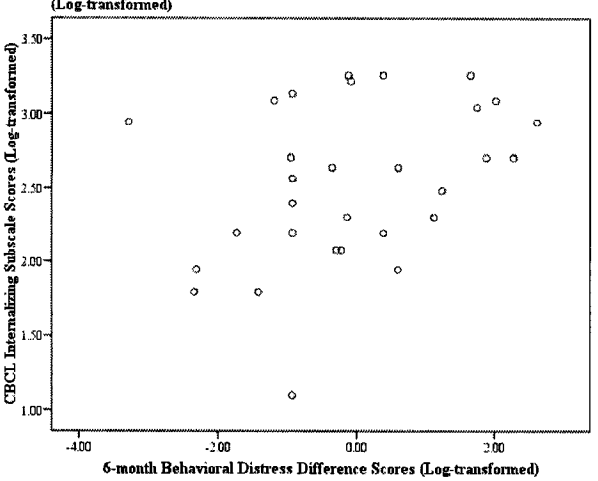


Figure A1.36. Scatterplot of the Relation between Third-trimester BDI Scores (Log-transformed) and the Composite of Health Indicators (Log-transformed)

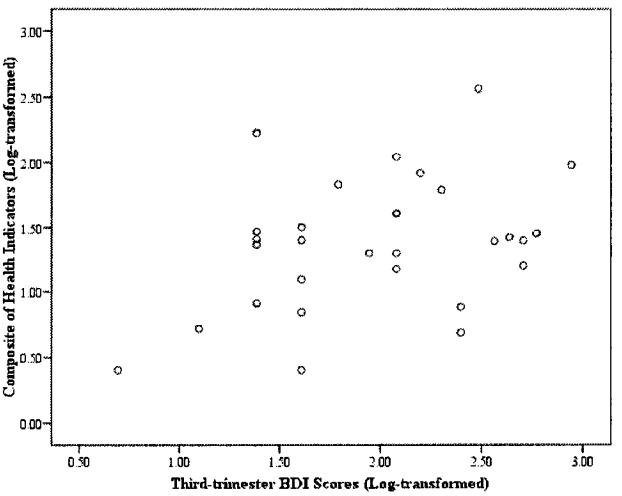


Figure A1.37. Scatterplot of the Relation between Third Trimester BDI Scores (Log-transformed) and CBCL Internalizing Subscale Scores (Log-transformed)

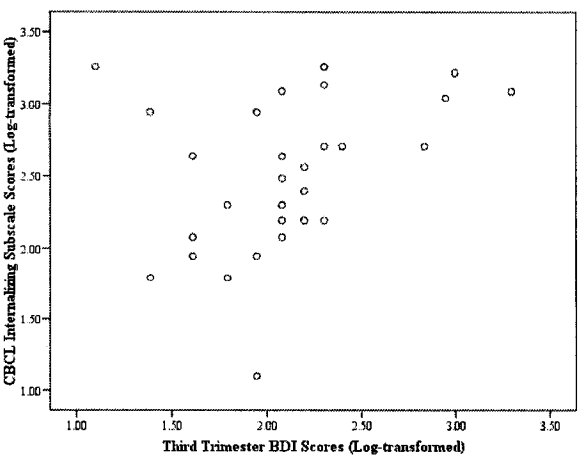


Table A2.1

Distribution Statistics of all Impact of Event Scale Scores

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
3rd Trimester IES Scores	-.32 (.43)	-.90 (.85)	.95
6-Month IES Scores	-.15 (.41)	-.94 (.81)	.97
12-Month IES Scores	-.56 (.40)	.28 (.79)	.97

$^{**}p \leq .01$, $^{*}p \leq .05$, $^{+}p \leq .10$

Table A2.2

Distribution Statistics of 12-Month and 18-Month Cortisol Parameters

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Baseline (12-Month)	1.06 (.43)	.83 (.85)	.91*
Post-challenge (12-Month)	.58 (.45)	.04 (.87)	.96
Difference Score (12-Month)	-1.25 (.47)	2.37 (.92)	.92*
Standardized Residual of Post Challenge (12-Month)	.46 (.47)	-.09 (.92)	.97
Standardized Residual of Difference Scores (12-Month)	.46 (.47)	-.09 (.92)	.97
Baseline (18-Month)	2.00 (.40)	5.05 (.79)	.81**
Post-challenge (18-Month)	1.20 (.43)	1.79 (.83)	.91*
Difference Score (18-Month)	-.04 (.42)	1.70 (.83)	.95
Standardized Residual of Post Challenge (18-Month)	1.50 (.43)	3.87 (.83)	.89**
Standardized Residual of Difference Scores (18-Month)	1.50 (.43)	3.87 (.83)	.89**

**$p \leq .01$, *$p \leq .05$, $^{+}p \leq .10$

Table A2.3

Distribution Statistics of 12-Month and 18-Month Log-ransformed Cortisol Parameters

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Baseline (12-Month)	-1.05 (.43)	1.31 (.85)	.93*
Post-challenge (12-Month)	-1.33 (.45)	2.47 (.87)	.91*
Difference Score (12-Month)	-.96 (.47)	1.88 (.92)	.91*
Standardized Residual of Post Challenge (12-Month)	-1.31 (.47)	2.28 (.92)	.91*
Standardized Residual of Difference Scores (12-Month)	-1.31 (.47)	2.28 (.92)	.91*
Baseline (18-Month)	-.13 (.40)	.97 (.79)	.97
Post-challenge (18-Month)	-.17 (.43)	-.29 (.83)	.98
Difference Score (18-Month)	.03 (.43)	-.21 (.83)	.99
Standardized Residual of Post Challenge (18-Month)	.03 (.43)	-.06 (.83)	.97
Standardized Residual of Difference Scores (18-Month)	-.36 (.43)	2.15 (.83)	.96

**$p \leq .01$, *$p \leq .05$, $^{+}p \leq .10$

Table A2.4

Distribution Statistics of Inverse-Transformed 12-Month Cortisol Parameters

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Baseline (12-Month)	-.26 (.43)	.04 (.83)	.97
Post-challenge (12-Month)	3.76 (.43)	15.54 (.83)	.50**
Difference Score (12-Month)	.69 (.43)	.95 (.83)	.96
Standardized Residual of Post Challenge (12-Month)	5.25 (.43)	28.22 (.83)	.28**
Standardized Residual of Difference Scores (12-Month)	5.25 (.43)	28.22 (.83)	.28**

**$p \leq .01$, *$p \leq .05$, $^{+}p \leq .10$

Table A2.5

Distribution Statistics of 6-month and 24--month ICQ Subscale and Total Scores

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Difficultness (6-Month)	.32 (.41)	.20 (.81)	.98
Inadaptability (6-Month)	.07 (.41)	-.47 (.81)	.98
Activity (6-Month)	.18 (.42)	-.19 (.82)	.98
Unpredictability (6-Month)	-.26 (.42)	-.86 (.82)	.96
Total Scores (6-Month)	-.38 (.43)	.15 (.83)	.98
Difficultness (24-Month)	.23 (.44)	-1.04 (.86)	.94
Inadaptability (24-Month)	-.37 (.43)	-.63 (.85)	.96
Activity (24-Month)	-.33 (.43)	1.02 (.85)	.97
Unpredictability (24-Month)	-.70 (.43)	-.10 (.85)	.92*
Total Scores (24-Month)	-.56 (.44)	-.01 (.86)	.97

**$p \leq .01$, *$p \leq .05$, $^{+}p \leq .10$

Table A2.6

Intercorrelations between IES Scores and Inverse-transformed 12-month and Log-transformed 18-month cortisol Parameters

	Baseline (12-M)	Post-challenge (12-M)	Difference Score (12-M)	Standardized Residual of Post-challenge (12-M)	Standardized Residual of Difference Score (12-M)	Baseline (18-M)	Post-challenge (18-M)	Difference Score (18-M)	Standardized Residual of Post-challenge (18-M)	Standardized Residual of Difference Score (18-M)
3rd Trimester IES Scores	-.28	.22	.36*	.22	.21	-.49 *	-.11	.40*	.00	.24
6-Month IES Scores	.10	.09	-.02	.07	.07	-.23	.16	.20	.25	.16
12-Month IES Scores	.35*	-.18	-.35*	-.24	-.24	-.14	-.22	-.38*	-.25	-.38*

**$p \leq .01$, *$p \leq .05$, $^{+}p \leq .10$

Table A2.7

Interrelations between IES Scores and ICQ Subscale and Total Scores

	3rd Trimester IES Scores	6-month IES Scores	12-month IES Scores
6-month ICQ Difficultness	.37*	.48**	.59**
6-month ICQ Inadaptability	.17	.11	.15
6-month ICQ Activity	.23	.24	.45
6-month ICQ Unpredictability	.36*	.13	.42
6-month ICQ Total Scores	.42*	.33^{+}	.59**
24-month ICQ Difficultness	.29	-.01	.01
24-month ICQ Inadaptability	.20	-.01	.11
24-month ICQ Activity	.17	.06	.20
24-month ICQ Unpredictability	.17	.05	-.03
24-month ICQ Total Scores	.43*	.04	.20

**$p \leq .01$, *$p \leq .05$, $^{+}p \leq .10$

Figure A2.1. Histogram of Third Trimester IES Total Scores

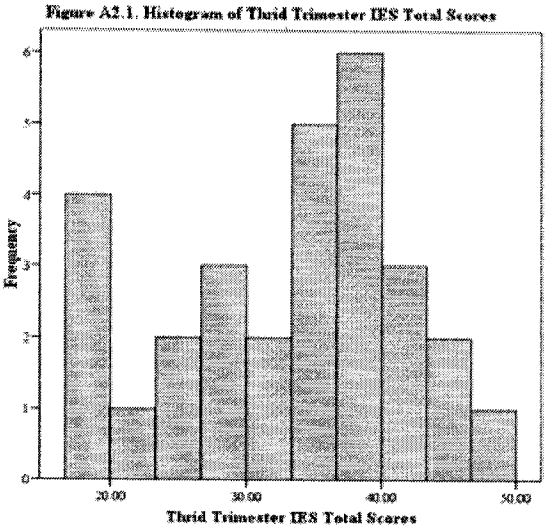


Figure A2.2. Histogram of 6-Month IES Total Scores

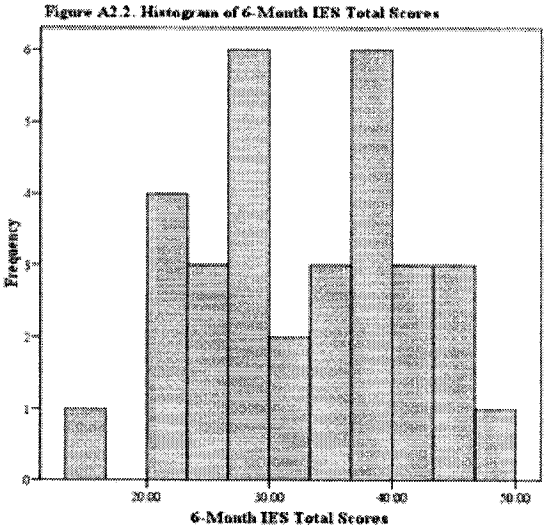


Figure A2.3. Histogram of 12-Month IES Total Scores

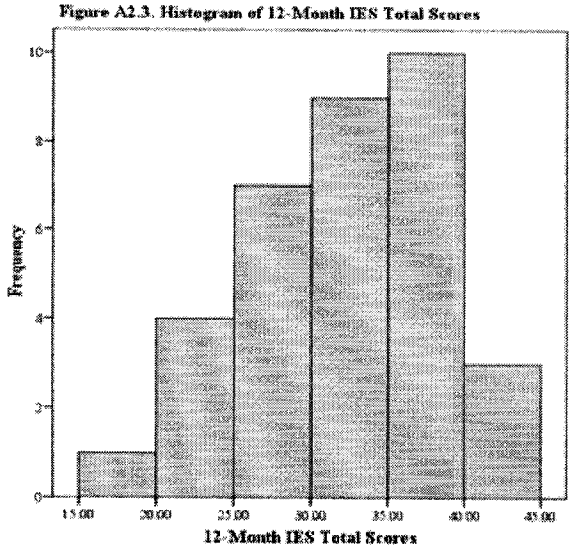


Figure A2.4. Histogram of 12-Month Cortisol Levels at Baseline (Raw Scores)

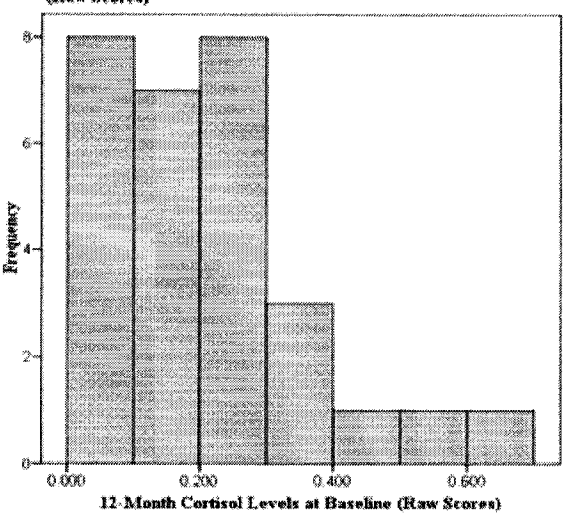


Figure A2.5. Histogram of 12-Month Post-Challenge Cortisol Levels (Raw Scores)

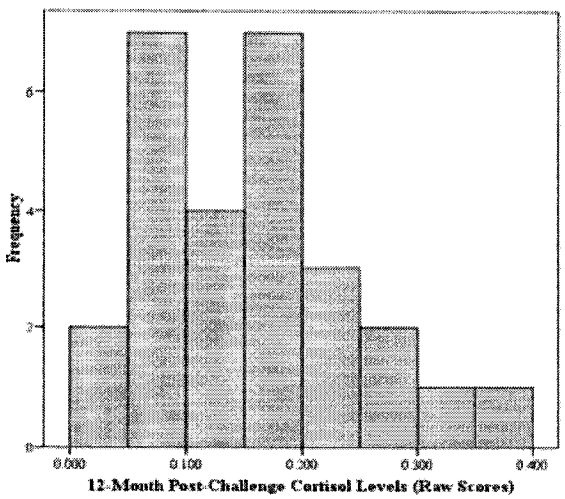


Figure A2.6. Histogram of the Difference Scores between Baseline and Post-Challenge Cortisol Levels at 12 Months (Raw Scores)

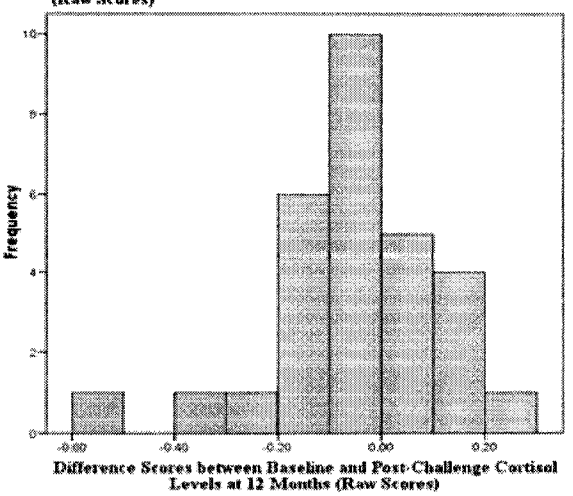


Figure A2.7. Histogram of the Standardized Residual of 12-Month Post-challenge Cortisol Levels Controlling for Baseline (Raw Scores)

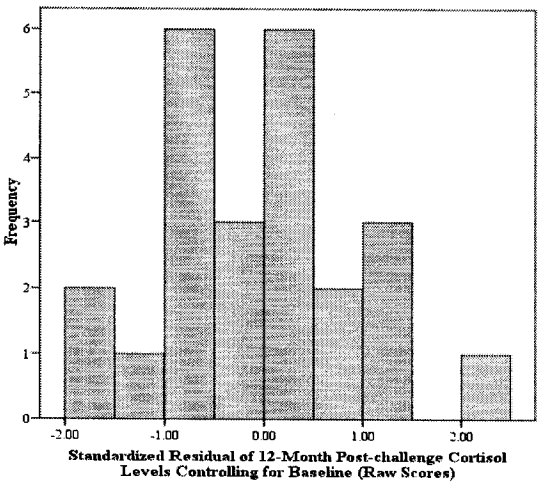


Figure A2.8. Histogram of the Standardized Residual of 12-Month Difference Scores Controlling for Baseline (Raw Scores)

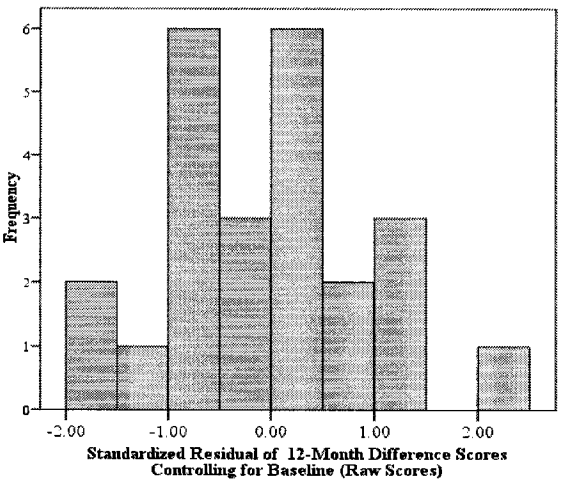


Figure A2.9. Histogram of 18-Month Cortisol Levels at Baseline (Raw Scores)

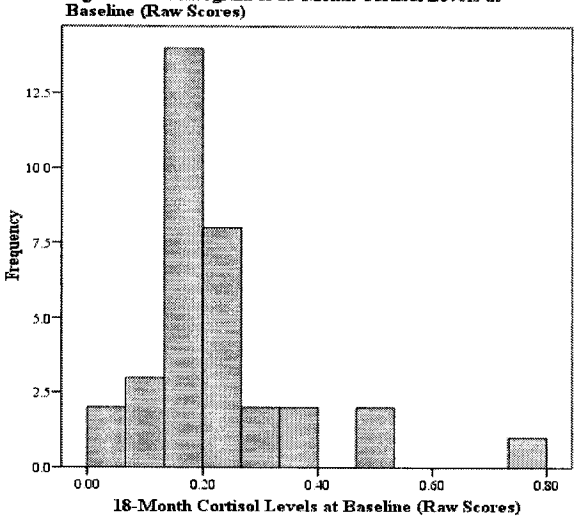


Figure A2.10. Histogram of 18-Month Post-Challenge Cortisol Levels (Raw Scores)

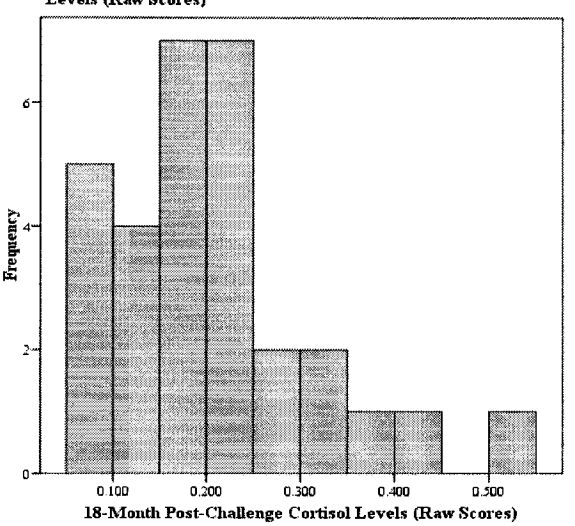


Figure A2.11. Histogram of Difference Scores between Baseline and Post-challenge Levels at 18-Month (Raw Scores)

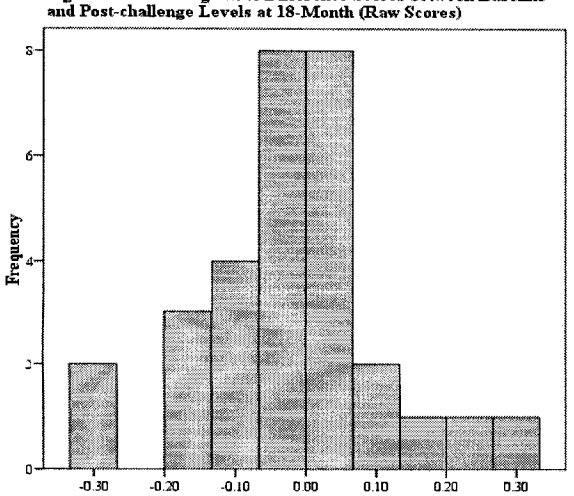


Figure A2.12. Histogram of the Standardized Residual of 18-Month Post-Challenge Cortisol Levels Controlling for Baseline (Raw Scores)

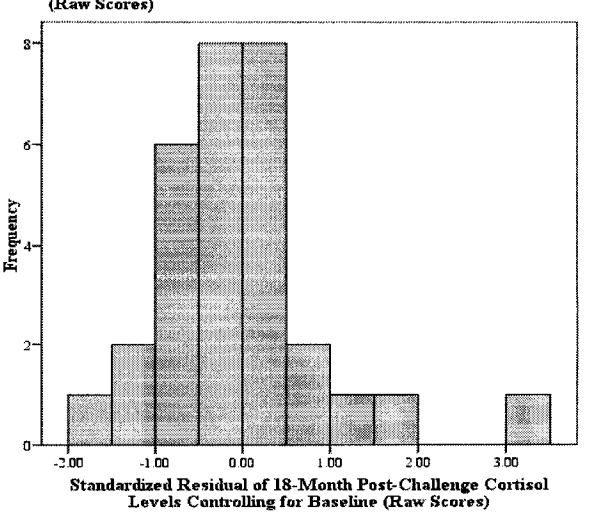


Figure A2.13. Histogram of the Standardized Residual of 18-Month Difference Scores Controlling for Baseline (Raw Scores)

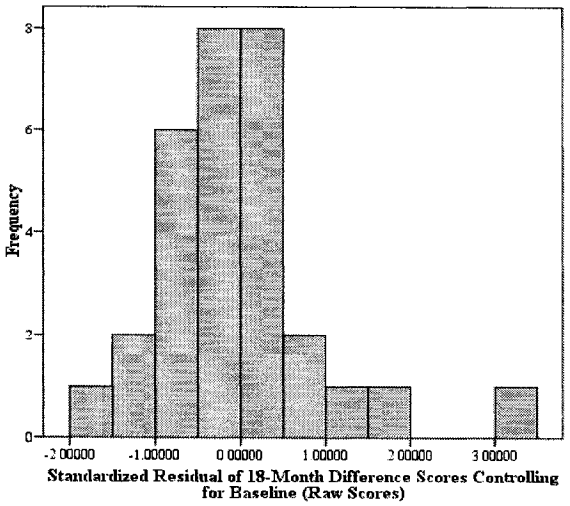


Figure A2.14. Histogram of 12-Month Cortisol Levels at Baseline (Log Scores)

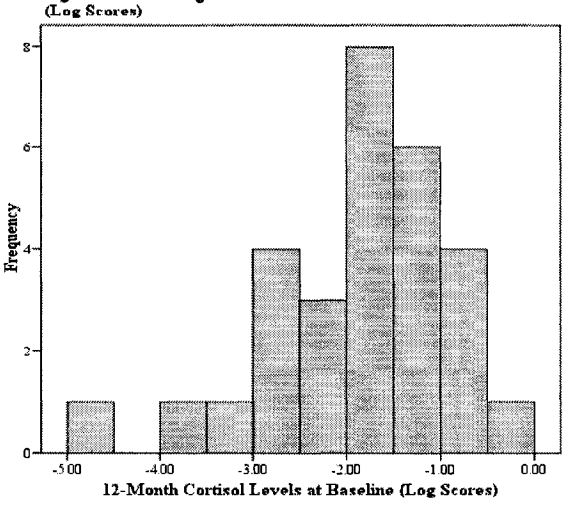


Figure A2.15. Histogram of 12-Month Post-Challenge Cortisol Levels (Log Scores)

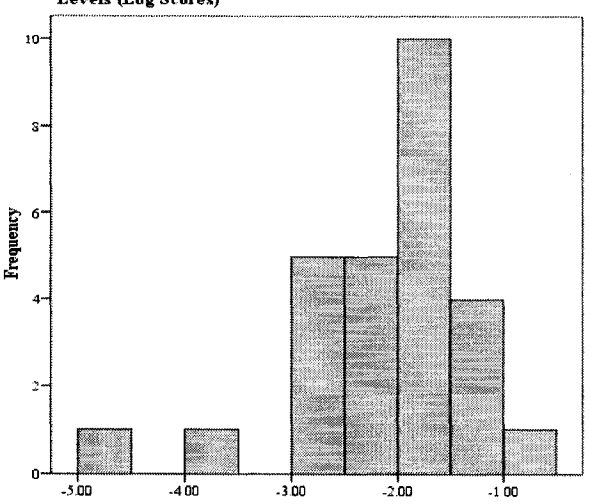


Figure A2.16. Histogram of the Difference Scores between Baseline and Post-Challenge Cortisol Levels at 12 Months (Log Scores)

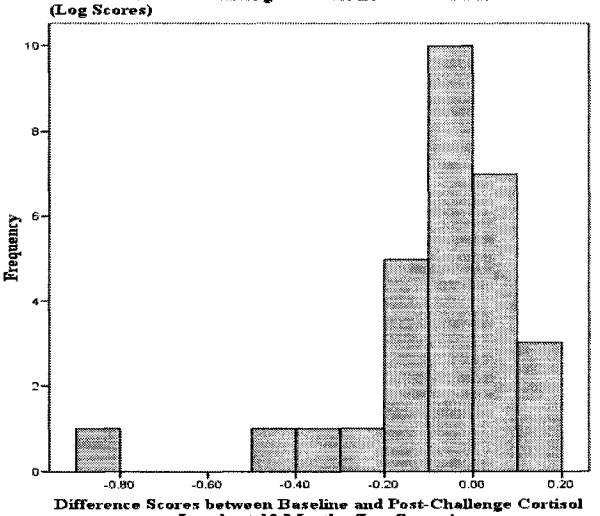


Figure A2.17. Histogram of the Standardized Residual of 12-Month Post-challenge Cortisol Levels Controlling for Baseline (Log Scores)

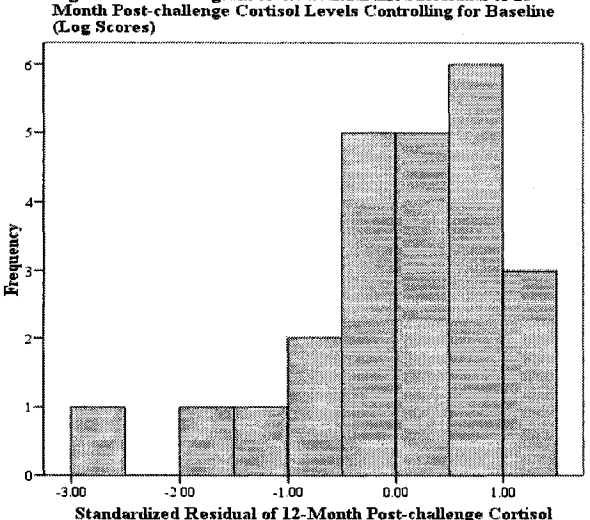


Figure A2.18. Histogram of the Standardized Residual of 12-Month Difference Scores Controlling for Baseline (Log Scores)

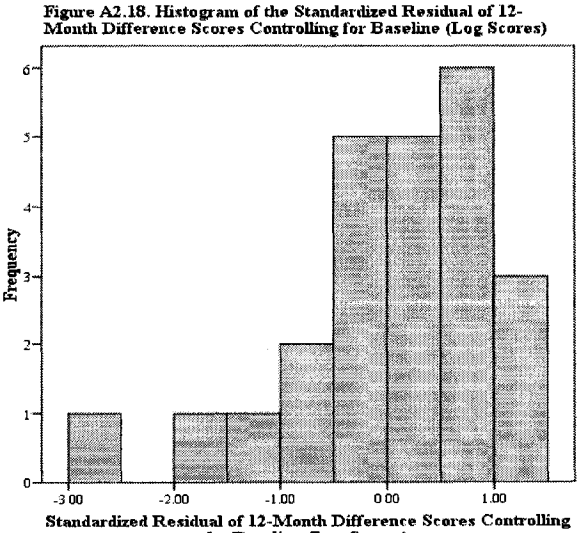


Figure A2.19. Histogram of 18-Month Cortisol Levels at Baseline (Log Scores)

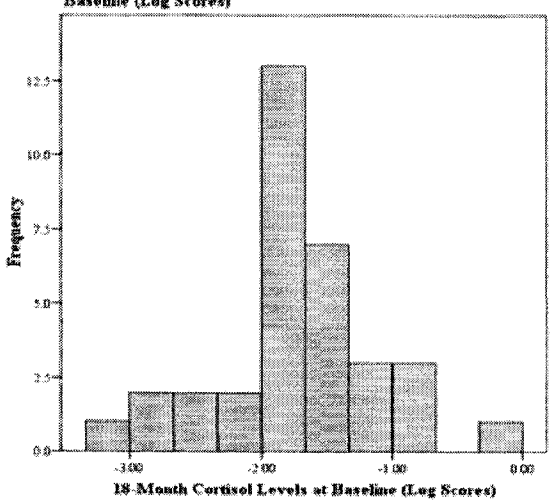


Figure A2.20. Histogram of 18-Month Post-Challenge Cortisol Levels (Log Scores)

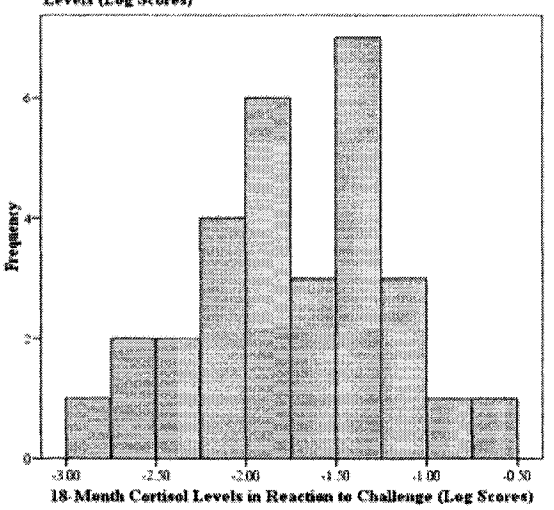


Figure A2.21. Histogram of Difference Scores between Basline and Post-challenge Cortisol Levels at 18 Months (Log Scores)

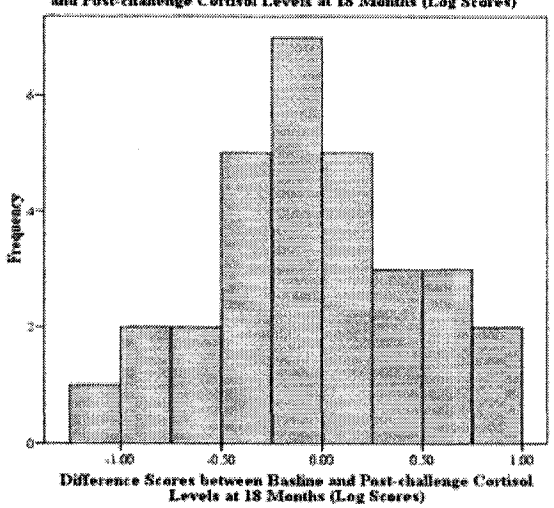


Figure A2.22. Histogram of Standardized Residual of 18-Month Post-challenge Cortisol Levels Controlling for Baseline (Log Scores)

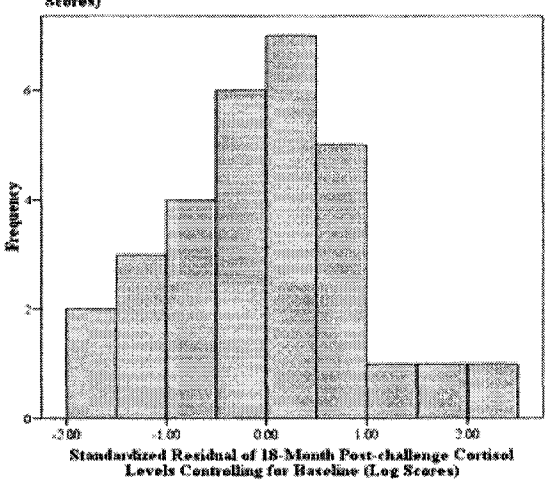


Figure A2.23. Histogram of Standardized Residual of 18-Month Difference Scores Controlling for Baseline (Log Scores)

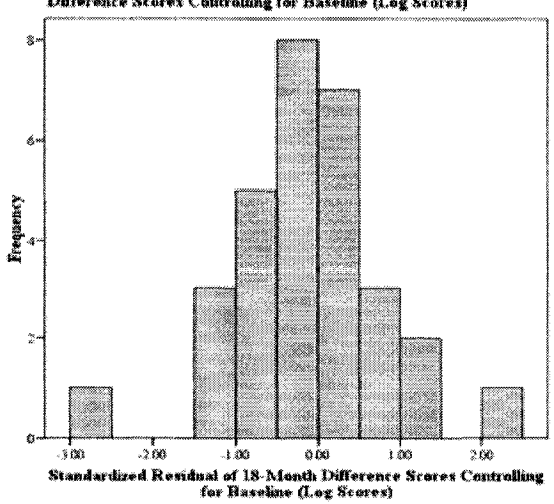


Figure A2.24. Histogram of 12-Month Cortisol Levels at Baseline (Inverse Scores)

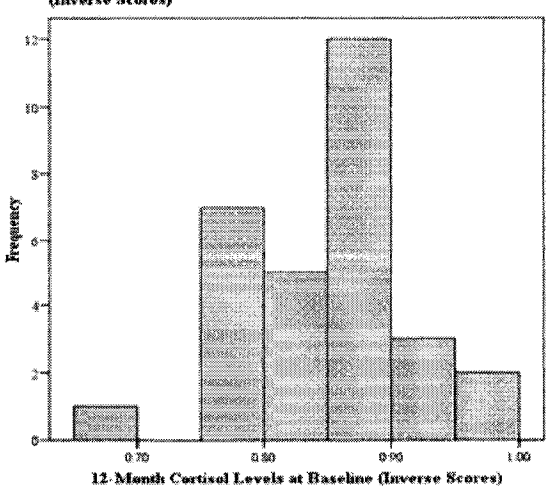


Figure A2.25. Histogram of 12-Month Post-Challenge Cortisol Level (Inverse Scores)

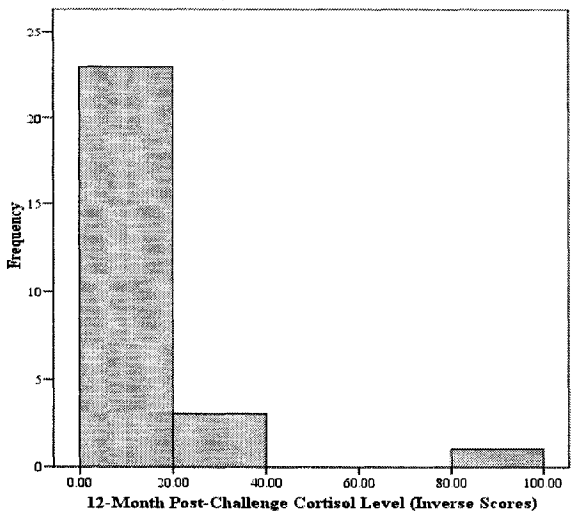


Figure A2.26. Histogram of the Difference Scores between Baseline and Post-Challenge Cortisol Levels at 12 Months (Inverse Scores)

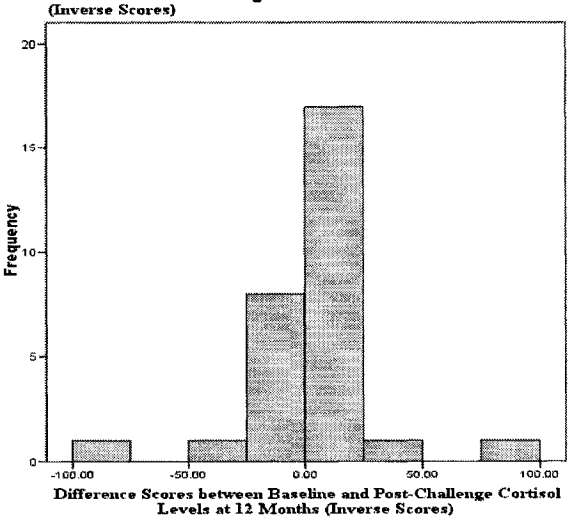


Figure A2.27. Histogram of the Standardized Residual of 12-Month Post-Challenge Cortisol Levels Controlling for Baseline (Inverse Scores)

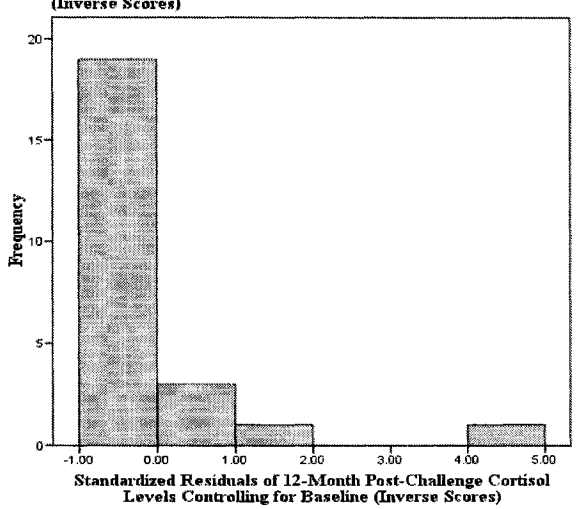


Figure A2.28. Histogram of the Standardized Residuals of 12-Month Difference Scores Controlling for Baseline (Inverse Scores)

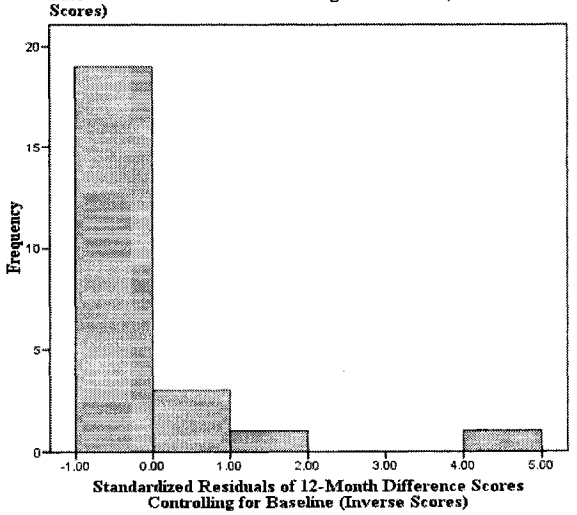


Figure A2.29 Histogram of 6-Month ICQ Difficultness Subscale (Raw Scores)

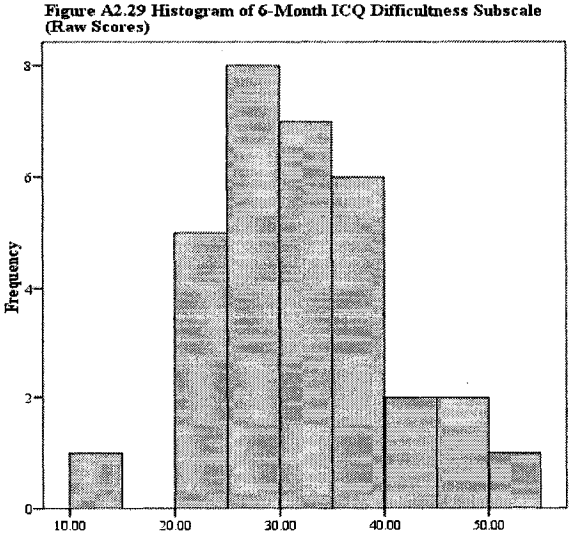


Figure A2.30. Histogram of 6-Month ICQ Adaptability Subscale (Raw Scores)

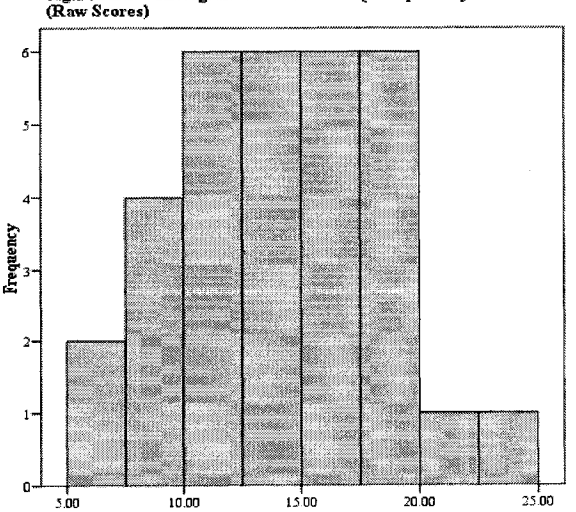


Figure A2.31. Histogram of 6-Month ICQ Activity Subscale (Raw Scores)

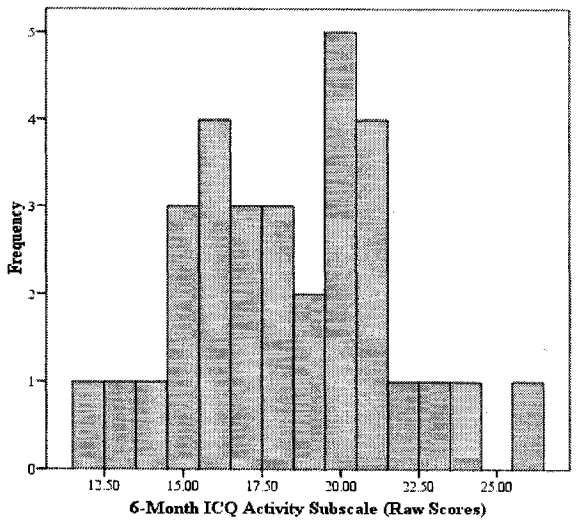


Figure A2.32. Histogram of 6-Month ICQ Unpredictability Subscale (Raw Scores)

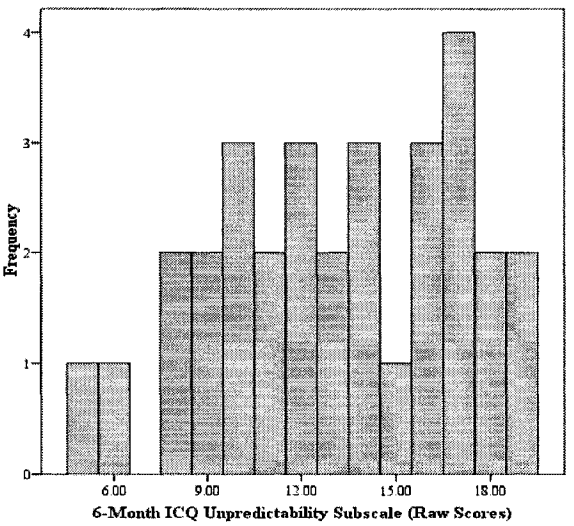


Figure A2.33. Histogram of 6-Month ICQ Total Scores (Raw Scores)

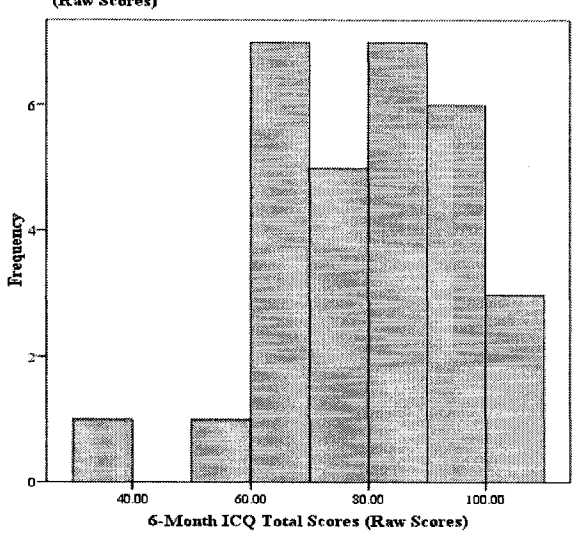


Figure A2.34. Histogram of 24-Month ICQ Difficultness Subscale (Raw Scores)

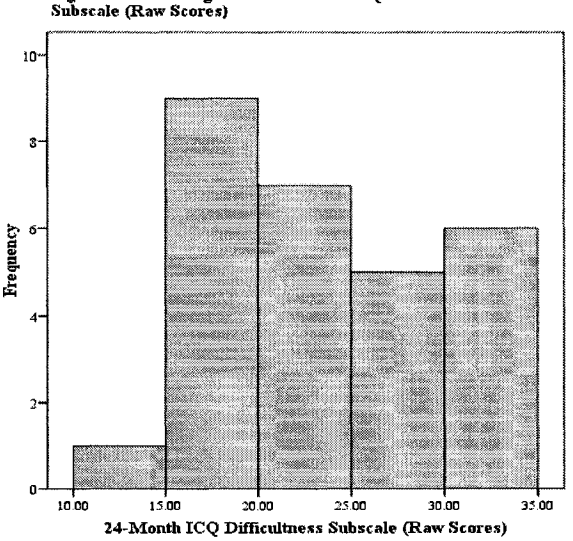


Figure A2.35. Histogram of 24-Month ICQ Adaptability Subscale (Raw Scores)

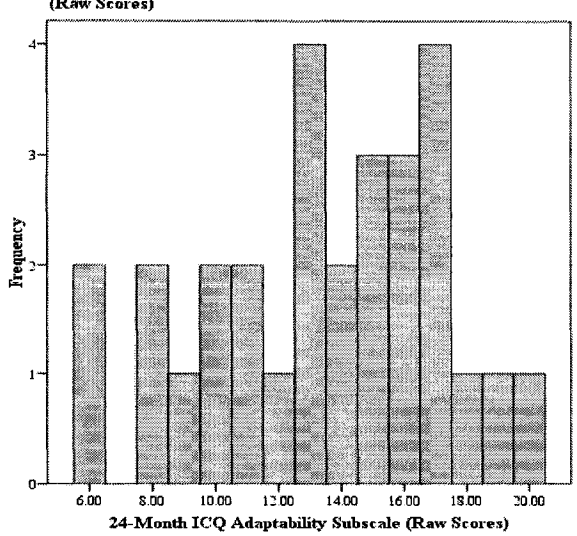


Figure A2.36. Histogram of 24-Month ICQ Activity Subscale (Raw Scores)

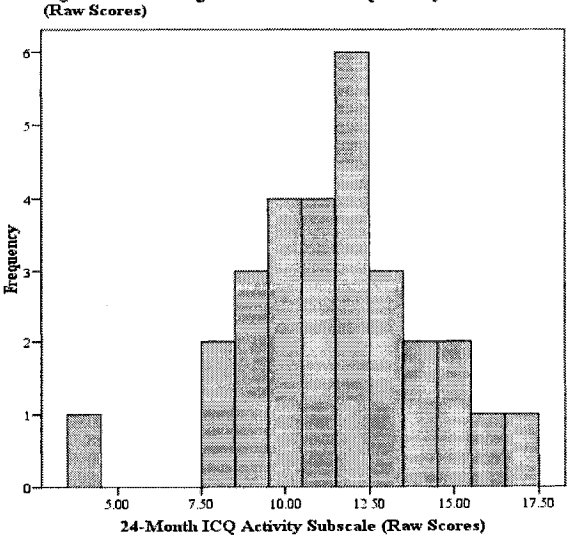


Figure A2.37. Histogram of ICQ Unpredictability Scores at 24 Months

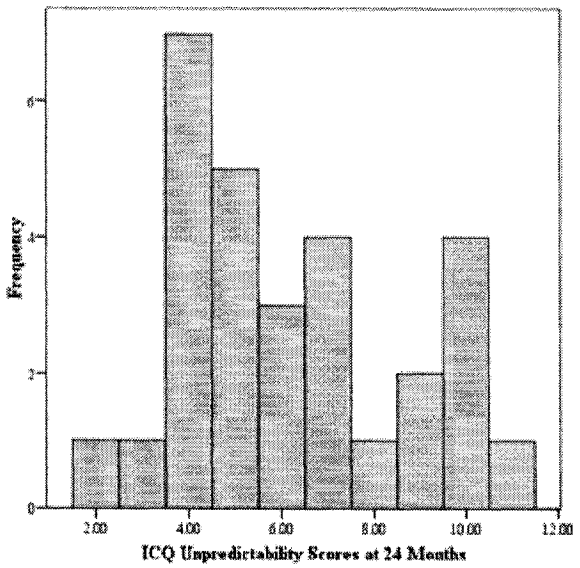


Figure A2.38. Histogram of 24-Month ICQ Total Scores (Raw Scores)

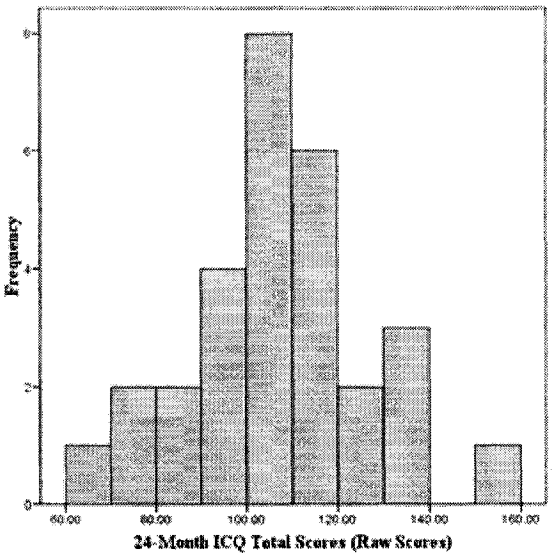


Figure A2.39. Scatterplot of the Relation between Third Trimester IES Scores and 12-month Cortisol Difference Scores (Inverse Scores, Reflected)

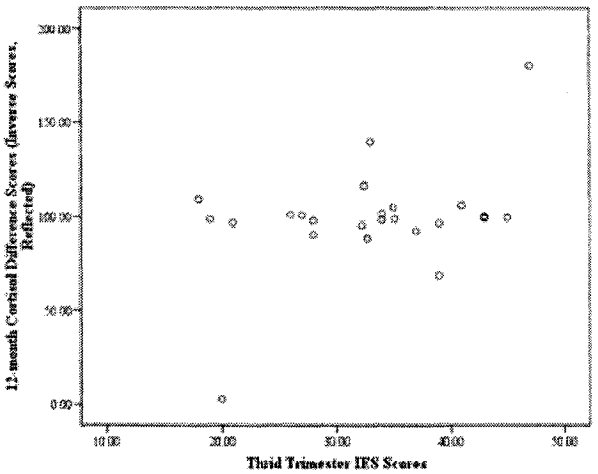


Figure A2.40. Scatterplot of the Relation between Third Trimester IES Scores and 18-month Cortisol Difference Scores (Log Scores)

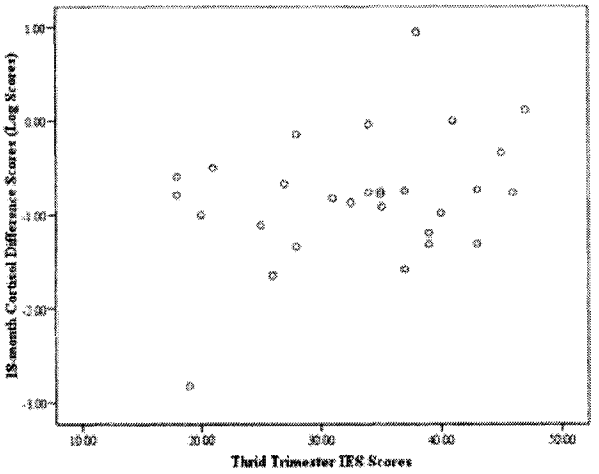


Figure A2.41. Scatterplot of the Relation between Third Trimester IES Scores and 6-Month ICQ Total Scores

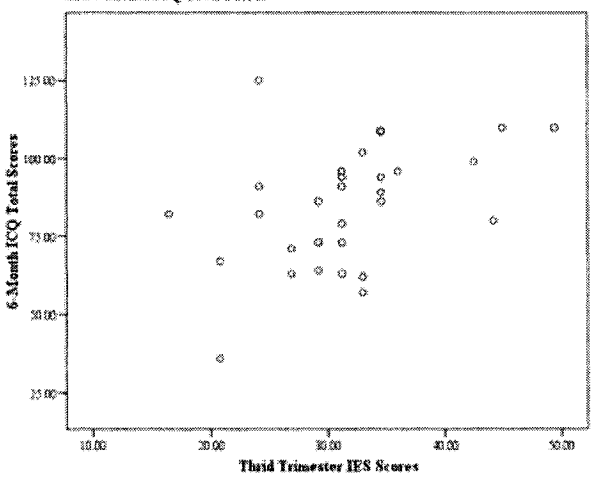


Figure A2.42. Scatterplot of the Relation between Third Trimester IES Scores and 24-Month ICQ Total Scores

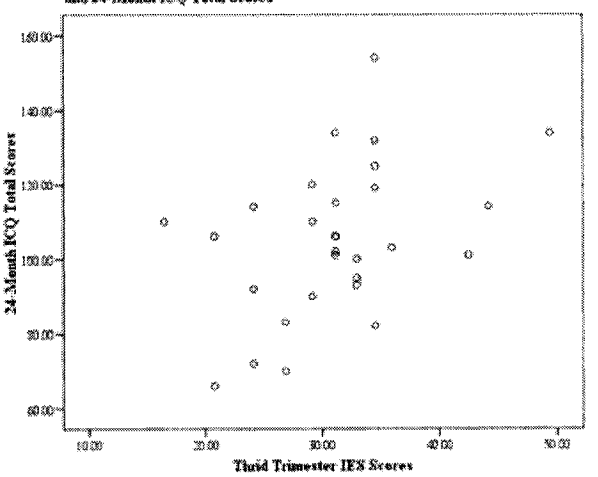


Table A3.1.

Distribution Statistics of Perinatal APS Subscale and Total scores

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Anxiety	-.74 (.46)	.46 (.89)	.94
Depression	-.19 (.46)	-.60 (.89)	.89**
Self-esteem	-2.06 (.46)	5.12 (.89)	.79**
Mastery	-.36 (.46)	-.75 (.89)	.95
Subjective Stress	.00 (.46)	.42 (.89)	.93**
Total	-1.35 (.46)	3.53 (.89)	.91*

Table A3.2.

Intercorrelation between Subscales of the Perinatal Abbreviated Psychosocial Scale

	Depression	Mastery	Anxiety	Self-esteem	Total
Stress	.35	.46*	.47**	.50**	.77**
Depression		.31	.63**	.43*	.63**
Mastery			.39*	.27	.64**
Anxiety				.63**	.85**
Self-esteem					.78**

**$p \leq .01$, *$p \leq .05$, $^{+}p \leq .10$

Table A3.3.

Distribution Statistics of 10-month APS Subscale and Total scores

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Anxiety	-.91 (.46)	.68 (.89)	.91*
Depression	-.83 (.46)	1.06 (.89)	.94
Self-esteem	-1.24 (.46)	1.12 (.89)	.85**
Mastery	-.43 (.46)	-.89 (.89)	.92*
Subjectiive Stress	-.45 (.46)	-.31 (.89)	.93
Total	-.91 (.46)	.12 (.89)	.92*

Table A3.4.

Intercorrelation between Subscales of the 10-month Abbreviated Psychosocial Scale

	Depression	Mastery	Anxiety	Self-esteem	Total
Stress	.62**	.46*	.21	.22	.58**
Depression		.46*	.39*	.52**	.81**
Mastery			.35	.49*	.71**
Anxiety				.60**	.73**
Self-esteem					.82**

**$p \leq .01$, *$p \leq .05$, $^{+}p \leq .10$

Table A3.5

Distribution Statistics of Perinatal Cortisol Parameters

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Baseline	1.99 (.46)	6.26 (.89)	.83**
Post-challenge	.70 (.46)	.21 (.89)	.96
Difference Score	-.80 (.46)	1.44 (.89)	.92*
Standardized residual of post-challenge	.03 (.46)	.17 (.89)	.99
Standardized residual of Difference Score	.03 (.46)	.17 (.89)	.99

Table A3.6

Distribution Statistics of Log-transformed Perinatal Cortisol Parameters

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Baseline	1.17 (.46)	2.55 (.89)	.91*
Post-challenge	.39 (.46)	-.24 (.89)	.98
Difference Score	-.40 (.46)	.86 (.89)	.94
Standardized residual of post-challenge	-.13 (.46)	.16 (.89)	.99
Standardized residual of Difference Score	-.13 (.46)	.16 (.89)	.99

Table A3.7

Intercorrelation among Different Log-transformed Perinatal Cortisol Parmeters

	Post-challenge	Difference Score	Standardized Residuals of Post-challenge	Standardized Residuals of Difference Score
Baseline	.56**	-.68**	.00	.00
Post-challenge		.23	.80**	.80**
Difference Score			.74**	.74**
Standardized Residuals of Post-challenge				1.00

**p≤.01, *p≤.05, +p≤.10

Table A3.8

Distribution Statistics of Postnatal Cortisol Parameters

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Baseline	2.35 (.46)	4.74 (.89)	.59**
Post-chllenge	1.63 (.46)	1.24 (.89)	.66**
Difference Score	3.42 (.46)	12.77 (.89)	.54**
Standardized residual of post-challenge	3.43 (.46)	12.78 (.89)	.54**
Standardized residual of Difference Score	3.43 (.46)	12.78 (.89)	.54**

Table A3.9

Distribution Statistics of Log-transformed Postnatal Cortisol Parameters

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Baseline	1.86 (.46)	2.49 (.89)	.70**
Post-challenge	1.28 (.46)	.19 (.89)	.75**
Difference Score	2.31 (.46)	6.20 (.89)	.73**
Standardized residual of post-challenge	2.29 (.46)	5.93 (.89)	.74**
Standardized residual of Difference Score	2.29 (.46)	5.93 (.89)	.74**

Table A3.10

Distribution Statistics of Inverse-transformed Postnatal Cortisol Parameters

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Baseline	-2.00 (.46)	3.03 (.89)	.66**
Post-challenge	-1.39 (.46)	.43 (.89)	.71**
Difference Score	-2.74 (.46)	8.23 (.89)	.65**
Standardized residual of post-challenge	-2.72 (.46)	8.06 (.89)	.66**
Standardized residual of Difference Score	-2.72 (.46)	8.06 (.89)	.66**

Table A3.11

Intercorrelation among Log-transformed Postnatal Cortisol Parameters

	10-month Post-challenge	10-month Difference Score	10-month Standardized Residuals of Post-Challenge	10-month Standardized Residuals of Difference Score
10-month Baseline	.88**	.15	.00	.00
10-month Post-challenge		.61**	.48**	.48**
10-month Difference Score			.99**	.99**
10-mongh Standardized Residuals of post-challenge				1.00

**$p \leq .01$, *$p \leq .05$, $^{+}p \leq .10$

Table A3.12

Distribution Statistics of Infant Behavioral Parameters

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Disengagement (mean differences)	3.87 (.46)	17.11 (.89)	.51**
Self-comfort (mean differences)	2.85 (.46)	8.64 (.89)	.55**
Negative Vocalization (mean differences)	.79 (.46)	.62 (.89)	.92*
Disengagement (difference score)	3.59 (.46)	14.89 (.89)	.67**
Self-comfort (difference score)	3.39 (.46)	11.77 (.89)	.54**
Negative Vocalization (difference score)	1.27 (.46)	.67 (.89)	.47**
Sum of Difference Scores	3.32 (.46)	11.82 (.89)	.83**
Sum of Mean Differences	3.15 (.46)	12.55 (.89)	.60**

Table A3.13

Distribution Statistics of Log-transformed Infant Behavioral Parameters

	Statistics (Standard Error)		
	Skewness	Kurtosis	Shapiro-Wilk
Disengagement (mean differences)	3.09 (.46)	11.73 (.89)	.61**
Self-comfort (mean differences)	2.41 (.46)	5.77 (.89)	.61**
Negative Vocalization (mean differences)	.51 (.46)	.05 (.89)	.94
Disengagement (difference score)	1.70 (.46)	4.65 (.89)	.89**
Self-comfort (difference score)	2.57 (.46)	6.69 (.89)	.78**
Negative Vocalization (difference score)	.94 (.46)	-.20 (.89)	.61**
Sum of Difference Scores	1.53 (.46)	2.55 (.89)	.88**
Sum of Mean Differences	1. 40 (.46)	3.11 (.89)	.86**

Table A3.14

Intercorrelation among log-transformed Infant Behavior Parameters

	Self-comfort (mean differences)	Negative Vocalization (mean differences)	Sum of Mean Differences	Disengagement (difference score)	Self-comfort (difference score)	Negative Vocalization (difference score)	Sum of Difference Scores
Disengagement (mean differences)	.76**	-.20	.87**	.79**	.71**	.10	.80**
Self-comfort (mean differences)		-.38*	.82**	.71**	.89**	-.15	.75**
Negative Vocalization (mean differences)			.11	-.42*	-.47*	.63**	-.21
Sum of Mean Differences				.63**	.68**	.26	.76**
Disengagement (difference score)					.76**	.04	.91**
Self-comfort (difference score)						-.16	.83**
Negative Vocalization (difference score)							.31^{+}

**p≤.01, *p≤.05, +p≤.10

Table A3.15

Intercorrelations between Antenatal APS Subscale Scores and Log-transformed Perinatal Cortisol Parameters

	Perinatal Cortisol Difference Score (log score)	Post-challenge (log score)	Baseline (log score)	Standardized Residuals of Post-challenge (log score)	Standardized Residuals of Difference Score (log scores)
Stress	.44*	-.10	-.60**	-.41*	-.41*
Depression	.19	.10	-.13	-.01	-.01
Mastery	.05	-.11	-.15	-.16	-.16
Anxiety	.31^{+}	.11	-.27	-.08	-.08
Self-esteem	.08	-.27	-.33^{+}	-.38^{+}	-.38^{+}
Total	.31^{+}	-.08	-.44*	-.31^{+}	-.31^{+}

**p≤.01, *p≤.05, +p≤.10

Table A3.16

Interrelations between Log-transformed Perinatal Cortisol Parameters and log-transformed Infant Behavioral Parameters

	Perinatal Cortisol Difference Score	Post-challenge	Baseline	Standardized Residuals of Post-challenge	Standardized Residuals of Difference Score
Disengagement (mean differences)	-.25	-.15	.17	-.01	-.01
Self-comfort (mean differences)	-.32^{+}	-.27	.13	-.11	-.11
Negative Vocalization (mean differences)	.57**	.71**	-.04	.47**	.47**
Sum of Mean Differences	-.11	.07	.19	.16	.16
Disengagement (difference score)	.31^{+}	-.33^{+}	.07	-.19	-.19
Self-comfort (difference score)	-.38*	-.46*	.04	-.30	-.30
Negative Vocalization (difference score)	.38*	.47*	-.03	.31^{+}	.31^{+}
Sum of Difference Scores	-.24	-.25	.07	-.13	-.13

**$p \leq .01$, *$p \leq .05$, +$p \leq .10$

Table A3.17

Intercorrelations between APS Subscale and Total Scores and log-transformed Infant Behavioral Parameters

	Stress	Depression	Mastery	Anxiety	Self-esteem	Total
Disengagement (mean differences)	.00	-.08	-.35^{+}	-.14	-.09	-.19
Self-comfort (mean differences)	-.17	-.23	-.36^{+}	-.18	.05	-.24
Negative Vocalization (mean differences)	.41*	.18	.12	.28	.07	.32^{+}
Sum of Mean Differences	.05	-.11	-.35^{+}	-.07	.02	-.12
Disengagement (difference score)	-.03	.01	.34^{+}	-.25	.06	-.18
Self-comfort (difference score)	-.05	-.28	.38*	-.23	.15	-.21
Negative Vocalization (difference score)	.37	.05	.07	-.06	-.05	.12
Sum of Difference Scores	.10	-.15	-.32^{+}	-.28	.07	-.16

**p≤.01, *p≤.05, +p≤.10

Figure A3.1. Historgram of the Antenatal APS Anxiety Subscale (Raw Scores)

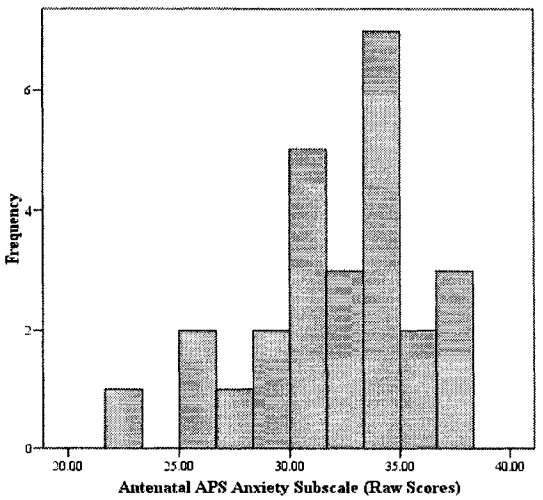


Figure A3.2. Histogram of the Antenatal APS Depression Subscale (Raw Scores)

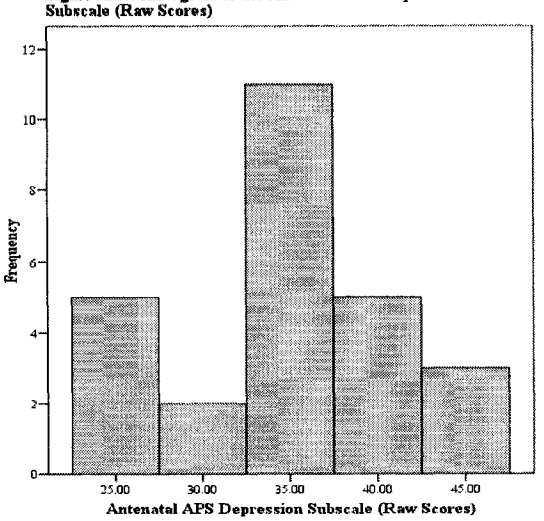


Figure A3.3. Histogram of the Antenatal APS Self-esteem Subscale (Raw Scores)

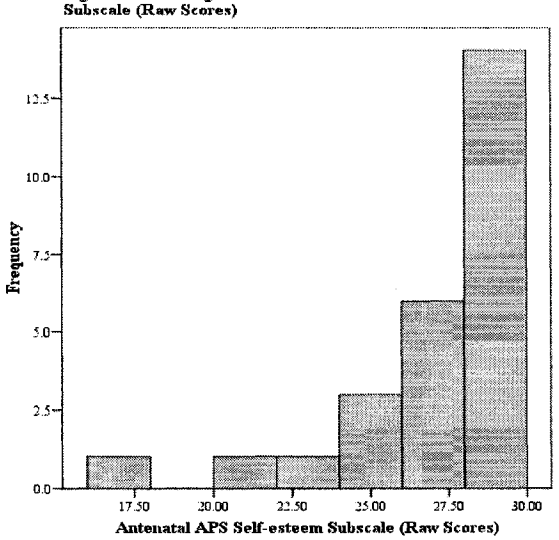


Figure A3.4. Histogram of the Antenatal APS Mastery Subscale (Raw Scores)

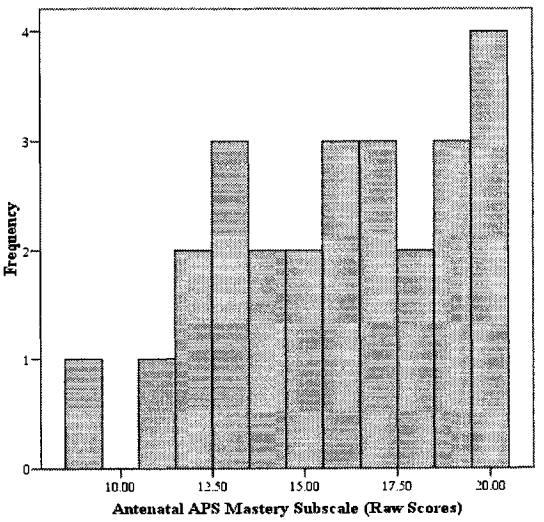


Figure A3.5. Histogram of the Antenatal APS Subjective Stress Subscale (Raw Scores)

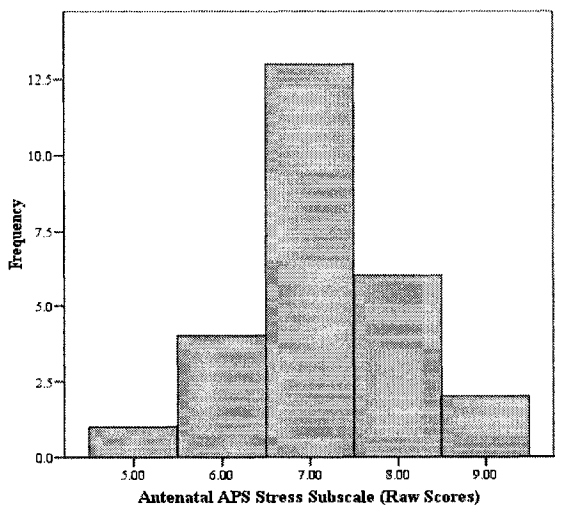


Figure A3.6. Histogram of the Antenatal APS Total (Raw Scores)

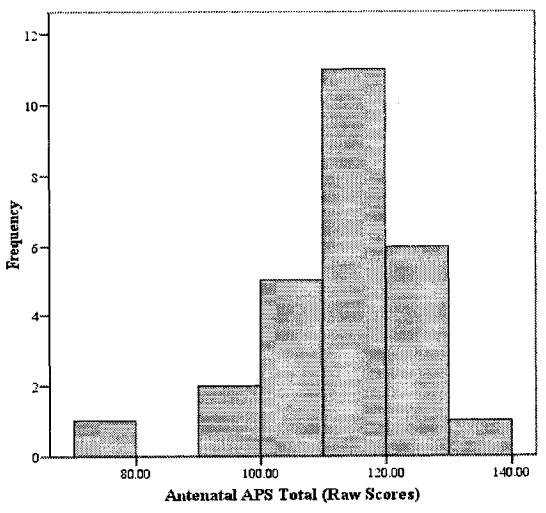


Figure A3.7. Histogram of the 10-month APS Anxiety Subscale (Raw Scores)

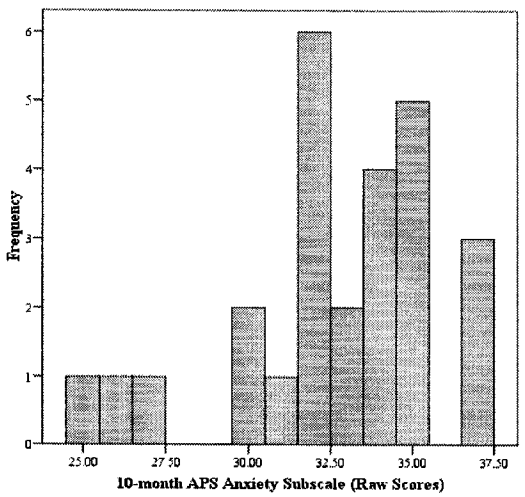


Figure A3.8. Histogram of the 10-month APS Depression Subscale (Raw Scores)

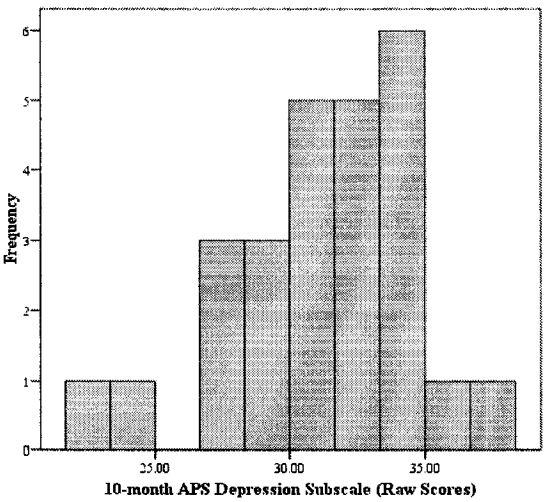


Figure A3.9. Histogram of the 10-month APS Self-esteem Subscale (Raw Scores)

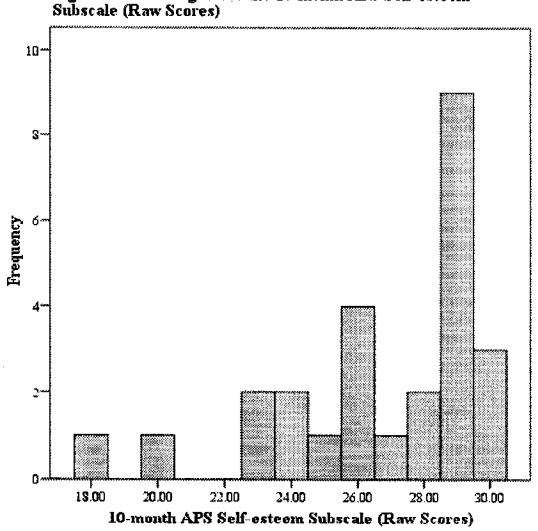


Figure A3.10. Histogram of the 10-month APS Mastery Subscale (Raw Scores)

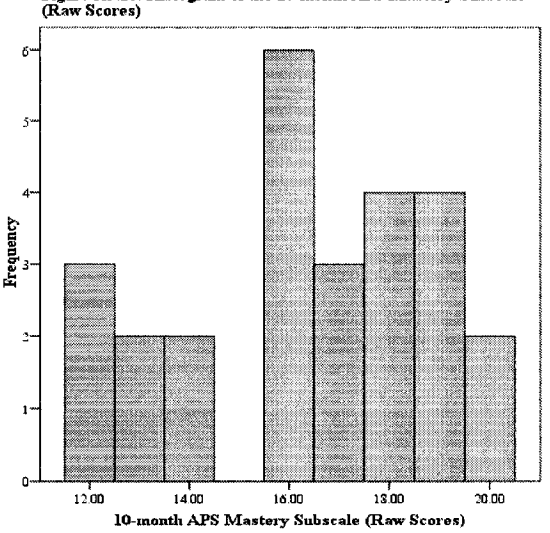


Figure A3.11. Histogram of the 10-month APS Subjective Stress Subscale (Raw Scores)

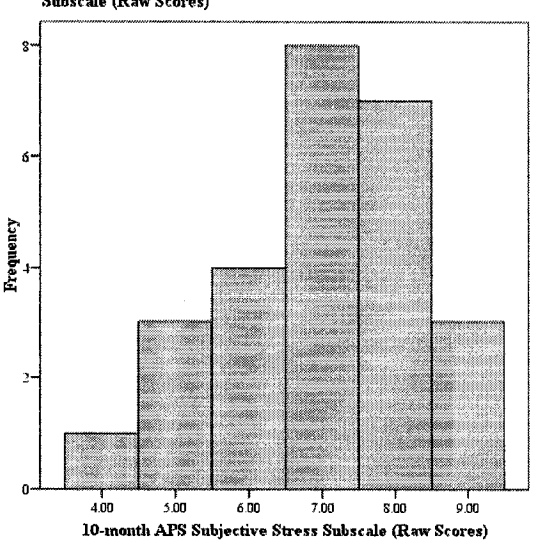


Figure A3.12. Histogram of the 10-month APS Total (Raw Scores)

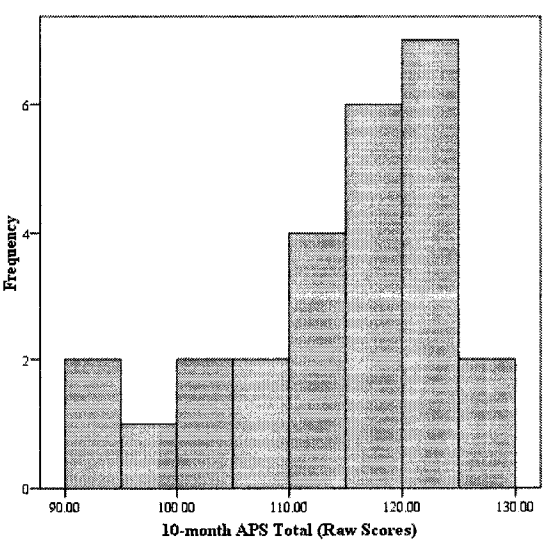


Figure A3.13. Histogram of the Perinatal Cortisol Baseline Levels (Raw Scores)

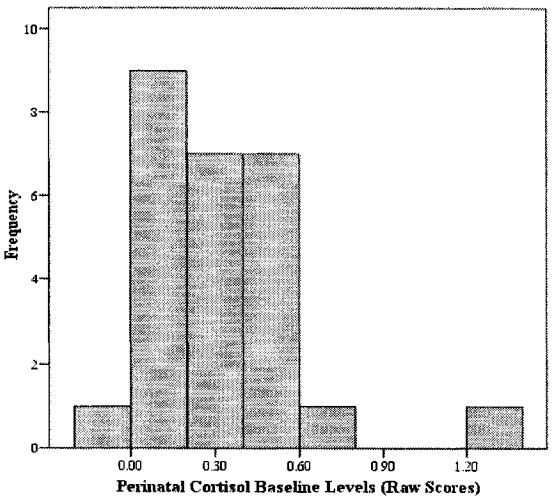


Figure A3.14. Histogram of the Perinatal Post-challenge Cortisol Levels (Raw Scores)

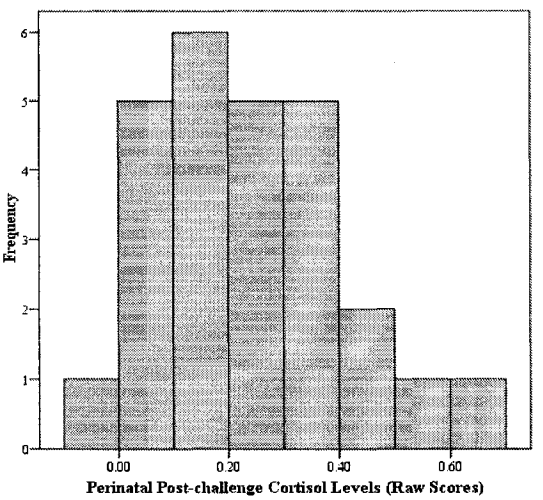


Figure A3.15. Histogram of the Perinatal Cortisol Difference (Raw Scores)

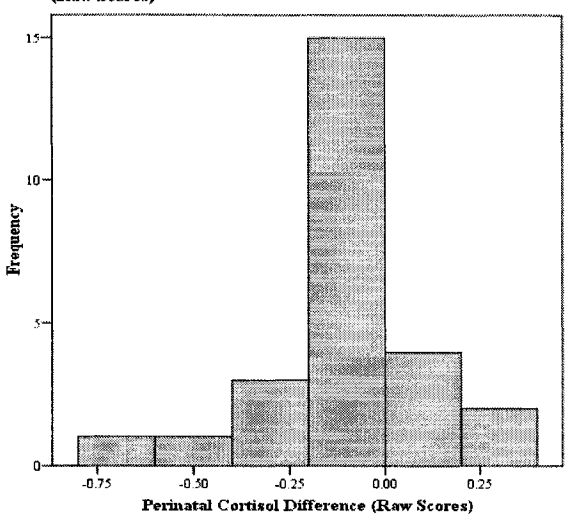


Figure A3.16. Histogram of the Perinatal Standardized Residuals of Post-challenge Cortisol Levels Controlling for Baselines (Raw Scores)

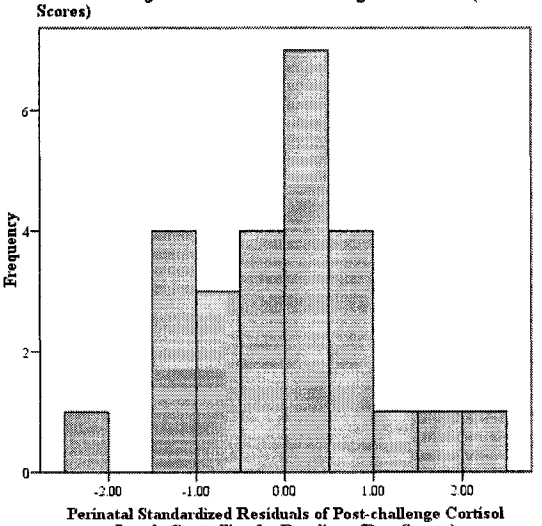


Figure A3.17. Histogram of the Perinatal Standardized Residuals of Cortisol Difference Scores Controlling for Baselines (Raw Scores)

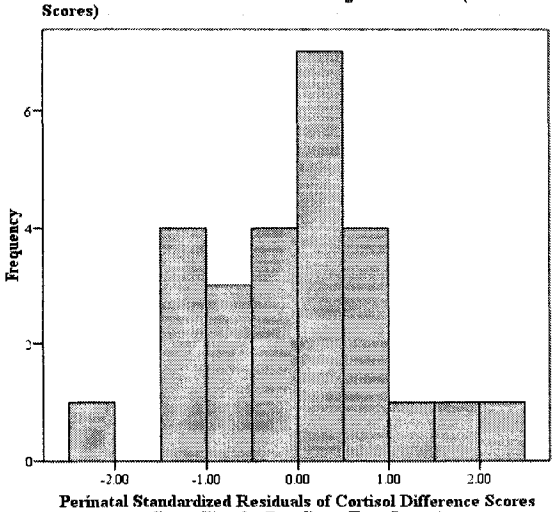


Figure A3.18. Histogram of the Perinatal Cortisol Baseline Levels (Log Scores)

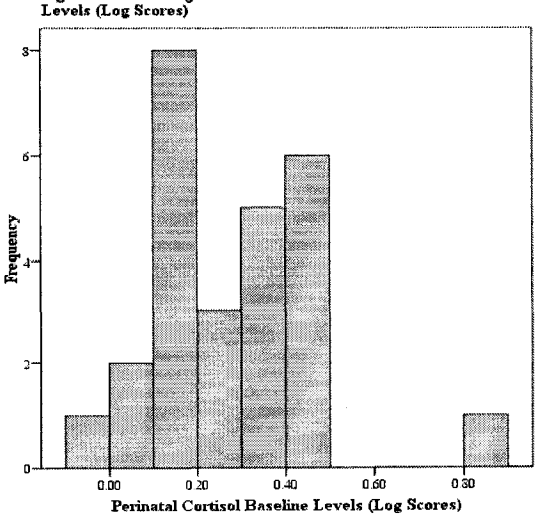


Figure A3.19. Histogram of the Perinatal Post-challenge Cortisol Levels (Log Scores)

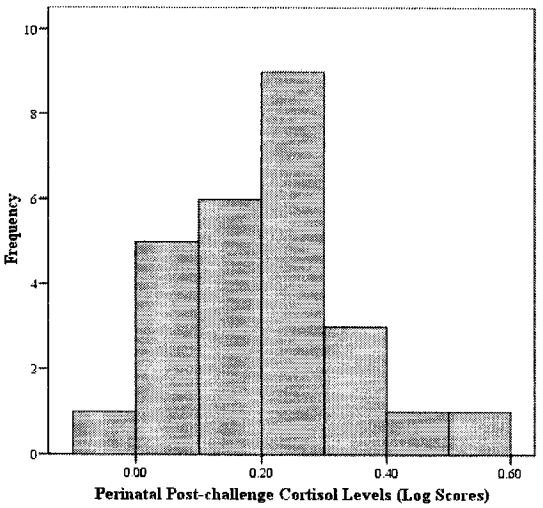


Figure A3.20. Histogram of the Perinatal Cortisol Difference (Log Scores)

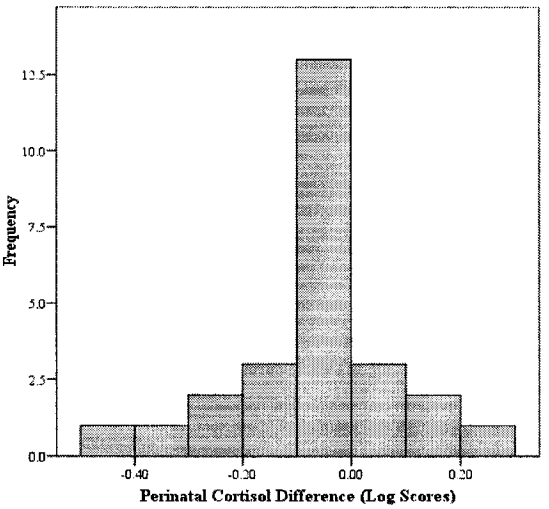


Figure A3.21. Histogram of the Perinatal Standardized Residuals of Post-challenge Cortisol Levels Controlling for Baselines (Log Scores)

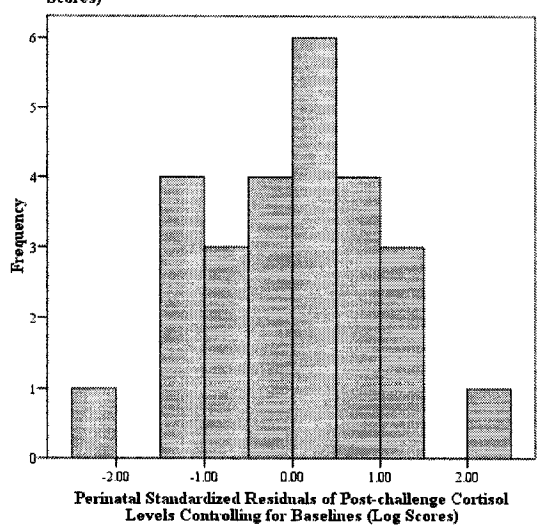


Figure A3.22. Histogram of the Perinatal Standardized Residuals of Cortisol Difference Scores Controlling for Baselines (Log Scores)

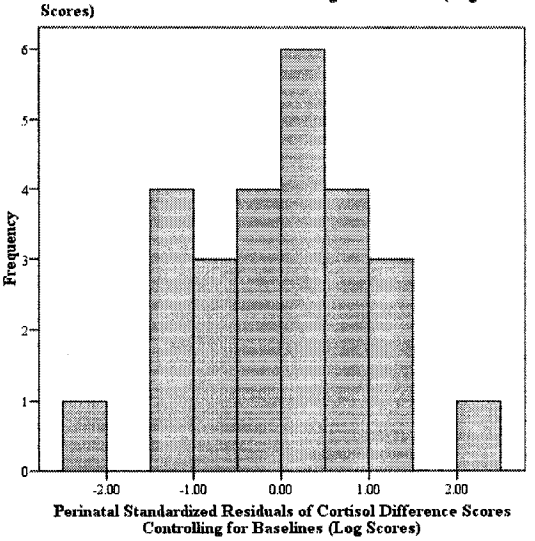


Figure A3.23. Histogram of the 10-month Cortisol Baseline Levels (Raw Scores)

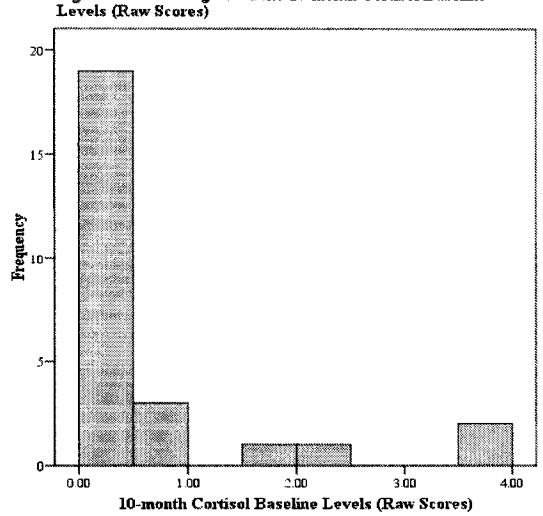


Figure A3.24. Histogram of the 10-month Post-challenge Cortisol Levels (Raw Scores)

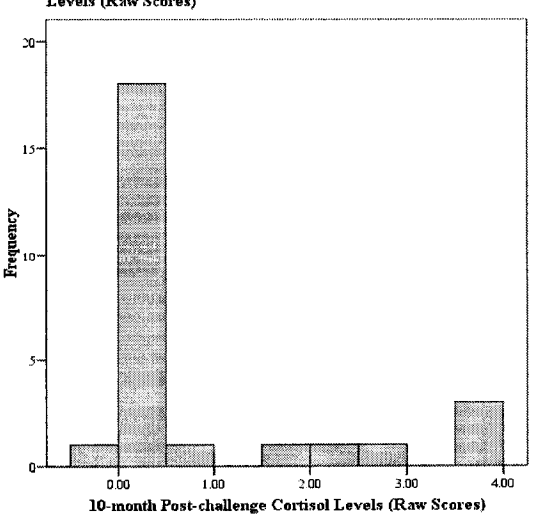


Figure A3.25. Histogram of the 10-month Cortisol Difference (Raw Scores)

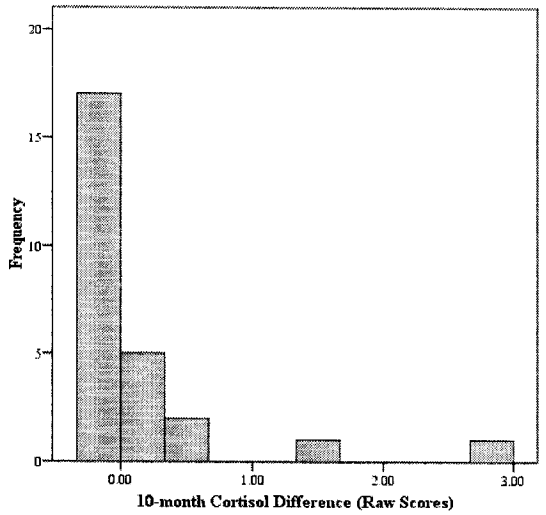


Figure A3.26. Histogram of the 10-month Standardized Residuals of Post-challenge Cortisol Levels Controlling for Baselines (Raw Scores)

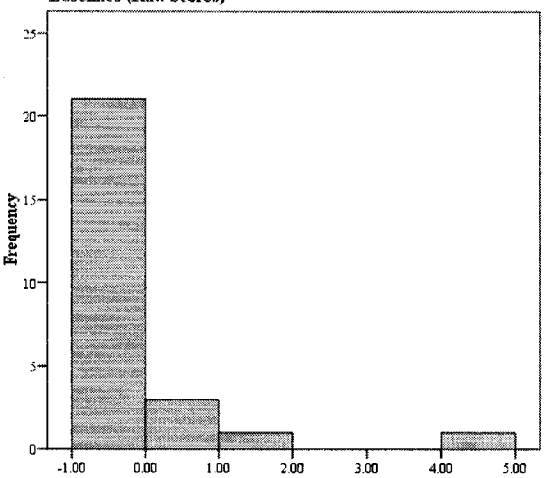


Figure A3.27. Histogram of the 10-month Standardized Residuals of Cortisol Difference Scores Controlling for Baselines (Raw Scores)

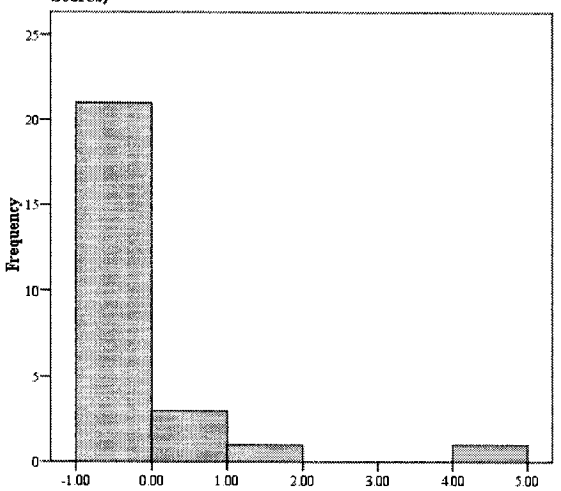


Figure A3.28. Histogram of the 10-month Cortisol Baseline Levels (Log Scores)

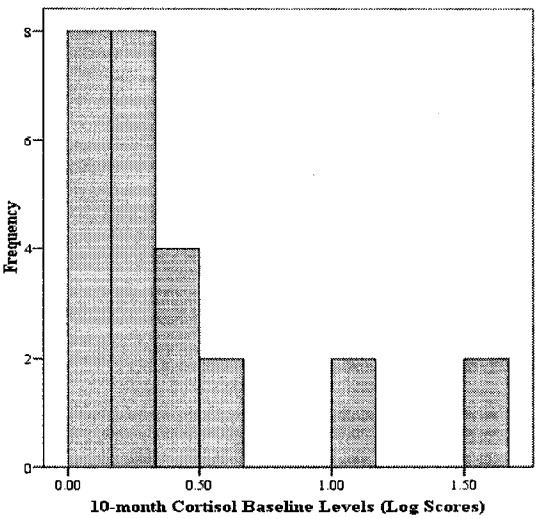


Figure A3.29. Histogram of the 10-month Post-challenge Cortisol Levels (Log Scores)

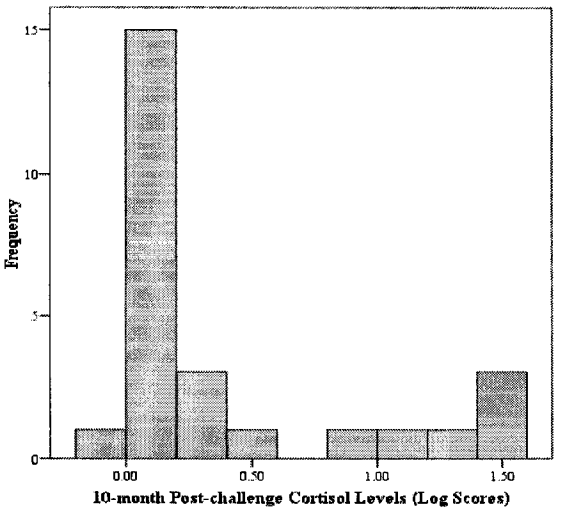


Figure A3.30. Histogram of the 10-month Cortisol Difference (Log Scores)

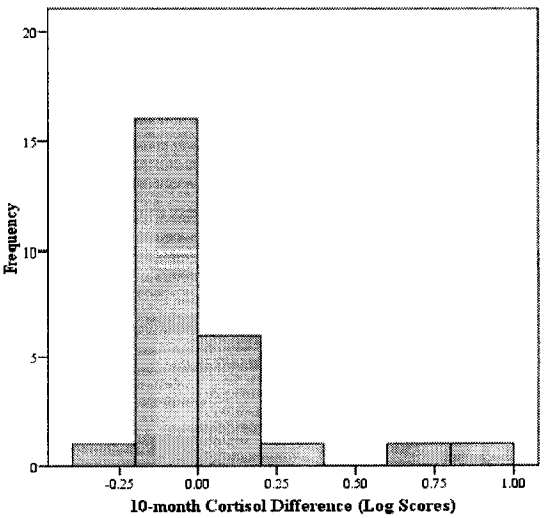


Figure A3.31. Histogram of the 10-month Standardized Residuals of Post-challenge Cortisol Levels Controlling for Baselines (Log Scores)

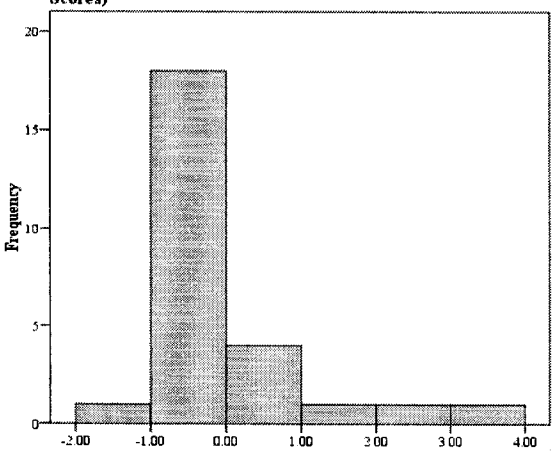


Figure A3.32. Histogram of the 10-month Standardized Residuals of Cortisol Difference Scores Controlling for Baselines (Log Scores)

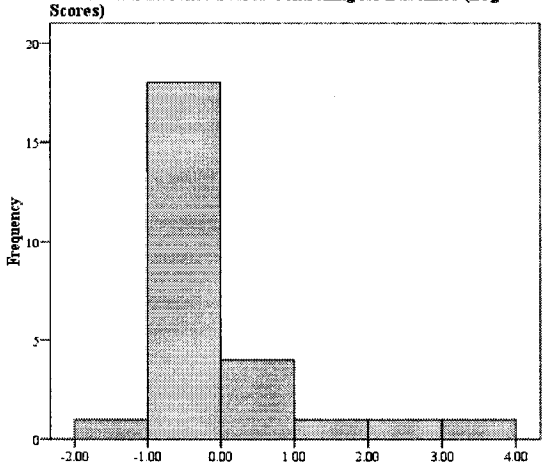


Figure A3.33. Histogram of the 10-month Cortisol Baseline Levels (Inverse Scores)

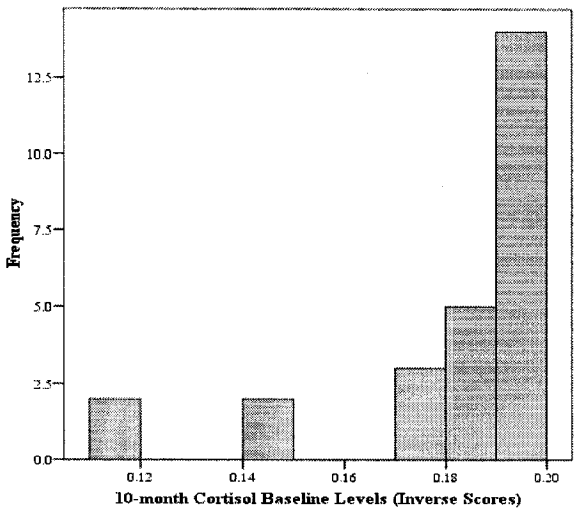


Figure A3.34. Histogram of the 10-month Post-challenge Cortisol Levels (Inverse Scores)

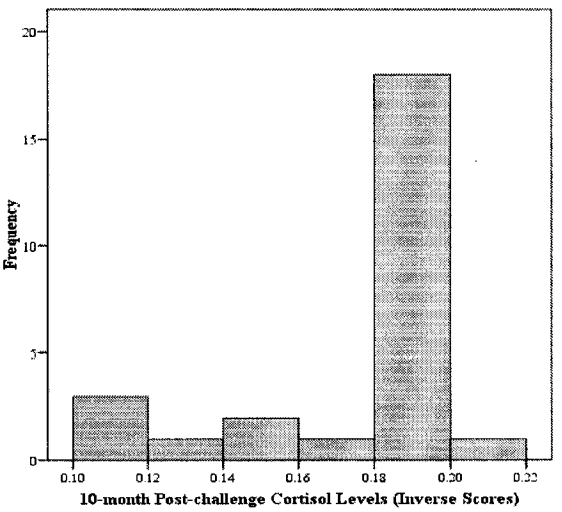


Figure A3.35. Histogram of the 10-month Cortisol Difference (Inverse Scores)

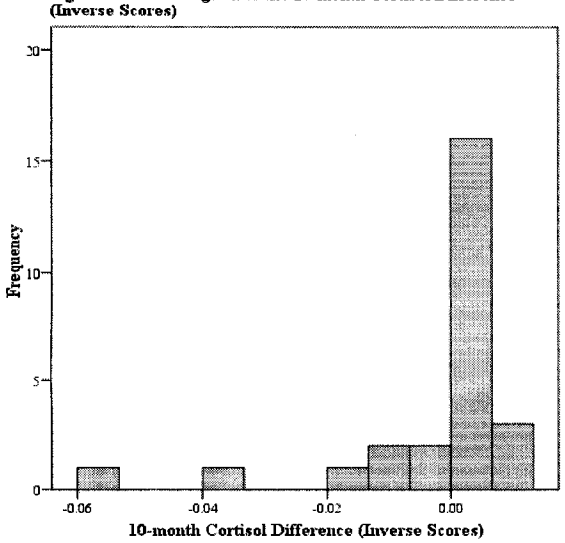


Figure A3.36. Histogram of the 10-month Standardized Residuals of Post-challenge Cortisol Levels Controlling for Baselines (Inverse Scores)

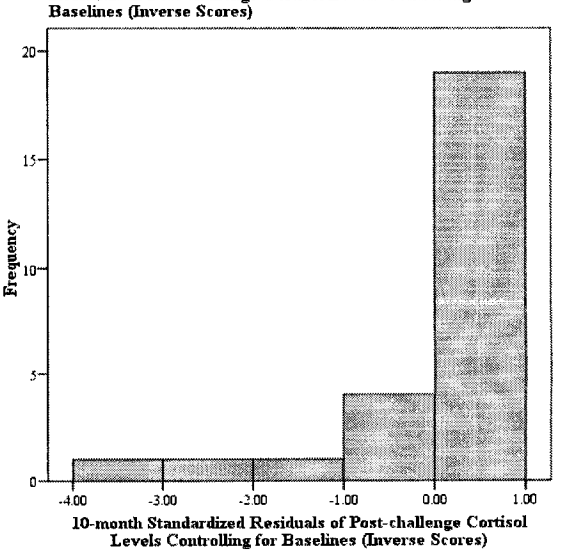


Figure A3.38. Histogram of the Mean Differences in the duration of Visual-behavioral Disengagement between Consecutive Epochs after Toy Removal (Raw Scores)

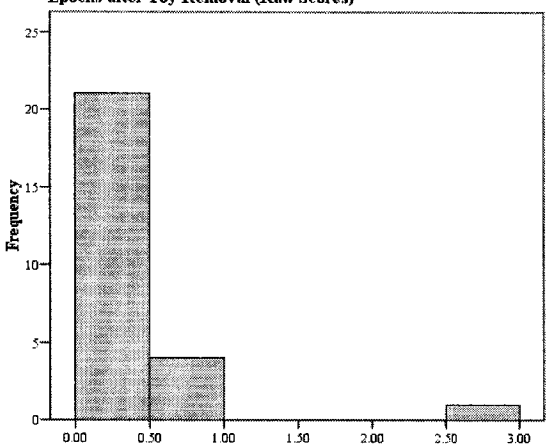


Figure A3.39. Histogram of the Mean Differences in the duration of Self-comforting Behaviors between Consecutive Epochs after Toy Removal (Raw Scores)

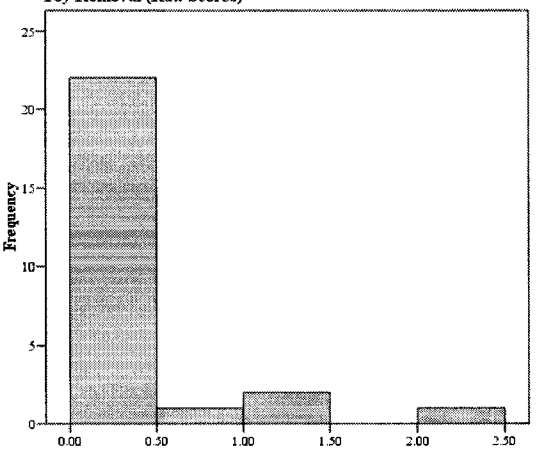


Figure A3.40. Histogram of the Mean Differences in Negative Vocalization Intensity between Consecutive Epochs after Toy Removal (Raw Scores)

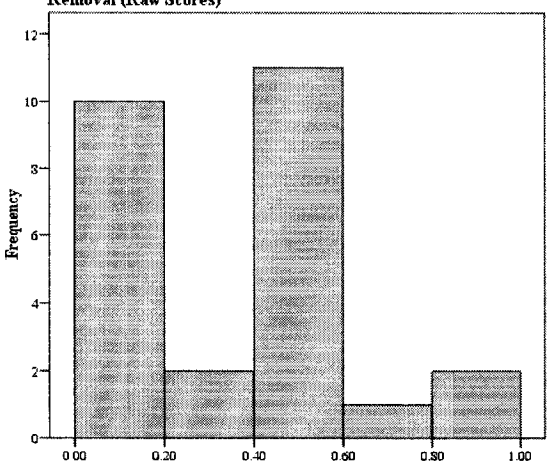


Figure A3.41. Histogram of the Sum of the Mean Differences in Negative Vocalizations Intensity, duration of Visual-behavioral Disengagement and duration of Self-comforting Behaviors between Consecutive Epochs after Toy Removal (Raw Scores)

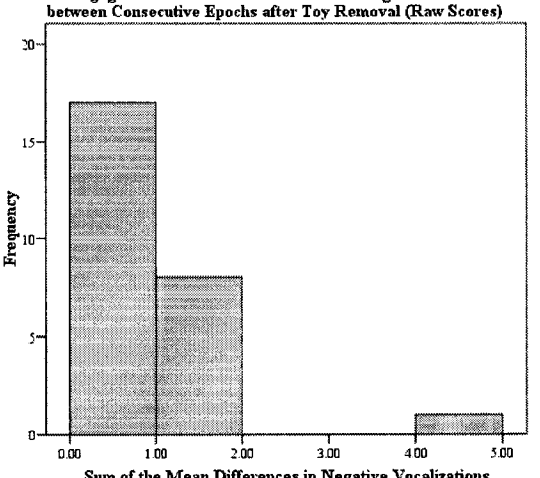


Figure A3.42. Histogram of the Difference Score between the Total number of Visual-behavioral Disengagement observed at Baseline and after Toy Removal (Raw Scores)

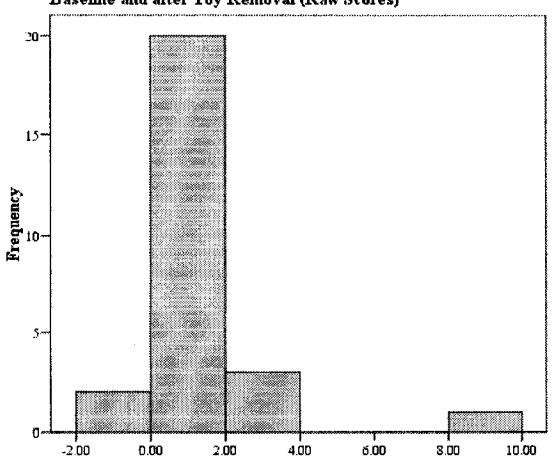


Figure A3.43. Histogram of the Difference Score between the Total number of Self-comforting Behaviors observed at Baseline and after Toy Removal (Raw Scores)

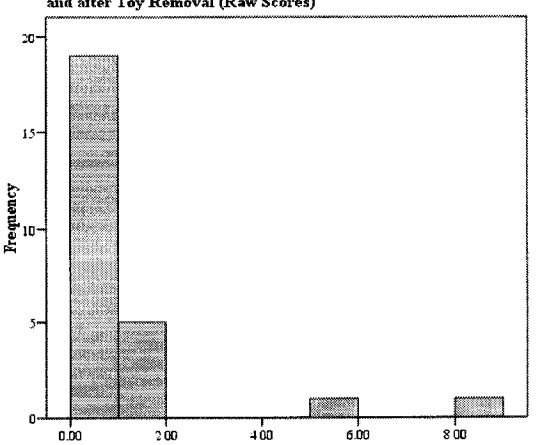


Figure A3.44. Histogram of the Difference Score between Total Negative Vocalization Intensity at Baseline and after Toy Removal (Raw Scores)

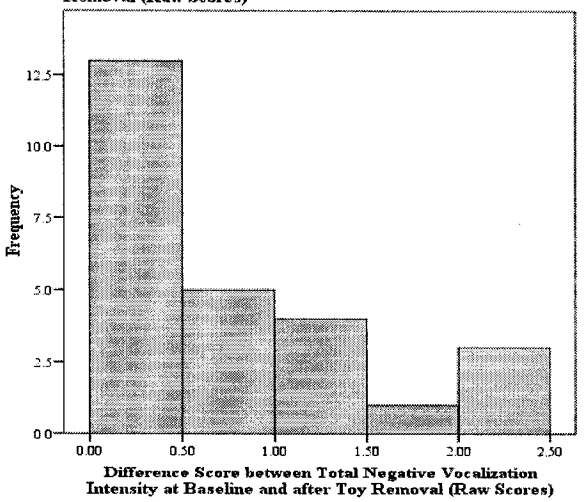


Figure A3.45. Histogram of the Sum of Difference Scores on Negative Vocalization, Visual-behavioral Disengagement and Self-comforting Behaviors between Baseline and the Episode after Toy Removal (Raw Scores)

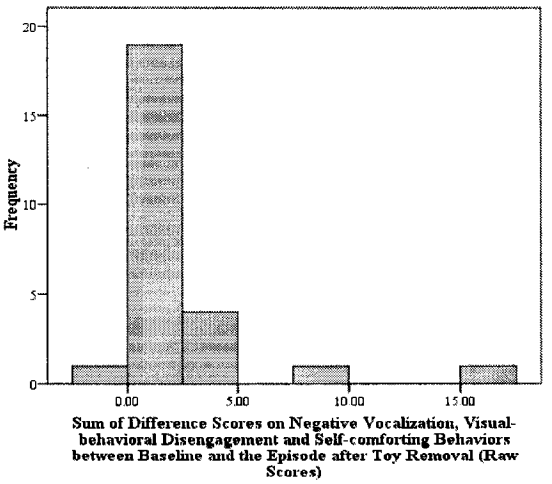


Figure A3.46. Histogram of the Mean Differences in the duration of Visual-behavioral Disengagement between Consecutive Epochs after Toy Removal (Log Scores)

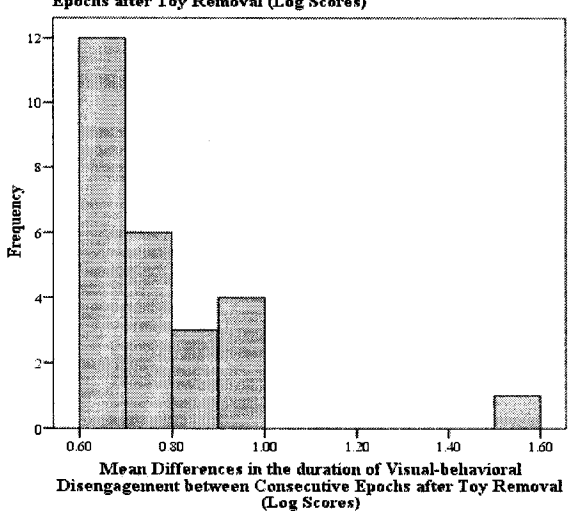


Figure A3.47. Histogram of the Mean Differences in the duration of Self-comforting Behaviors between Consecutive Epochs after Toy Removal (Log Scores)

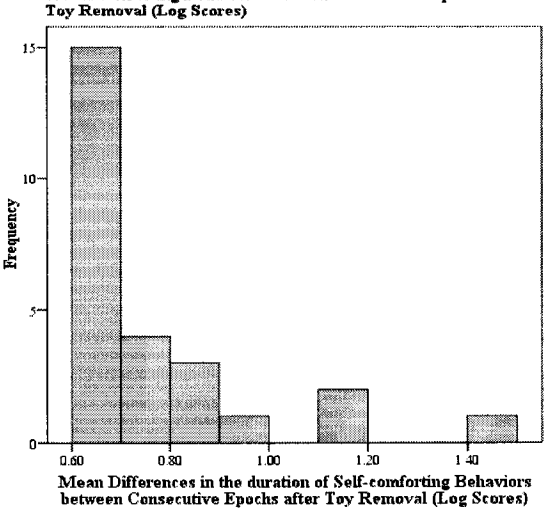


Figure A3.48. Histogram of the Mean Differences in Negative Vocalization Intensity between Consecutive Epochs after Toy Removal (Log Scores)

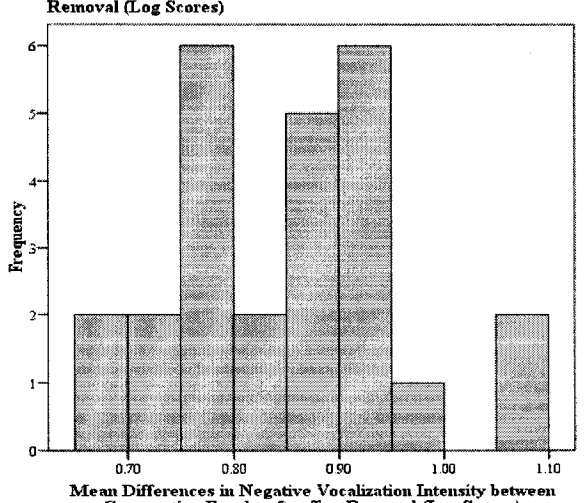


Figure A3.49. Histogram of the Sum of the Mean Differences in Negative Vocalizations Intensity, duration of Visual-behavioral Disengagement and duration of Self-comforting Behaviors between Consecutive Epochs after Toy Removal (Log Scores)

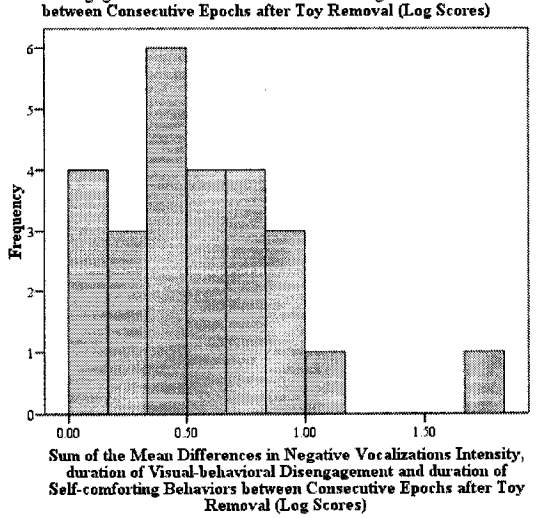


Figure A3.50. Histogram of the Difference Score between the Total number of Visual-behavioral Disengagement observed at Baseline and after Toy Removal (Log Scores)

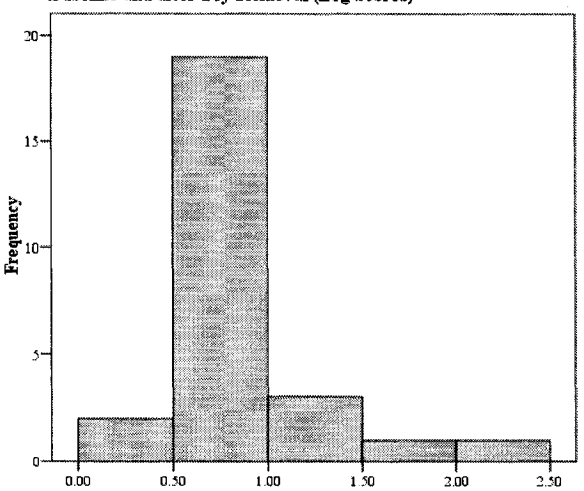


Figure A3.51. Histogram of the Difference Score between the Total number of Self-comforting Behaviors observed at Baseline and after Toy Removal (Log Scores)

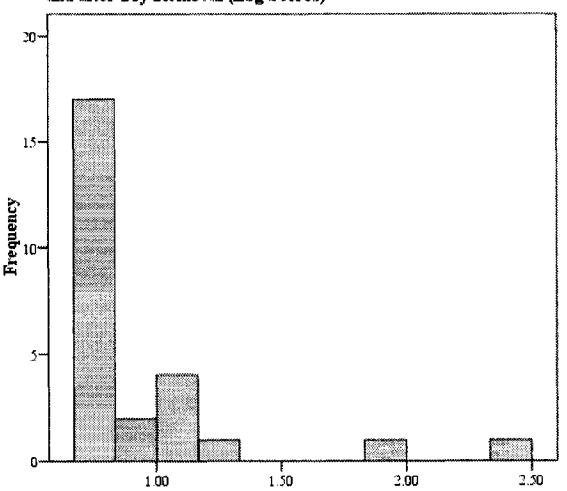


Figure A3.52. Histogram of the Difference Score between Total Negative Vocalization Intensity at Baseline and after Toy Removal (Log Scores)

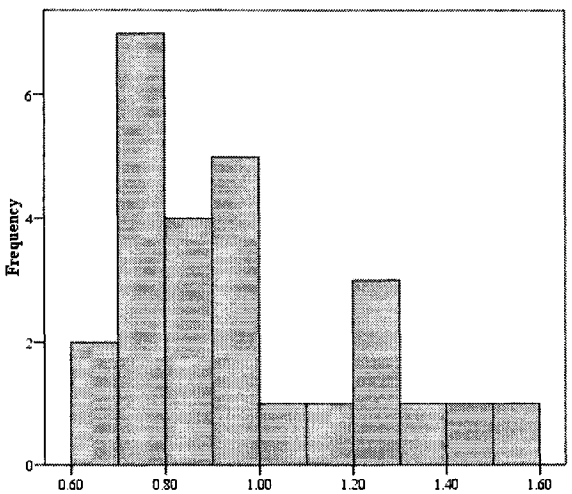


Figure A3.53. Histogram of the Sum of Difference Scores on Negative Vocalization, Visual-behavioral Disengagement and Self-comforting Behaviors between Baseline and the Episode after Toy Removal (Log Scores)

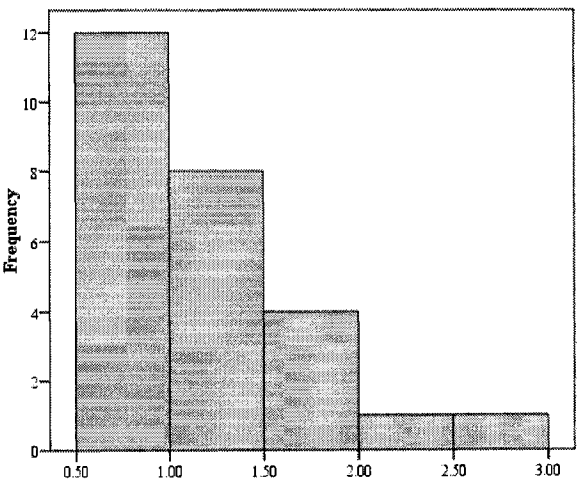


Figure A3.50. Scatterplot of the relation between APS subjective stress subscale and perinatal cortisol difference score

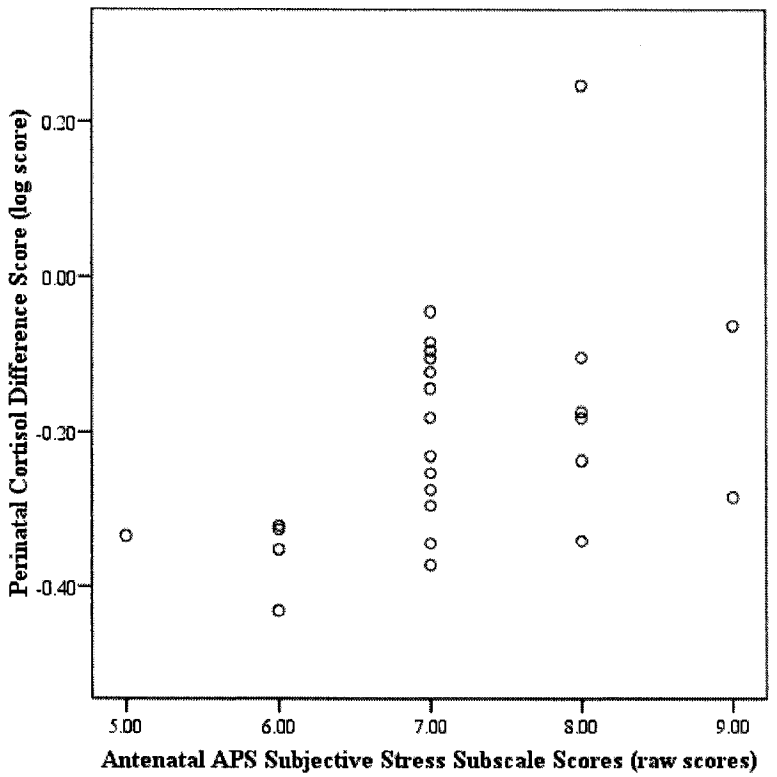


Figure A3.55. Scatterplot of the Relation between Perinatal Cortisol Difference Score and mean differences in Negative Vocalization following Toy Removal

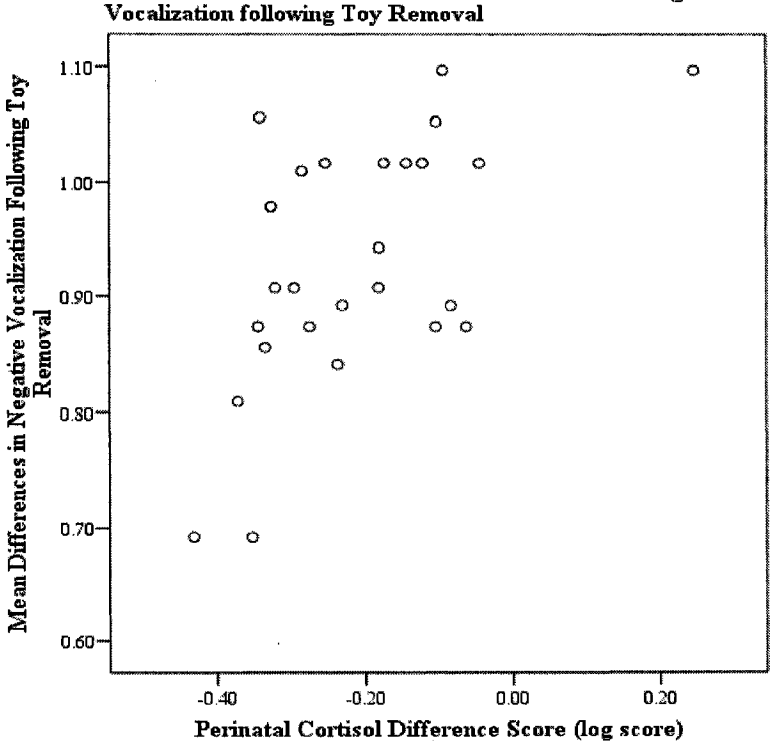


Figure A3.56. Scatterplot of the Relation between Antenatal APS Subjective Stress Subscale score and Mean Differences in Negative Vocalization Following Toy Removal