Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Danielle A. Swales April 6, 2015

Timing and Trajectories of Maternal Depression during Pregnancy and Emotional Reactivity Outcomes in the Preschool Period

by

Danielle A. Swales

Patricia Brennan, Ph.D.
Adviser

Department of Psychology

Patricia Brennan, Ph.D.

Adviser

Elaine Walker, Ph.D.

Committee Member

Azhar Nizam, M.S.

Committee Member

Anne Winiarski, M.A.

Committee Member

2015

Timing and Trajectories of Maternal Depression during Pregnancy and Emotional Reactivity Outcomes in the Preschool Period

By

Danielle A. Swales

Patricia A. Brennan, Ph.D.

Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Arts with Honors

Department of Psychology

2015

Abstract

Timing and Trajectories of Maternal Depression during Pregnancy
and Emotional Reactivity Outcomes in the Preschool Period

By Danielle A. Swales

Background: The intrauterine environment can have a profound effect on the developing fetus, shaping the developmental trajectory of the child and producing fetal programming effects which may be sustained well into the postnatal period. Prenatal exposure to maternal depression within this sensitive period of development has been linked to a variety of adverse physiological, neurological, and behavioral outcomes across infancy and early childhood. The current study investigated the relationship between the timing and trajectory of maternal depression during pregnancy and emotional reactivity outcomes of the offspring in the preschool period. The sex of the child was also considered as a potential moderator of this relationship. **Methods:** 180 mothers were recruited for participation in the study during pregnancy, and they then returned to the laboratory for a follow up visit when the child reached preschool age. Maternal depressive symptoms during pregnancy were measured using self-report. Emotional reactivity was assessed through multiple measures, including cortisol reactivity and laboratory observational measures of emotional reactivity following exposure to a stressor task. Maternal and alternative caregiver report of the child's emotional reactivity were obtained as well. **Results:** Growth Mixture Modeling was used to empirically define three trajectory classes of maternal depression during the prenatal period within the sample. A trajectory class representing high but decreasing levels of maternal depression across pregnancy, as well as a separate measure of maternal depression in the first trimester, were found to be significantly and positively related to offspring cortisol reactivity. Maternal depression in the first and second trimester was also found to be positively related to alternative caregiver report of emotional reactivity in girls but not boys. Laboratory observational measures and maternal report of offspring emotional reactivity were not found to be significantly related to maternal depression during pregnancy. **Conclusions:** Findings support a link between the timing and trajectory of maternal depression during pregnancy and markers of emotional reactivity in the preschool period. The clinical implications of study findings were considered and future research directions were discussed.

Timing and Trajectories of Maternal Depression during Pregnancy and Emotional Reactivity Outcomes in the Preschool Period

By

Danielle A. Swales

Patricia Brennan, Ph.D.

Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Arts with Honors

Department of Psychology

2015

Table of Contents

Timing and Trajectories of Maternal Depression during Pregnancy and Emotional Reactivity Outcomes in the Preschool Period

The risk for depression is relatively high during pregnancy, as studies suggest that between 10% and 20% percent of women experience depression while they are pregnant (Marcus, Flynn, Blow, & Barry, 2003; Marcus, 2009). Maternal depression is an especially problematic public health concern, as it not only indicates an overall impairment in maternal mental health and wellbeing, but is implicated in higher risk for negative physiological, psychological, and behavioral outcomes in the mother's offspring (Davis et al., 2004; Deave, Heron, Evans, & Emond, 2008; Field, Diego, & Hernandez-Reif, 2006; Sohr-Preston & Scaramella, 2006). These risk factors may be conferred upon the developing neonate during gestation, as alterations to the uterine environment may produce programming effects, shaping the development of the offspring and impacting long term health and behavioral outcomes. This fetal programming hypothesis argues that maternal depression leads to harmful alterations to the fetal system, impacting the development of critical physiological and neurological structures, and influencing behavioral and health outcomes well into the postnatal period (Godfrey & Barker, 2007; Kapoor, Dunn, Kostaki, Andrews, & Matthews, 2006; Kinsella & Monk, 2009).

The link between maternal depressive symptoms during pregnancy and altered neonatal and child outcomes has been well supported throughout the literature, as prenatal exposure to maternal depression and stress have been associated with an elevated risk of obstetric complications such as premature delivery, low birth weight, and preeclampsia (Field et al., 2006; Grote, Bridge, & Gavin, 2014), as well as later impairments in offspring cognitive and language skills (Sohr-Preston & Scaramella, 2006). Negative behavioral outcomes have also been observed in offspring throughout development, including increased behavioral reactivity and

negative affect in infancy, as well as greater levels of externalizing problems in childhood (Davis et al., 2004; Luoma et al., 1998).

Maternal Depression and Emotional Reactivity

Prenatal exposure to maternal depression has also been linked to an impairment in emotional reactivity in offspring (Davis et al., 2007). Calkins, Gill, Johnson, and Smith (1999) conceptualize emotional reactivity as "an individual's characteristic threshold, intensity and duration of affective arousal" (p. 312) . This construct has been measured through various means including cortisol reactivity, behavioral observation of emotional reactivity, and caregiver report of child behavior (Davis et al., 2004; Kaplan, Evans, & Monk, 2009; Zuckerman, Bauchner, Parker, & Cabral, 1990). Studies have linked prenatal exposure to maternal depression to a variety of emotional reactivity outcome measures, including increased behavioral reactivity (Davis et al., 2004), elevated cortisol reactivity (Kaplan et al., 2009), and reports of greater irritability in offspring (Zuckerman et al., 1990). Evidence of high emotional reactivity may therefore manifest itself across these various physiological and behavioral dimensions. A highly emotionally reactive child would therefore be expected to display elevated intensity and duration of physiological and behavioral markers of distress, such as cortisol and affective change, following exposure to a frustration stimulus (Calkins et al., 1999). While typically investigated separately, behavioral and cortisol markers both fall within this shared construct of emotional reactivity, a key component of the child's temperament. Consideration of measurements across these various facets of emotional reactivity therefore allows for the formation of a more complete picture of this complex construct.

Prenatal exposure to maternal depression and stress has been linked to increased risk of irritability in newborns, and greater cortisol and behavioral reactivity in infancy (Davis et al.,

2004, 2007; Zuckerman et al., 1990). The vast majority of the literature, however, has focused primarily on outcomes in infancy; therefore the study of whether and how such effects progress later into the postnatal period merits further investigation. The preschool period is an interesting and important time period in which to examine the relationship between prenatal maternal depression and offspring emotional reactivity, as children begin to develop more effective emotional regulation strategies (Cole, Martin, & Dennis, 2014). The current study focuses on outcomes during the preschool phase of development.

Emotional reactivity is an outcome of particular interest as it is a predictor of prosocial behaviors throughout development (Eisenberg & Fabes, 1992). Emotional reactivity within the preschool period has been linked with markers of social competence, including conflict and cooperation (Calkins et al., 1999). Abnormalities in a child's emotional reactivity may therefore lead to a significant impairment of social and psychological functioning (Bradley et al., 2011; Calkins et al., 1999). High emotional reactivity may also lead to emotional dysregulation, which has been identified as a key component of various psychiatric disorders in later adulthood (Bradley et al., 2011). Given the importance of the preschool period in the development of emotion regulation, as well as the established links between emotion regulation and behavioral outcomes in late childhood, research that helps better elucidate the early life predictors of emotional reactivity could help in the development of useful clinical interventions for at-risk children.

Effect of Maternal Depression on the Fetal Environment

Mother and child cohabitate in a shared physical system during gestation; therefore the biochemistry of the mother can have profound impact on the uterine environment of the developing fetus (Field et al., 2004). Studies have found that levels of cortisol, a key stress

hormone and glucocorticoid produced by the hypothalamic-pituitary-adrenal (HPA) axis, are often elevated in expecting mothers suffering from depression (Davis et al., 2007). Activation of this stress response system can be adaptive to cope with moderate, short term stress, and it is advantageous from an evolutionary perspective, as it prepares the individual for a flight or fight response in the face of a perceived danger or threat. Exposure to significant chronic stress however can be harmful to the individual, as it may lead to the dysregulation and overactivity of the HPA axis (Pariante & Lightman, 2008). Among depressed individuals, these elevations in cortisol production, elicited by exposure to a stressor, are both heightened and prolonged (Davis et al., 2007). This finding of the dysregulation of physiological stress responses among depressed individuals can be extended to pregnant women suffering from depression as well because pregnancy does not protect the mother from these detrimental physiological effects (Davis et al., 2007).

The heightened cortisol production that accompanies depression impacts the immediate environment of the developing fetus as well the mother (Lundy et al., 1999). The structure that facilitates the interaction between the maternal and fetal systems is the placenta (Ponder et al., 2011). The placenta acts as a partial barrier between the maternal and fetal environment, preventing an estimated 50-90% of circulating maternal cortisol from passing through to the fetal system (Mulder et al., 2002). This protective function of the placenta is achieved through the activity of 11 β –Hydroxysteroid dehyodrogenase (11β-HSD2), a placental enzyme that oxidizes maternal cortisol as it crosses the placental barrier, thus turning it into its inactive form (Buss et al., 2012; Glover, O'Connor, & O'Donnell, 2010; Ponder et al., 2011). However when mothers are in a depressive state, this protective function may become impaired due to the downregulation of 11β-HSD2 (Buss et al., 2012). This allows for more maternal cortisol to cross

the placental barrier and enter into the uterine environment (Buss et al., 2012). In addition to an increase in the amount of maternal cortisol entering the fetal environment, fetal production of cortisol becomes elevated as well due to an enhanced production of placental corticotrophin-releasing hormone (CRH), which acts on the fetal HPA axis to increase production of cortisol (Buss et al., 2012). The combined effect of these two mechanisms, the down regulation of 11β-HSD2 and increase in fetal CRH, results in an overall elevation in cortisol within the fetal environment, shaping the surrounding fetal biochemical environment to reflect that of the depressed mother (Field et al., 2006).

Fetal Programming Hypothesis

Alterations to the fetal environment as a result of maternal depression may have prolonged effects on the neonate. As previously noted, the fetal programming hypothesis argues that a change or stressor in the prenatal environment can result in developmental adaptations that permanently alter the structure, physiology, and metabolism of the fetus (Godfrey & Barker, 2007). Such alterations may be caused by exposure to teratogens, severe chronic stress, maternal psychopathology, or a lack of necessary resources (e.g., malnourishment). These insults to the uterine environment may impact the development of critical neurological structures within the fetal brain as well as important physiological systems, thus shifting the course of development and predisposing the child to later health risks, as well as deficits in behavioral, neurological, and psychological functioning.

Impact on Developing Neurological Structures

The development of the amygdala and HPA axis, structures which have been implicated in emotional reactivity, may be particularly sensitive to alterations in fetal glucocorticoid exposure as a result of maternal depression (Buss et al., 2012). Fetal programming of the HPA

axis and amygdala may in turn lead to dysregulation of emotional reactivity in later childhood. While the current study does not directly measure or investigate such underlying mechanisms of fetal programming, it is important to explore this pathway linking maternal mental health status during pregnancy and long term developmental outcomes in the offspring, so as to better understand the mechanisms of this predicted relationship.

Empirical data link changes in offspring HPA axis functioning to prenatal exposure to maternal depression (Charil, Laplante, Vaillancourt, & King, 2010). More specifically, prenatal exposure to maternal stress and depression alters the glucocorticoid and mineralocorticoid receptor expression in fetal brain areas such as the hippocampus, paraventricular nucleus, and the amygdala (Davis, Glynn, Waffarn, & Sandman, 2011; Kapoor et al., 2006). Animal models have been used to demonstrate this mechanism, finding reduced expression of glucocorticoid and mineralocorticoid receptors in the offspring hippocampus, dampening its inhibitory function and negative feedback action, and thus prolonging elevations in cortisone following exposure to a stressor (Mulder et al., 2002). Elevated cortisone exposure has also been shown to impact glucocorticoid receptors in the amygdala as well, which impacts the activity of the stress response system as this brain structure activates HPA axis activity (Tottenham & Sheridan, 2009). Prenatal amygdala development may be sensitive to early exposure to glucocorticoids, as Buss and colleagues found that elevated maternal cortisol 15 weeks into gestation was associated with an increase in right amygdala volumes in 7 year year-old children (Buss et al., 2012). This increase in amygdala volume was suggested to mediate the relationship between prenatal maternal cortisol and greater affective problems in girls, and is consistent with findings of other negative outcomes including elevated stress reactivity in offspring and a lowering of the threshold of response to emotional events (Buss et al., 2012; Tottenham & Sheridan, 2009).

Although these findings are preliminary, they suggest physiological mechanisms that might link prenatal maternal depression to offspring difficulties in emotional reactivity.

Long Term Emotional Reactivity Outcomes

Empirical research lends support for an association between prenatal maternal depression and both behavioral and physiological measures of emotional reactivity throughout early infancy and childhood (Davis et al., 2004, 2007; Kaplan et al., 2009). Studies utilizing behavioral measures of emotional reactivity have found that offspring of mothers experiencing depression during pregnancy tend to show elevated behavioral reactivity, increased irritability and emotional problems, and greater externalizing problems (Davis et al., 2004, 2007; Luoma et al., 1998; Zuckerman et al., 1990). These outcomes have been measured through various methods, including maternal report of infant negative reactivity and externalizing behaviors, as well as assessment of infant consolability and excessive crying by a pediatrician in infancy (Davis et al., 2007; Luoma et al., 1998; Zuckerman et al., 1990). These findings indicate the presence of various behavioral markers that may point towards a dysregulation of emotional reactivity in the offspring. Prenatal exposure to maternal depression and elevated cortisol have also been linked to physiological markers of emotional reactivity, such as elevated cortisol reactivity and high cortisol levels in offspring as well (Davis et al., 2011; Field, Diego, & Hernandez-Reif, 2006b; Kofman, 2002). A study conducted by Davis and colleagues (2011), for example, found that maternal cortisol levels during pregnancy were able to predict infant cortisol reactivity following the stress of a heelstick procedure.

Timing and Trajectory of Maternal Depression

The timing and trajectory of maternal depressive symptoms across the course of pregnancy may play a role in determining the fetus's vulnerability to developing poor emotional

reactivity outcomes in childhood. Thus far, studies that have aimed to identify periods of vulnerability have yielded mixed results. Charil and colleagues (2010) proposed that stress exposure in mid-gestation was associated with worse behavioral outcomes in childhood, while Davis and colleagues (2007) found elevated cortisol at 30-32 weeks of gestation, but not earlier in gestation, to be associated with greater maternal report of negative reactivity in infancy. Natural variations in maternal cortisol production throughout the course of a typical pregnancy can also confound timing effects, as 11β-HSD2 expression decreases in late pregnancy prior to the birth of the child (Ponder et al., 2011). The current study aims to add to this area of research, investigating how the timing and trajectory of maternal depressive symptoms in pregnancy may influence the development of poor emotional reactivity outcomes in offspring.

Sex Differences

Sex differences may also moderate the relationship between maternal depression during pregnancy and offspring emotional reactivity. Sex differences have been noted in vulnerability to maternal cortisol, effect of maternal depression during pregnancy on neurological structures, and emotional reactivity outcomes in later childhood. Studies examining sex differences in the effectiveness of the placenta in protecting the fetus from maternal cortisol found that males tend to exhibit decreased expression of placental 11β-HSD2 (Charil et al., 2010). These findings suggest that male fetuses may be exposed to more maternal cortisol during gestation and perhaps may have an overall greater vulnerability to prenatal stress in the fetal period (Charil et al., 2010). However others studies examining outcomes later in development have demonstrated evidence of greater vulnerability among female as opposed to male offspring. For example, a study conducted by Buss and colleagues (2012), found that elevated maternal cortisol levels in early pregnancy were associated with greater affective problems and larger right amygdala

volume in females but not males. Sandman, Glynn, and Davis (2013) suggest that both observations of greater fetal vulnerability to cortisol among male fetuses and elevated risk of affective problems among females in the postnatal period can be explained by a sex-dependent viability-vulnerability tradeoff. According to this model, while males may be more at risk prenatally, they are also less likely to survive, thus resulting in impairment in prenatal viability. The surviving population of males, however, may be different from female offspring in that they are less susceptible to sustain the long-term effects of prenatal stress exposure. On the other hand, while female offspring demonstrate greater viability and a higher chance of survival in the fetal period, they are at greater risk of being affected by stress during the postnatal period. This model, in addition to several empirical findings, suggests that vulnerability to maternal stress, depression, and cortisol is sexually dimorphic (Sandman et al., 2013). The role of offspring sex in moderating the relationship between prenatal exposure to maternal depression and emotional reactivity outcomes in childhood will therefore be examined as an exploratory hypothesis within the current study.

The Current Study

The current study utilizes longitudinal, prospective data to examine the impact of varying patterns of maternal depression during pregnancy on emotion reactivity outcomes in the preschool period. We will use multiple assessments of maternal depression over the course of pregnancy to identify trajectories of depressive symptoms throughout gestation and to assess timing of exposure, thereby attempting to identify windows of fetal vulnerability to prenatal maternal depression. Child emotional reactivity outcomes will be assessed through multiple measures, including cortisol reactivity, behavioral observation in a laboratory setting, and

primary and alternative caregiver (e.g., father, grandparent, babysitter) reports of emotional reactivity. The study will also examine the role of child sex in moderating this relationship.

Hypotheses.

(1) It is hypothesized that the timing and trajectory of maternal depression during pregnancy will be associated with offspring cortisol reactivity in the preschool period.

(2) It is hypothesized that the timing and trajectory of maternal depression during pregnancy will be associated with laboratory observational measures of offspring emotional reactivity in the preschool period.

(3) It is hypothesized that the timing and trajectory of maternal depression during pregnancy will be associated with maternal and alternative caregiver report of offspring emotional reactivity in the preschool period.

(4) Exploratory analyses will be conducted to examine the potential role of the sex of the child in moderating the relationship between the timing and trajectory of maternal depression during pregnancy and offspring emotional reactivity outcomes in the preschool period.

Methods

Participants

The study utilizes data collected from participants of the Preschool Outcomes Study, conducted by the Biosocial Underpinnings Involved in Learning & Development (BUILD) Lab at Emory University. The Preschool Outcomes Study (NIH Grant RC1 MH088609, Brennan/Smith PIs) investigated the impact of prenatal exposure to psychotropic medications on the behavioral and cognitive outcomes of the children once they reached preschool age. The

longitudinal cohort consists of 180 mother-child dyads (Mean maternal age=37 years, *SD*=2.7; Mean child age=45 months, *SD*=11.1; 48% female; see Table 1). Mothers were recruited for participation in the study from the Emory Women's Mental Health Program (WMHP) at Emory University School of Medicine in the Department of Psychiatry. The WMHP is a mental health treatment center for women, with an emphasis on clinical care in pregnancy and the postpartum period. The center also provides psychiatric care for women experiencing depression prior to pregnancy; therefore mothers could be recruited for participation in the study early in pregnancy. The mother-child dyads were then followed from the prenatal to the preschool period (ages 2.5 to 5.5 years of age).

Procedure

Mothers were recruited for participation in the study through the WMHP in the early stages of pregnancy, and researchers obtained full informed consent from participants prior to testing. The Beck Depression Inventory, Second Edition (BDI-II) (Beck, Steer, & Brown, 1997) was administered to mothers during their visits to the WMHP over the course of their pregnancy (approximately every 4-6 weeks), providing maternal self-report of depressive symptoms throughout gestation. When the children reached preschool age, both mother and child returned to the lab for a 2 hour assessment visit to participate in a follow-up study. Mothers provided additional informed consent for both themselves and their child for this postnatal, preschool follow-up study. During the preschool assessment, researchers administered a battery of tests and questionnaires to both the mother and child, including multiple measure of the children's emotional reactivity, which are described below.

Measures

Maternal Prenatal Depression. The Beck Depression Inventory, Second Edition (BDI-II) is a 21-item self-administered questionnaire that was used in the study to assess maternal depressive symptoms throughout pregnancy (Beck, Steer, & Brown, 1997). For each item, the mother indicated the intensity of the symptom over the past two weeks. A score of 1-10 is considered to fall within normative range, while 11-16 indicates a mild mood disturbance, 17-20 is borderline clinical depression, 21-30 is moderate depression, 31-40 is severe depression, and over 40 indicates extreme depression (Beck, Steer, & Brown, 1997). Previous literature demonstrates good internal consistency of the BDI among psychiatric populations, as well as high internal reliability and strong discriminant, concurrent, and construct validity (Beck, Steer, & Garbin, 1988).

The current study utilizes BDI scores over each trimester of pregnancy to assess timing and determine trajectories of maternal depression within the sample. Specifically, BDI scores obtained within each trimester were combined to yield an Area Under the Curve (AUC) measure by trimester. These scores were used to assess the potential differential impact of the timing of maternal depression by trimester. In addition, trimester AUC scores were used to create trajectory classes of maternal prenatal depression as described in detail below.

Growth mixture modeling using Mplus 7.0 (Muthén & Muthén, 2012) was used to identify trajectory classes of maternal depressive symptoms throughout pregnancy. This methodology allowed for the trajectory classes to be determined empirically, as it was unclear what patterns of maternal depression would be present within the sample. After the trajectory classes were determined, the groupings were used for subsequent hypothesis testing to investigate if certain progressions of maternal depression over the course of pregnancy were linked to elevated risk of poor child emotional reactivity outcomes.

Child Emotional Reactivity. The child's behavioral ratings of emotional reactivity and cortisol reactivity were assessed through two frustration tasks from the preschool version Laboratory Temperament Assessment Battery (Lab-TAB)—Preschool Version (Goldsmith, Reilly, Lemery, Longley, & Prescott, 1999). Both tasks were designed to elicit moderate levels of stress or frustration in the child and allow for standardized coding and empirical observation of the child's behavior and temperament. In the first task, called the "Attractive Toy in a Transparent Box" task, the child chose 1 of 2 toys which the tester then placed it in a clear box. The box was then locked and the tester gave the child a set of keys to open it (none of which fit the lock) and instructed the child to unlock the box. Prior to exiting the room, the tester told the child that "most kids do this fast." After the tester left the room, the child's behavior was monitored via video recording. After 2 minutes had passed, the research assistant reentered the room and provided the child with the correct key and informed them that the task was in fact impossible as the RA had the correct key. The tester and child then unlocked the box so that the child could play with the toy.

In the second frustration task, called the "Impossibly Perfect Green Circle" task, the tester instructed the child to draw a perfect green circle. After each attempt, the research assistant critiqued the drawing and instructed the child to draw it again. Such feedback included statements such as "that one's too pointy" "that circle has an edge", and was provided regardless of the quality of the drawing. After the first two attempts, the tester showed the child two drawings of perfect (traced) circles and said, "the last two kids that were here drew these circles. These circles are perfect. They thought this was easy." The task then continued as the child resumed drawing circles for the remainder of the 3 minutes task interval. The tester then ended

the task by telling the child one of the circles they drew looked really good and affirmed that they did a good job.

Video footage of the child's behavior during both frustration tasks was later coded by a team of research assistants using standardized protocols to assess the child's behavior in response to the frustration tasks. The footage was broken up into 60-second intervals and then further divided into 10-second epochs. Each epoch was then coded by a trained researcher to analyze the child's behavior among the following dimensions for the Clear Box task: intensity of anger expression, presence of bodily anger, peak intensity of frustration, intensity of sadness expression, presence of bodily sadness, and peak intensity of gaze aversion. For the Green Circles Task, the following dimensions were also used to code the child's behavior: intensity of anger expression, presence of bodily anger, peak intensity of protest, peak intensity of opposition, peak intensity of sadness, presence of bodily sadness, and intensity of resignations. Following initial coding of the video footage, a graduate student recorded ten percent of all videos to assess for inter-rater reliability, which was high for each task ($\alpha>0.8$). Principal Components Analyses were then conducted for the behaviors observed in each task; these analyses yielded a factor for anger and a factor for sadness for both the Clear Box and the Green Circles tasks. Those factors were then used for later hypothesis testing.

Cortisol reactivity. Salivary cortisol samples were collected when the child arrived at the lab and following the frustration tasks in order to assess the child's physiological stress response following participation in the Lab-TAB frustration tasks. The collection of the second, post-stressor cortisol sample occurred 20 minutes following the conclusion of both frustration tasks, in order to account for an approximate 15 to 20 minutes delay in peak cortisol response following exposure to a stressor (Kirschbaum & Hellhammer, 1989). Saliva samples were

collected by having the child chew on a cotton roll, covered in 0.025 grams of Kool-Aid™ to aid the child in producing saliva. Saliva samples were then extracted from the cotton roll using a syringe and were transferred to a plastic vial. Cortisol samples were frozen and stored at -20°C, until they were sent to the Endocrine Core Laboratory at the Yerkes National Primate Research Center to be assayed.

Cortisol reactivity was calculated by finding the difference between pre and post-task salivary cortisol levels. Previous literature has shown that salivary cortisol is a common and reliable method of assessing glucocorticoid levels and HPA axis activity in humans (Kirschbaum & Hellhammer, 1989). Its noninvasive method of collection also makes it an appropriate measure for cortisol in young children.

Caregiver and Teacher Ratings of Emotional Reactivity. Mothers completed the Preschool-Age Child Behavioral Checklist (CBCL) for children ages 1.5 to 5 years, providing primary caregiver reports of their child's behavioral functioning, including their emotional reactivity through an emotional reactivity subscale (Achenbach & Rescorla, 2000). Additional alternative caregiver CBCL's, completed by alternate caregivers (e.g., father, grandparent, etc.), were also sent via mail to obtain additional report of the child's behavior. The CBCL is a 100-item questionnaire that assesses a child's behavioral, emotional, and social problems through caregiver report. For each item the rater indicated on a 3 point Likert scale if the statement was "not true" (0), "somewhat or sometimes true" (1), or "very true or often true" (2) over the past 2 months. The responses were then scored on seven different syndrome subscales, including a 9 item "emotionally reactive scale" which was the subscale of interest used in analyses for the current study (Sikora, Hall, Hartley, Gerrard-Morris, & Cagle, 2008; Tan, Dedrick, & Marfo, 2007). Items in the emotionally reactive subscale include the following statements describing the

child's behavior: "disturbed by any change in routine", "nervous movements or twitching", "shows panic for no good reason", "rapid shifts between sadness and excitement", "sulks a lot", "upset by new people or situations", "whining", "worries". The CBCL has been shown to have high test-retest reliability for the emotionally reactive subscale, and good criterion related and construct validity (Achenbach & Rescorla, 2000). Internal consistency was adequate for both the maternal CBCL (α=.66) and the alternative caregiver CBCL (α=.64).

Results

Preliminary Analyses

Descriptive statistics for the study's primary measures of emotional reactivity are presented in Table 2. As a preliminary analysis, bivariate correlations were run to assess the relationships between the various emotional reactivity outcomes measures (see Table 3). Maternal and alternative caregiver emotional reactivity rating scores on the CBCL were found to be significantly correlated to one another, $r(143)=.30$, $p<.01$. Mean anger scores in the Green Circles task was also associated with mean anger scores in the Clear Box task, $r(148)=.21$, $p=.01$, and the mean PCA sadness score in the Green Circles task, $r(165)=.30$, $p<.01$; while cortisol reactivity was not significantly associated with the other measures. None of the remaining measures were significantly correlated with one another.

Identifying Trajectory Classes

Growth mixture modeling (GMM) was conducted using MPlus (Muthén & Muthén, 2012) to empirically identify trajectory classes for maternal depression over the course of gestation. Severity of maternal depression within each trimester of pregnancy was calculated by taking the area under the curve (AUC) for the BDI scores within each time period. GMM was

then conducted using the AUC BDI data across all three trimesters of pregnancy to identify trajectory classes.

GMM analyses followed the guidelines laid out in Jung and Wikrama (2008), beginning by specifying a single-class latent growth curve model. In order to determine the appropriate number of trajectory classes that best fit the data, GMM was run to create several models, varying the number of classes. The number of classes was increased for each model and tests of model fit were compared in order to determine which model provided the best fit for the data (see Table 4). Ultimately, the three class model was selected, for it had a low BIC (BIC=5584) when compared to a 1 and 2 class model, high entropy(.80), and an adequate number of women in each class to run reliable analyses. Proportions for the latent classes were all also above .01, and posterior probabilities for the likelihood of latent class membership within each of the three classes was high.

Descriptive statistics for the three trajectory classes are presented in Tables 5 and 6 (see Figure 1). Of the three trajectory classes, the first class fell within the normal range of BDI scores for depression and stayed fairly constant across all three trimesters; the second trajectory class was within the category of subthreshold depression, with a very slight decline in depressive symptomatology over the course of gestation; while the third trajectory class followed a path that fell within the clinical range of depression, although symptoms improved over pregnancy. These trajectory classes were used for hypothesis testing to investigate their relationship with the outcome variables of interest.

Assessing for Potential Confounds

Potential confounds were identified prior to hypothesis testing by assessing their association with the dependent variables using correlational analyses (see Table 7 for a full list of

potential confounds that were tested). Maternal report of the child's emotional reactivity was found to be significantly correlated with the total duration of the mother's psychiatric illness, $r(176)=.20$, $p=.01$; maternal SRI usage during pregnancy $r(176)=.15$, $p=.04$; total maternal BDI score at the time of the postnatal lab visit $r(178)=.23$, $p<.01$; and maternal tobacco usage during pregnancy, $r(171)=.24$, $p<.01$. Emotional reactivity of the child, reported by an alternative caregiver was found to be significantly associated with maternal age, $r(141)=-.22$, $p=.01$; maternal education, $r(141)=-.25$, $p<.01$; total duration of maternal psychiatric illness during the postnatal period, $r(141)=.17$, $p=.04$; and maternal antidepressant usage during pregnancy, $r(141)=-.25$, $p=.03$. Variables found to be significantly correlated with the child's cortisol reactivity in the preschool period include the time of day in which the first cortisol sample was collected, $r(150)=-.17$, $p=.04$; as well as the race of the child (if they were a minority or not), $r(120)=-.31$, $p<.01$. Potential confounds of the laboratory behavioral measures of the child's emotional reactivity during the Lab-TAB tasks were also assessed using correlational analyses. Maternal antidepressant use during pregnancy was found to be significantly associated with mean sadness score in the Clear Box task, $r(149)=.24$, $p<.01$; and child age, $r(165)=-.22$, $p=.01$, and maternal education, $r(165)=.19$, $p=.01$, were both significantly correlated with mean sadness score in the Green Circles task. All variables that were significantly associated with the outcome variables of interest were controlled for in further analyses (see Table 8).

Cortisol Reactivity

The first hypothesis was that the timing and trajectories of maternal depression during pregnancy would be associated with children's cortisol reactivity in the preschool period. Cortisol reactivity was calculated by taking the difference between the first and second cortisol samples. This difference score was then winsorized, a process in which extreme values beyond 3

standard deviations from the mean were brought in closer to the mean value to prevent outliers from creating a strong skew in the data.

The relationship between trajectories of maternal depression and cortisol reactivity was tested using Univariate Analysis of Covariance (ANCOVA), controlling for the time of day in which the first cortisol sample was collected, the race of the child (if they were a minority or not), as well as baseline cortisol levels (see Table 9). The trajectory classes were found to have a significant effect on cortisol reactivity, $F(2)=3.17$, $p=.046$, $R^2=.63$. Post hoc analyses using Fisher's LSD test, revealed that mean cortisol reactivity within the third trajectory class ($M=.025$, $SE=-.021$), in which maternal depression was the highest in pregnancy, was significantly higher than cortisol reactivity within class 1 ($M=-.031$, $SE=.014$) and class 2 ($M=-.033$, $SE=.011$) (see Table 10 and Figure 2). Sex moderator effects for cortisol reactivity were also tested for using ANCOVA; however no significant moderator effect was found.

To assess the impact of the timing of maternal depression on cortisol reactivity, regression analyses were conducted examining AUC scores for each trimester of pregnancy separately (see Table 11). The AUC for maternal BDI scores in the first trimester significantly predicted offspring cortisol reactivity, $\beta=.14$, $F(1,103)=4.79$, $p=.031$, $R^2=.017$. Regression analyses in the second and third trimester were not significant.

Laboratory Behavioral Measures of Child's Emotional Reactivity

The second hypothesis was that the timing and trajectory of maternal depression during pregnancy would be associated with laboratory observational measures of children's emotional reactivity in response to a stressor task during the preschool period. ANCOVA's were conducted to determine if anger and sadness scores in the Green Circle and Clear Box tasks (derived from principal component analyses) significantly differed between trajectory classes. In analyses for

sadness in the Clear Box task, antidepressant usage was controlled for, while in analyses for the sadness measure in the Green Circles task the child's age and maternal education were controlled. No significant effect was found between trajectory classes and any of the laboratory behavioral measures of emotional reactivity, and no sex moderator effects were observed (see Table 9).

Maternal and Alternative Caregiver Report of Child's Emotional Reactivity

The third hypothesis was that the timing and trajectory of maternal depression during pregnancy would be associated with maternal and alternate caregiver ratings of children's emotional reactivity. This outcome variable was measured using the emotional reactivity subscale of the CBCL, completed by both the mother and an alternative caregiver. ANCOVA's were conducted to determine if emotional reactivity scores on the CBCL differed among trajectory classes; controlling for total duration of maternal postpartum psychiatric illness, maternal SRI and tobacco use during pregnancy, and maternal BDI score on the day of testing for the maternal CBCL; and maternal age, maternal education, total duration of maternal postpartum psychiatric illness, and maternal antidepressant usage during pregnancy for the alternative caregiver CBCL. No significant relationship between the trajectory groups was found for either maternal or alternative caregiver emotional reactivity scores on the CBCL (see Table 9).

The sex of the child was then included into ANCOVA analyses to test for moderator effects. When sex was included in the model, there was evidence of a trend between trajectory classes and alternative caregiver report of emotional reactivity, $F(2) = 3.07$, $p = .050$, $R^2 = .14$; but no significant relationship was found with maternal report (see Table 9 and Figure 3). Regression analyses were then conducted to examine the relationship between AUC of BDI scores in each

trimester and alternative caregiver report of the child's emotional reactivity, with sex as a moderator. The interaction between child sex and maternal depression in first trimester, β=-.23, F(1,121)=6.78, p=.01, R^2= .049, and in the second trimester, β=-.19, F(1,121)=4.92, p=.03, R^2=.036, but not the third, significantly predicted alternative caregiver reports of emotional reactivity (Table 12). Post hoc analyses using Fisher's LSD test, splitting the file by sex, demonstrated that maternal depression in the first trimester, β=.29, F(1,56)=4.89, p=.03; R^2=.068, and second trimester, β=.27, F(1,56)=4.44, p=.04; R^2=.062; was significantly predictive of alternative caregiver report of emotional reactivity on the CBCL for girls, but not for boys (see Table 13).

Discussion

The purpose of the current study was to investigate the impact of the timing and trajectory of maternal depression during pregnancy on offspring emotional reactivity outcomes in the preschool period, as well as the potential role of sex in moderating this relationship. Growth Mixture Modeling was used to successfully identify three distinct trajectory classes of maternal depression over the course of pregnancy within the study sample. While each trajectory class was unique in its severity and progression of symptoms across pregnancy, all of the classes demonstrated either the maintenance or a reduction of depression severity; none of the trajectory classes indicated the worsening of depressive symptoms throughout gestation. This shared feature of all three trajectory classes can likely be attributed to the fact that study participants were recruited from the Emory Women's Mental Health Program, and were thus receiving treatment for their depression during their pregnancy. The three trajectory classes however did uniquely vary both in the mother's report of the severity of her depressive symptoms as well as the rate in which they changed over time; therefore the use of trajectory classes appears to be an

effective way to investigate and explore the complex relationship between prenatal exposure to maternal depression and child emotional reactivity outcomes in the preschool period.

Our findings suggest that the maternal psychological profile of high depression throughout gestation, even with symptom reduction in the final trimester, is associated with elevated cortisol reactivity in offspring. Analyses of the timing of maternal depression demonstrated that increased severity of symptoms in the first trimester is associated with elevated cortisol reactivity in the preschool period, whereas severity of symptoms in the second or third trimester was not. Taken together, these findings suggest that perhaps exposure to maternal depression early in gestation may uniquely shape cortisol reactivity in the postnatal period. The potential sensitivity of this window of development may be attributed to the early fetal programming of critical neurological structures that underlie emotional reactivity. The absence of a significant association between cortisol reactivity outcomes and maternal depression in the second and third trimester could also perhaps be accounted for or influenced by the reduced variability and severity of depressive symptoms reported by women in the sample during those time periods. Because the literature remains mixed on the topic of timing effects, conclusive identification of a sensitive window for fetal programming has yet to be determined (Charil et al., 2010; Davis & Sandman, 2010; Davis et al., 2007). Nevertheless, our results suggest that the first trimester may be a sensitive period for the development of the HPA axis and the influence of maternal depression.

Although our laboratory stressor task successfully elicited an elevation in cortisol that was higher among children prenatally exposed to more severe maternal depression early in pregnancy, this physiological change was not accompanied by an observable behavioral or affective change. Perhaps this discrepancy between measures can be explained by an internalized

emotional reaction that was not expressed in an overt manner, and was therefore not measurable by the Lab-TAB coding system. Emotional reactivity includes both internal and external response systems; therefore some emotional reactions, while present, may not manifest in an observable behavioral change.

Methodological issues may also account for our lack of findings concerning behavioral observations of emotional reactivity. Our laboratory task was conducted in a setting that was new to the child, with individuals with whom they were not familiar. These added pressures may have potentially dampened any observable behavioral changes that would otherwise have been displayed. The task is also relatively brief; therefore this short observational window may have prevented behavioral markers of emotional reactivity from surfacing. It could be the case that cortisol reactivity was just a more sensitive indicator of emotional reactivity, whereas our behavioral measures were not able to pick up on more discrete emotional changes that may have been occurring.

Secondary caregiver reports of emotional reactivity problems also suggest that girls prenatally exposed to maternal depression may be at an elevated risk for long term emotional reactivity deficits. This finding is consistent with the viability-vulnerability tradeoff hypothesis, which claims that fetal programming following prenatal exposure to maternal depression is sexually dimorphic, and that female offspring are more vulnerable to prenatal adversity in the long term (Sandman et al., 2013). Although there was a discrepancy between maternal and alternative caregiver report, it could be the case that the mother may be less equipped to provide an unbiased report of her child's behavior, especially as the mothers within the study come from a clinical sample. An alternative caregiver report of the child's emotional reactivity may therefore be more accurate in terms of the assessment of the child's behavior.

This sexual dimorphism in fetal programming however was not observed in cortisol reactivity and observational measures of emotional reactivity in a laboratory setting. Perhaps the secondary caregivers are more attuned to the behavior of the child, and therefore better equipped to pick up on the subtleties of emotional reactivity more typical of an internalizing child. Girls have been found to be at an elevated risk for internalizing problems, which may explain why a more subtle internalizing form of emotional reactivity may be disproportionally present among girls as opposed to in boys (Leadbeater, Kuperminc, Blatt, & Hertzog, 1999).

Study Limitations

A major limitation of the current study was the lack of diversity in the sample. The study sample consisted of mothers with high educations and from predominantly high socio-economic backgrounds. It is unclear if these findings would generalize to a sample of individuals from a lower socioeconomic status. As previously discussed, the fact that the mothers were all receiving treatment for their depression is also a unique characteristic of the sample that may limit the generalizability of the findings to mothers with untreated depression. Another limitation of the study was the small sample sizes within each trajectory class. While the number of participants within each group was acceptable, larger sample sizes would have allowed for higher power to detect group differences in child outcomes, as well as moderator effects of child sex.

Study Strengths

A major strength of the current study is its prospective longitudinal design, as it is best suited to examine long term outcomes of fetal programming and the research questions of interest. Another strength of the study is its use of multiple measures of emotional reactivity. The use of multiple measures allows for assessment of this outcome across various dimensions of emotional reactivity and facilitates better understanding of this complex construct. An additional

strength of the study is its focus on offspring outcomes in the preschool period. A great deal of the literature on maternal depression during pregnancy and child emotional reactivity has focused on outcomes during infancy (Davis et al., 2004; Kaplan et al., 2009; Zuckerman et al., 1990). Since there is a paucity of research on the impact of fetal programming on emotional reactivity outcomes in the preschool period and later childhood, the current study addresses this gap in the literature and provides evidence of the maintenance of fetal programming effects later in development.

Clinical Implications

The results of the current study have important clinical implications for both depressed mothers and their children. The study findings highlight the importance of having access to effective depression treatment during pregnancy, as depression during pregnancy may result in impairment not only in maternal wellbeing, but also in long-term child emotional reactivity outcomes. Early pregnancy was identified as a period in which the fetus may be particularly vulnerable to the effect of maternal depression; therefore preconception treatment may reduce the risk of these negative developmental outcomes in the postnatal period. While preventive preconception and prenatal treatment may be an effective intervention, prevention in the postnatal period may be necessary as well, especially as many offspring may be exposed to untreated maternal depression or depression that is resistant to treatment. The development and implementation of postnatal interventions may therefore be effective in ameliorating the effect of maternal depression on child emotional reactivity outcomes. Past studies have provided support for such interventions; as Kaplan, Evan, and Monk (2009) found that maternal factors in the postnatal period, such as sensitivity, modulated the effect of maternal psychological state on cortisol in infancy. Interventions targeting the development of adaptive maternal parenting styles

may therefore be effective in modifying fetal programming trajectories. Potential clinical interventions may also target the behavior of the child directly, with interventions that focus the development of more adaptive emotional regulation strategies.

Future Research Directions

The findings of the current study provide support for the fetal programming hypothesis, and highlight the importance of further investigating this process in future studies. Additional research is needed to assess the relationship between maternal depression during pregnancy and emotional reactivity outcomes beyond the preschool period and into adolescence and adulthood. Further work is also needed to incorporate measures of the proposed mechanisms of fetal programming into future analyses of this relationship, in order to test if the underlying pathway through which maternal psychiatric status impacts the fetus and its long term outcomes is consistent with the proposed theoretical explanations. The current study also highlights the importance of further investigating sex differences in fetal programming. The viability-vulnerability tradeoff hypothesis was partially supported, and this finding emphasizes the merit of examining this relationship at greater depth.

The study also brings up important questions concerning the methodology of assessing cortisol reactivity. For example, although baseline salivary cortisol levels at time 1 were controlled for in regression analyses, they were strongly and negatively associated with cortisol reactivity in the final models. This observation is consistent with findings throughout the literature, as the Law of Initial Values (LIV) proposes that baseline levels of physiological stress markers are often negatively associated with reactivity measurements (Burt & Obradović, 2013). Such findings highlight key concerns throughout the literature on the physiological assessment of

stress reactivity, and the topic thus necessitates additional consideration throughout future research (Burt & Obradović, 2013; Miller, Plessow, Kirschbaum, & Stalder, 2013).

Another topic which merits further investigation in future research is the impact of timing of exposure to maternal depression on fetal programming and emotional reactivity outcomes. As has been previously discussed, the identification of a sensitive window has varied across studies; therefore additional research would be needed to account for the variance in findings across the literature. Future studies may also benefit from the study of additional trajectory classes of maternal depression, including profiles of maternal depression in which symptoms worsen over gestation. Greater variability in trajectory classes as well as increased diversity in the study sample may improve the generalizability of results.

Conclusions

Prenatal exposure to maternal depression has previously been linked to various markers of emotional reactivity in children, and the current study expanded upon this area of research, employing multiple measures of emotional reactivity in the context of a longitudinal prospective design. In addition to taking a more comprehensive approach to measuring the construct of emotional reactivity, this study added to the literature by investigating the degree to which both the timing and trajectory of maternal depression in pregnancy were able to predict emotional reactivity outcomes in the preschool period. Findings indicated that both the timing and trajectory of maternal depression during pregnancy were significantly related to cortisol reactivity in response to a laboratory stressor among preschool age children. Timing of maternal depression was also found to be significantly related to alternative caregiver report of the child's emotional reactivity among girls but not boys. Maternal depression early in pregnancy was associated with emotional reactivity outcomes, while maternal depression in later gestation had

no significant effect on these long term outcomes. Study findings may have important clinical implications for both depressed mothers and their children, as they identify children who may be at risk for later emotional reactivity problems and highlight the importance of access to effective preventive depression treatment and early targeted interventions.

References

Achenbach, T. M., & Rescorla, L. A. (2000). *Manual for the ASEBA Preschool Forms & Profiles*. Burlington, VT: University of Vermont Research Center for Children, Youth, & Families.

Beck, A. T., Steer, R. A., & Brown, G. K. (1997). Beck Depression Inventory: Second Edition. San Antonio: The Psychological Corporation.

Beck, A. T., Steer, R. A., & Garbin, M. G. (1988). Psychometric properties of the Beck Depression Inventory: Twenty-five years of evaluation. *Clinical Psychology Review, 8*(1), 77–100. http://doi.org/10.1016/0272-7358(88)90050-5

Bradley, B., DeFife, J. A., Guarnaccia, C., Phifer, J., Fani, N., Ressler, K. J., & Westen, D. (2011). Emotion dysregulation and negative affect: Association with psychiatric symptoms. *The Journal of Clinical Psychiatry*, *72*(5), 685–91. http://doi.org/10.4088/JCP.10m06409blu

Burt, K. B., & Obradović, J. (2013). The construct of psychophysiological reactivity: Statistical and psychometric issues. *Developmental Review, 33*(1), 29-57. http://doi.org/10.1016/j.dr.2012.10.002

Buss, C., Davis, E. P., Shahbaba, B., Pruessner, J. C., Head, K., & Sandman, C. a. (2012). Maternal cortisol over the course of pregnancy and subsequent child amygdala and hippocampus volumes and affective problems. *Proceedings of the National Academy of Sciences of the United States of America, 109*(20), 1312–1319. http://doi.org/10.1073/pnas.1201295109

Calkins, B. S. D., Gill, K. L., Johnson, M. C., & Smith, C. L. (1999). Emotional reactivity and emotional regulation strategies as predictors of social behavior with peers during toddlerhood. *Social Development*, *8*, 310–341. http://doi.org/10.1111/1467-9507.00098

Charil, A., Laplante, D. P., Vaillancourt, C., & King, S. (2010). Prenatal stress and brain development. *Brain Research Reviews*, *65*(1), 56–79. http://doi.org/10.1016/j.brainresrev.2010.06.002

Cole, P. M., Martin, S. E., & Dennis, T. A. (2014). Emotion regulation as a scientific construct : Methodological challenges and directions for child development research. *Child Development, 75*(2), 317–333. http://doi.org/10.1111/j.1467-8624.2004.00673.x

Davis, E. P., Glynn, L. M., Schetter, C. D., Hobel, C., Chicz-Demet, A., & Sandman, C. A. (2007). Prenatal exposure to maternal depression and cortisol influences infant temperament. *Journal of the American Academy of Child and Adolescent Psychiatry*, *46*(6), 737–746. http://doi.org/10.1097/chi.0b013e318047b775

Davis, E. P., Glynn, L. M., Waffarn, F., & Sandman, C. a. (2011). Prenatal maternal stress programs infant stress regulation. *Journal of Child Psychology and Psychiatry*, *52*(2), 119–129. http://doi.org/10.1111/j.1469-7610.2010.02314.x

Davis, E. P., & Sandman, C. (2010). The timing of prenatal exposure to maternal cortisol and psychosocial stress is associated with human infant cognitive development. *Child Development*, *81*(1), 131–148. http://doi.org/10.1111/j.1467-8624.2009.01385.x.The

Davis, E. P., Snidman, N., Wadhwa, P. D., Glynn, L. M., Schetter, C. D., & Sandman, C. A. (2004). Prenatal maternal anxiety and depression predict negative behavioral reactivity in infancy. *Infancy*, *6*(3), 319–331. http://doi.org/10.1207/s15327078in0603_1

Deave, T., Heron, J., Evans, J., & Emond, A. (2008). The impact of maternal depression in pregnancy on early child development. *BJOG: An International Journal of Obstetrics and Gynaecology*, *115*(8), 1043–1051. http://doi.org/10.1111/j.1471-0528.2008.01752.x

Eisenberg, N., & Fabes, R. A. (1992). Emotion, regulation, and the development of social competence. *Review of Personality and Social Psychology*, *14*, 119-150. http://doi.org/ 10.1146/annurev.psych.51.1.665

Field, T., Diego, M., Dieter, J., Hernandez-Reif, M., Schanberg, S., Kuhn, C., … Bendell, D. (2004). Prenatal depression effects on the fetus and the newborn. *Infant Behavior and Development*, *27*(2), 216–229. http://doi.org/10.1016/j.infbeh.2003.09.010

Field, T., Diego, M., & Hernandez-Reif, M. (2006). Prenatal depression effects on the fetus and newborn: A review. *Infant Behavior & Development*, *29*(3), 445–455. http://doi.org/10.1016/j.infbeh.2006.03.003

Glover, V., O'Connor, T. G., & O'Donnell, K. (2010). Prenatal stress and the programming of the HPA axis. *Neuroscience and Biobehavioral Reviews*, *35*(1), 17–22. http://doi.org/10.1016/j.neubiorev.2009.11.008

Godfrey, K. M., & Barker, D. J. (2007). Fetal programming and adult health. *Public Health Nutrition*, *4*(2B), 611–624. http://doi.org/10.1079/PHN2001145

Goldsmith, H. H., Reilly, J., Lemery, K. S., Longley, S., & Prescott, A. (1999). The laboratory temperament assessment: Preschool version. *Unpublished Measure*.

Grote, N. K., Bridge, J. A., & Gavin, A. R. (2014). A Meta-analysis of depression during pregnancy and the risk of preterm birth, low birth weight, and intrauterine growth restriction. *Archives of General Psyciatry*, *67*(10), 1012–1024. doi:10.1001/archgenpsychiatry.2010.111

Jung, T., & Wickrama, K. A. S. (2008). An introduction to latent class growth analysis and growth mixture modeling. *Social and Personality Psychology Compass*, *2*(1), 302–317. http://doi.org/10.1111/j.1751-9004.2007.00054.x

Kaplan, L. A., Evans, L., & Monk, C. (2009). Caregiving on infant biobehavioral regulation: Can prenatal programming be modified? *Early Human Development*, *84*(4), 249–256. http://doi.org/10.1016/j.earlhumdev.2007.06.004.Effects

Kapoor, A., Dunn, E., Kostaki, A., Andrews, M. H., & Matthews, S. G. (2006). Fetal programming of hypothalamo-pituitary-adrenal function: Prenatal stress and glucocorticoids. *The Journal of Physiology*, *572*(1), 31–44. http://doi.org/10.1113/jphysiol.2006.105254

Kinsella, M., & Monk, C. (2009). Impact of maternal stress, depression & anxiety on fetal neurobehavioral development. *Clinical Obstetrics and Gynecology*, *52*(3), 1–15. http://doi.org/10.1097/GRF.0b013e3181b52df1.Impact

Kirschbaum, C., & Hellhammer, D. H. (1989). Salivary cortisol in psychobiological research: An overview. *Neuropsychobiology*, *22*, 150–169. http://doi.org/10.1159/000118611

Kofman, O. (2002). The role of prenatal stress in the etiology of developmental behavioural disorders. *Neuroscience and Biobehavioral Reviews*, *26*(4), 457–470.

Leadbeater, B. J., Kuperminc, G. P., Blatt, S. J., & Hertzog, C. (1999). A multivariate model of gender differences in adolescents' internalizing and externalizing problems. *Developmental Psychology*, *35*(5), 1268–1282. http://doi.org/10.1037//0012-1649.35.5.1268

Lundy, B. L., Jones, N. A., Field, T., Nearing, G., Davalos, M., Pietro, P. a., … Kuhn, C. (1999). Prenatal depression effects on neonates. *Infant Behavior and Development*, *22*(1), 119–129. http://doi.org/10.1016/S0163-6383(99)80009-5

Luoma, I., Tamminen, T., Laippala, P., Puura, K., Salmelin, R., & Almqvist, F. (1998). Longitudinal study of maternal depressive symptoms and child well-being. *Journal of the American Academy of Child & Adolescent Psychiatry*, *40*(12), 1367–1374.

Marcus, S. M. (2009). Depression during pregnancy: Rates , risks and consequences. *The Candadian Journal of Clinical Pharmacology*, *16*(1), 15–22.

Marcus, S. M., Flynn, H. A., Blow, F. C., & Barry, K. L. (2003). Depressive symptoms among pregnant women screened in obstetrics settings. *Journal of Women's Health*, *12*(4), 373-380. http://doi.org/10.1089/154099903765448880

Miller, R., Plessow, F., Kirschbaum, C., & Stalder, T. (2013). Classification criteria for distinguishing cortisol responders from nonresponders to psychosocial stress: Evaluation of salivary cortisol pulse detection in panel designs. *Psychosomatic Medicine*, *75*(9), 832-840. http://doi.org/10.1097/PSY.0000000000000002

Mulder, E. J. H., Robles de Medina, P. G., Huizink, a C., Van den Bergh, B. R. H., Buitelaar, J. K., & Visser, G. H. a. (2002). Prenatal maternal stress: Effects on pregnancy and the (unborn) child. *Early Human Development*, *70*(1-2), 3–14.

Pariante, C. M., & Lightman, S. L. (2008). The HPA axis in major depression: classical theories and new developments. *Trends in Neurosciences*, *31*(9), 464–468. http://doi.org/10.1016/j.tins.2008.06.006

Ponder, K. L., Salisbury, A., Ph, D., Mcgonnigal, B., Laliberte, A., Lester, B., & Padbury, J. F. (2011). Maternal depression and anxiety are associated with altered gene expression in the human placenta without modification by antidepressant use: Implications for fetal programming. *Developmental Psychobiology*, *53*(7), 711–723. http://doi.org/10.1002/dev.20549.Maternal

Sandman, C. A., Glynn, L. M., & Davis, E. P. (2013). Is there a viability-vulnerability tradeoff? Sex differences in fetal programming. *Journal of Psychosomatic Research*, *75*(4), 327–335. http://doi.org/10.1016/j.jpsychores.2013.07.009.IS

Sikora, D. M., Hall, T. A., Hartley, S. L., Gerrard-Morris, A. E., & Cagle, S. (2008). Does parent report of behavior differ across ADOS-G classifications: Analysis of scores from the CBCL and GARS. *Journal of Autism and Developmental Disorders*, *38*(3), 440–448. http://doi.org/10.1007/s10803-007-0407-z

Sohr-Preston, S. L., & Scaramella, L. V. (2006). Implications of timing of maternal depressive symptoms for early cognitive and language development. *Clinical Child and Family Psychology Review*, *9*(1), 65–83. http://doi.org/10.1007/s10567-006-0004-2

Tan, T. X., Dedrick, R. F., & Marfo, K. (2007). Factor structure and clinical implications of Child Behavior Checklist/1.5–5 ratings in a sample of girls adopted from China. *Journal of Pediatric Psychology*, *32*(7), 807–818.

Tottenham, N., & Sheridan, M. a. (2009). A review of adversity, the amygdala and the hippocampus: A consideration of developmental timing. *Frontiers in Human Neuroscience*, *3*(68), 1-18. http://doi.org/10.3389/neuro.09.068.2009

Zuckerman, B., Bauchner, H., Parker, S., & Cabral, H. (1990). Maternal depressive symptoms during pregnancy and newborn irritability. *Developmental and Behavioral Pediatrics*, *11*(4), 190–194.

Table 1

Sample Demographic Information

Variable		*Mean (SD)*	
Child Age		45.2 months (11.2)	
Mother Age		37.3 years (2.7)	
		% (N)	%(N)
		Mother	Child
Sex			
	Male		51(92)
	Female		48(86)
Race/Ethnicity			
	African American	7.2 (13)	6.7 (12)
	Asian	2.2 (4)	0 (0)
	Biracial	.6 (1)	9.4 (17)
	Caucasian	85.0 (153)	80.0 (144)
	Hispanic	2.8 (5)	1.7 (3)
	Missing	2.2 (4)	2.2 (4)
Marital Status			
	Married or living with someone as if married	83.3 (150)	
	Divorced or annulled	6.1 (11)	
	Separated	2.8 (5)	
	Never married	5.6 (10)	
	Missing	2.2 (4)	
Mother's Highest Level of Education			
	Graduated High School or GED	1.7 (3)	
	Part College	11.7 (21)	
	Graduated 2-year College	7.2 (13)	
	Graduated 4-year College	33.3 (60)	
	Part Graduate/Professional School	3.9 (7)	
	Completed Graduate/Professional School	40.0 (72)	

Table 2

Descriptive Statistics of Study Measures

Measure	*Mean*	*SD*	Max	Min	Range
Alternative Caregiver CBCL	1.88	2.03	9.00	.00	9.00
Maternal CBCL	2.37	2.14	10.00	.00	10.00
Cortisol Reactivity	-.02	.14	.39	-.55	.94
Green Circles Sadness	.00	.97	4.12	-.94	5.06
Green Circles Anger	.04	1.02	5.38	-.94	5.06
Clear Box Sadness	.03	1.04	5.38	-.83	6.22
Clear Box Anger	.05	1.04	4.82	-1.22	6.04
AUC BDI in the First Trimester	166.21	136.18	672.00	.00	672.00
AUC BDI in the Second Trimester	138.50	123.63	659.55	.00	659.55
AUC BDI in the Third Trimester	110.49	89.31	508.50	.00	508.50

Table 3

Bivariate Correlations between Measures of Emotional Reactivity

	1	2	3	4	5	6	7
1. Alternative Caregiver CBCL	--	.294**	.141	.107	.108	-.043	.021
2. Maternal CBCL	--	--	-.085	-.119	-.045	-.058	.005
3. Cortisol[a] Reactivity	--	--	--	.004	.147	.010	-.041
4. Green Circles Sadness[b]	--	--	--	--	.292**	.001	-.032
5. Green Circles Anger[b]	--	--	--	--	--	.075	.211*
6. Clear Box Sadness[b]	--	--	--	--	--	--	.132
7. Clear Box Anger[b]	--	--	--	--	--	--	--

Note. Baseline cortisol level was controlled for in correlation analyses. [a] Cortisol reactivity values are winsorized. [b] GC and CB scores are derived from Principle Components Analyses. *p<.05. **p<.01.

Table 4

Model Fit Information for GMM of Maternal Depression

Model	AIC	BIC	Entropy
1-class model	5698.732	5723.571	--
2-class model	5560.523	5604.006	.779
3-class model	5509.567	5584.118	.804
4-class model	5484.229	5564.983	.828

Note. AIC = Akaike Information Criterion. BIC = Bayesian Information Criterion.

Table 5

Trajectory Class Descriptive Statistics

Class	Descriptors	*N (%)*	Male	Female
1	Very Low Depression Score	59 (35.76)	29 (49.2%)	28 (47.5%)
2	Subthreshold Depression, Slight Decline	77 (46.67)	33 (42.9%)	44 (57.1%)
3	High Depression within Clinical Range	29 (17.57)	18 (62.1%)	11 (37.9%)

Table 6

Descriptive Statistics of BDI Scores within Trajectory Classes

Class	*Mean (SD)*		
	BDI Score during Pregnancy	Highest BDI Score during Pregnancy	BDI Score at Delivery
1	3.56 (1.73)	6.28 (3.31)	8.72 (7.95)
2	10.35 (3.74)	16.09 (4.67)	9.95 (5.02)
3	21.96 (9.41)	32.10 (9.09)	16.58 (13.15)

Note. BDI score of 1-10 is considered normal, 11-16 is mild mood disturbance, 17-20 is borderline clinical depression, 21-30 is moderate depression, 31-40 is severe depression and over 40 is extreme depression.

Table 7

Bivariate Correlations between Potential Covariates and Outcome Measures

Measure	Pearson's *r*	*p*
Maternal CBCL		
Total Duration of Maternal Psychiatric Illness (in months) Across All Diagnoses	.20	.01
Maternal BDI Score on the Day of Testing	.23	<.01
Prenatal SRI Use	.15	.04
Prenatal Tobacco Use	.24	<.01
Alternative Caregiver CBCL		
Total Duration of Maternal Psychiatric Illness (in months) Across All Diagnoses	.17	.04
Prenatal Antidepressant Use	.18	.03
Maternal Age	-.22	.01
Maternal Education	-.25	<.01
Cortisol Reactivity[a]		
Race/Ethnicity of the Child as Binary (minority yes/no)	-.31	<.01
Lab Cortisol Collection Time	-.17	.04
GC Sadness[b]		
Child's Age	-.22	.01
Maternal Education	.19	.01
CB Sadness[b]		
Prenatal Antidepressant Use	.24	<.01
Prenatal SRI Use	.17	.04

Note. Only the variables that were significantly correlated with the outcome variables at the $p<.05$ level of significance are reported. No significant correlations were found for GC Anger and CB Sadness. [a] Cortisol reactivity values are winsorized. [b] GC and CB scores are derived from Principle Components Analyses. All significant covariates were controlled for in further analyses.

Table 8

Variables Tested as Potential Covariates

Outcomes Variable	Variables tested as potential covariates
Cortisol Reactivity	Child's age, child's sex, race/ethnicity of the child as a binary (minority yes/no), number of children, maternal education, total Hollingshead rating, count of delivery complications; if the child took stimulants, antidepressants, anxiolytics, antihistamines, steroids, allergy medication, and cold medication on the day of testing or 3 days prior; if the child was sick with a cold on the day of testing or 3 days prior; cortisol collection time; total duration of maternal psychiatric illness (in months) across all diagnoses; prenatal exposure to antiepileptics, antipsychotics, antidepressants, SRI's, alcohol, and tobacco; if the child had caffeine or candy in the last 24 hours; child time since waking before cortisol collection; time since child brushed teeth and ate before cortisol collection; if the child drank before cortisol collection; and if the child exercised or had a loose tooth or mouth sore on the day of testing.
Laboratory Observational Measures of Emotional Reactivity (Green Circles and Clear Box Sadness and Anger)	Child's age, child's sex, race/ethnicity of the child as binary (minority yes/no), number of children, maternal education, total Hollingshead rating, count of delivery complications, total duration of maternal psychiatric illness (in months) across all diagnoses, prenatal exposure to antiepileptics, antipsychotics, antidepressants, SRI's, alcohol, and tobacco.
Maternal and Alternative Caregiver Report of Emotional Reactivity	Child's age, mother's age, child's sex, race/ethnicity of the child as binary (minority yes/no), number of children, maternal education, total Hollingshead rating, count of delivery complications, total duration of maternal psychiatric illness (in months) across all diagnoses, prenatal exposure to antiepileptics, antipsychotics, antidepressants, SRI's, alcohol, and tobacco.

Table 9

Summary ANCOVA Analyses for Trajectory Classes and Emotional Reactivity Outcomes

Variable	*df*	R^2	*F*	*p*
Alternative Caregiver CBCL				
Class	2	.088	.650	.524
Class*Sex	2	.137	3.072	.050
Maternal CBCL				
Class	2	.070	.022	.978
Class*Sex	2	.167	.929	.397
Cortisol Reactivity[a]				
Class	2	.634	3.172	.046*
Class*Sex	2	.640	.048	.953
Clear Box Sadness[b]				
Class	2	.091	2.389	.096
Class*Sex	2	.981	.091	.981
Clear Box Anger[b]				
Class	2	.012	.788	.457
Class*Sex	2	.025	.599	.551
Green Circles Sadness[b]				
Class	2	.088	.043	.957
Class*Sex	2	.101	.605	.548
Green Circles Anger[b]				
Class	2	.018	1.360	.260
Class*Sex	2	.036	.147	.864

Note. [a] Cortisol reactivity values are winsorized. [b] GC and CB scores are derived from Principle Components Analyses.
*$p<.05$. **$p\leq.01$.

Table 10

Descriptive Statistics and Pairwise Comparisons of Trajectory Classes and Cortisol Reactivity using Fisher's LSD

Descriptive Statistics		Pairwise Comparisons		
Trajectory Class	*Mean (SE)*	Trajectory Classes (I-J)		*Mean Difference (SE)*
Class 1	-.031 (.014)	Class	1 –2	.002 (.018)
Class 2	-.033 (.011)		1 – 3	-.056 (.025)*
Class 3	.025 (.021)		2 – 3	-.057 (.024)*

Note. Mean Difference = I – J. Cortisol reactivity values are windsorized.
*p<.05. **p≤.01.

Table 11

Summary of Regression Analyses for Variables Predicting Cortisol Reactivity

	Trimester 1 Model		Trimester 2 Model		Trimester 3 Model	
Variable	*β*	*p*	*β*	*p*	*β*	*p*
Constant						
Cortisol Level at Time 1	-.82	.00**	-.81	.00**	-.79	.00**
Cortisol Collection Time 1	-.01	.87	-.01	.91	.01	.90
Race/ethnicity of the child as binary (minority yes/no)	-.01	.93	.00	.98	.01	.86
AUC BDI Trim. 1	.141	.031*				
AUC BDI Trim. 2			.113	.081		
AUC BDI Trim. 3					.01	.87
R^2 change	.017		.011		.000	
F change	4.787*		3.109		.029	

Note. Cortisol values are winsorized.
*$p < .05$. **$p<.01$.

Table 12

Summary of Regression Analyses for Variables Predicting Alternative Caregiver CBCL Emotional Reactivity Scores

	Trimester 1 Model		Trimester 2 Model		Trimester 3 Model	
Variable	*β*	*p*	*β*	*p*	*β*	*p*
Constant						
Maternal Age	-.17	.07	-.17	.08	-.17	.09
Maternal Education	-.08	.41	-.09	.32	-.11	.24
Total Duration of Maternal Psychiatric Illness	.11	.24	.09	.38	.08	.41
Prenatal Antidepressant Exposure	.12	.17	.13	.15	.14	.13
Sex	-.05	.54	-.05	.59	-.07	.44
AUC BDI 1	.01	.93				
AUC BDI 2						
AUC BDI 3			.06	.53	.07	.44
Sex*AUC BDI 1	-.23	.01*				
Sex*AUC BDI 2						
Sex*AUC BDI 3			-.19	.03*	-.09	.32
R^2 change	.049		.036		.008	
F change	6.783*		4.922*		1.009	

Note. Sex and AUC BDI levels were centered.
*$p < .05$. **$p<.01$.

Table 13

Summary of Regression Analyses for Variables Predicting Alternative Caregiver CBCL Emotional Reactivity Scores Split by the Sex of the Child

	Trimester 1 Model		Trimester 2 Model		Trimester 3 Model	
Variable	*β*	*p*	*β*	*p*	*β*	*p*
Females						
Constant						
Maternal Age	-.16	.21	-.17	.19	-.19	.15
Maternal Education	.02	.91	-.01	.94	-.04	.79
Total Duration of Maternal Psychiatric Illness	.29	.03*	.28	.03*	.32	.02
Prenatal Antidepressant Exposure	-.11	.40	-.10	.44	-.09	.51
AUC BDI 1	.29	.03*				
AUC BDI 2			.27*	.04*		
AUC BDI 3					.16	.21
R^2 change	.068		.062		.024	
F change	4.890*		4.437*		1.638	
Males						
Constant						
Maternal Age	-.16	.24	-.15	.26	-.14	.28
Maternal Education	.22	.10	-.22	.10	-.21	.11
Total Duration of Maternal Psychiatric Illness	-.14	.30	-.18	.19	-.21	.11
Prenatal Antidepressant Exposure	.42	.00	.43	.00	.43	.00
AUC BDI 1	-.09	.48				
AUC BDI 2			-.03	.85		
AUC BDI 3					.05	.69
R^2 change	.007		.001		.002	
F change	.514		.039		.162	

Note. AUC BDI levels were centered.
*$p < .05$. **$p<.01$.

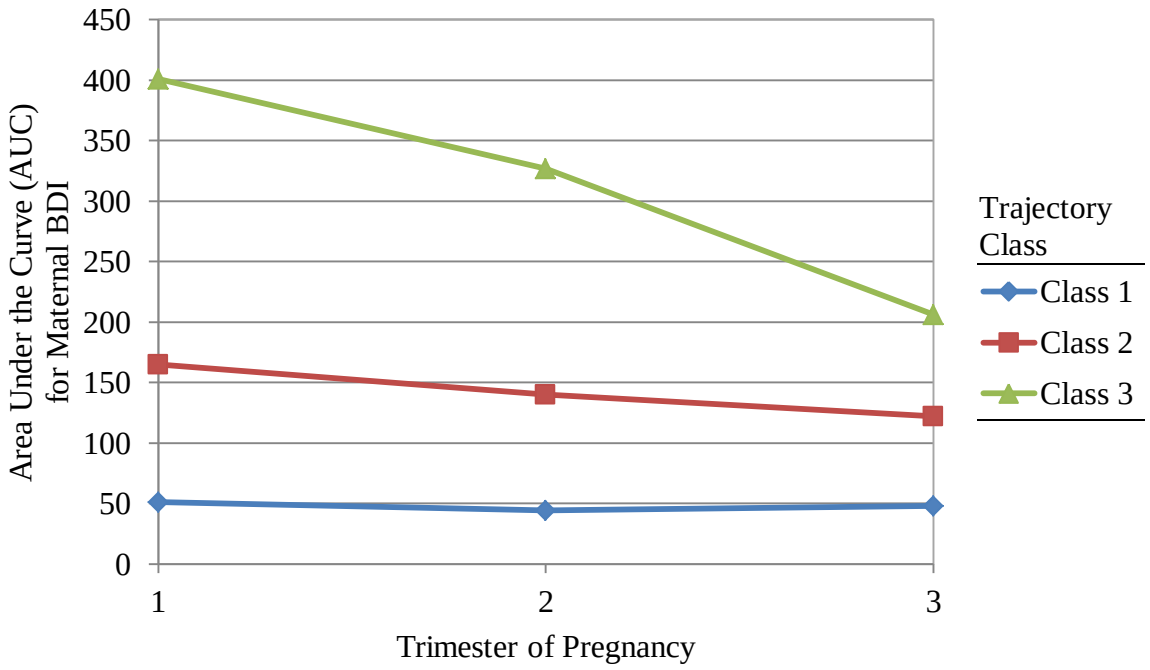

Figure 1. Average trajectories for maternal depression during pregnancy obtained from Growth Mixture Modeling.

Figure 2. Mean cortisol reactivity split by trajectory classes.

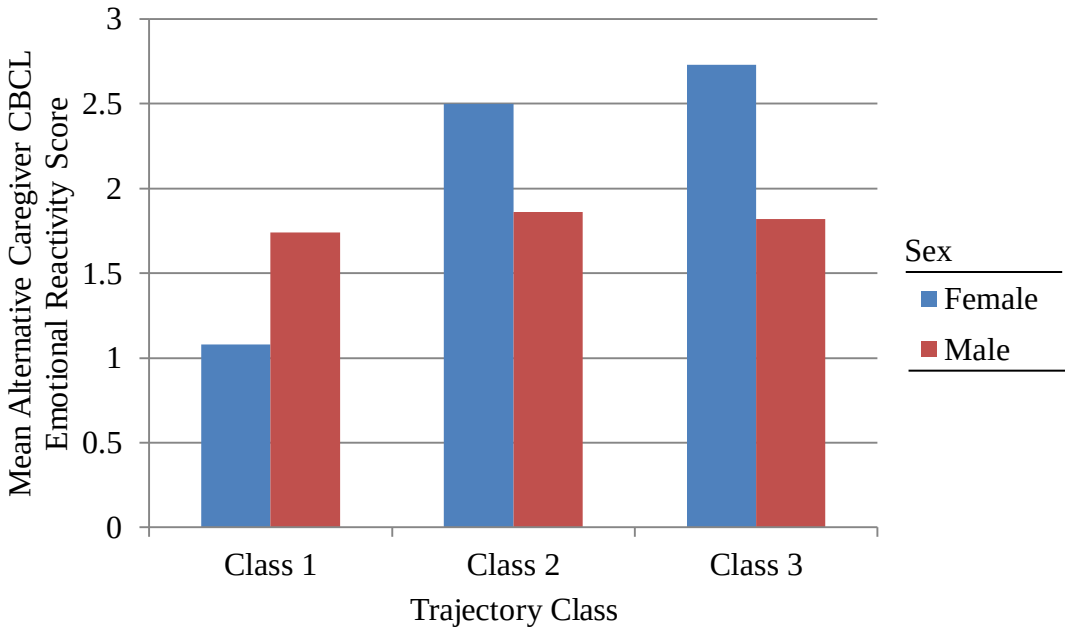

Figure 3. Mean alternative caregiver CBCL emotional reactivity rating split by class membership and sex of the child.