Maternal prenatal stress predicts internalizing behavior in healthy 10-year-old children

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Master Science Thesis

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July 10, 2018
Abstract

Prenatal maternal stress (PNMS) has been related to a number of adverse emotional and behavioral outcomes in early life, some of which persist throughout childhood and into adulthood. However, the majority of studies that have investigated long-term child outcomes have not considered the potential differences between psychosocial PNMS (i.e. experienced stress) and physiological PNMS (i.e. circadian cortisol) or examined additional factors within this relationship, such as cortisol activity, parental care, and the child’s sex. The aim of this study was to investigate the association between these two types of PNMS and 10-year-old child internalizing and externalizing behavior within the prospective BIBO study (n=193), as mediated by toddler cortisol and moderated by maternal sensitivity and the child’s sex. Structural equation modeling analyses revealed that PNMS predicted child internalizing behavior. While physiological PNMS significantly predicted self-reported internalizing behavior, psychosocial PNMS significantly predicted maternal-reported internalizing behavior. Conclusions about the potential mediation and moderation of these relationships could not be made based on the current analyses. These results suggest that future research should approach psychosocial and physiological PNMS as independent predictors of child outcomes and consider the perspectives of multiple reporters for a comprehensive assessment of child behavior.
Maternal prenatal stress predicts internalizing behavior in healthy 10-year-old children

There is a growing body of research indicating that early life experiences can profoundly impact emotional and behavioral development across the lifespan. The prenatal period in particular has been identified as a critical time in which the developing child is vulnerable to maternal stress, independent of genetics and the postnatal environment (Rice et al., 2010; Baibazarova et al., 2013). Prenatal maternal stress (PNMS) reaches clinically-recognized levels in approximately one in six expecting mothers (Fairbrother, Janssen, Antony, Tucker, & Young, 2016; Kang et al., 2016) and has been estimated to explain as much as 15% of subsequent child behavior problems (Talge, Neal, & Glover, 2007). Previous literature has established the prospective relationship between PNMS and many long-term child emotional and behavioral outcomes, but the majority of these studies have not considered additional explanations for adverse child outcomes or potential resilience factors in conjunction with PNMS. Moreover, most prior research has not concurrently examined psychosocial and physiological stress in the context of child development. Therefore, the current research intends to prospectively investigate the association between prenatal maternal psychosocial and physiological stress and 10-year-old child internalizing and externalizing behavior, as mediated by toddler cortisol and moderated by maternal sensitivity and the child’s sex (Figure 1). Note that an additional novel aspect of this study is the inclusion of both child- and maternal-reports of child behavioral outcomes.
Late pregnancy has been predominantly established as a time in which PNMS has long-term associations with the child’s development (Van den Bergh et al., 2017). Prior research has provided a considerable amount of evidence for the link between PNMS in late pregnancy and child behavioral outcomes during toddlerhood (Sharp, Hill, Hellier, & Pickles, 2015), early childhood (Loomans et al., 2011; Pickles, Sharp, Hellier, & Hill, 2017), and mid-childhood (Davis & Sandman, 2012; Braithwaite et al., 2013; O’Donnell, Glover, Holbrook, & O’Connor, 2014). Some studies have found that these associations extend to late childhood and early adolescence as well (Braithwaite et al., 2013; O’Donnell et al., 2014; Isaksson, Lindblad, Valladares, & Högberg, 2015). The persistent relationship between PNMS and child behavior has often been considered to be indicative of dysfunctional child self-regulation strategies.

These unfavorable self-regulation and behavioral outcomes could arise via a number of biopsychosocial mechanisms following both psychosocial and physiological PNMS. Psychosocial PNMS, or the mother’s own experience of stress, is typically assessed via a
questionnaire or structured interview while physiological PNMS is measured through a biological marker, such as cortisol. The vast majority of previous studies have examined only psychosocial or physiological PNMS as a predictor of later child behavior rather than including both measurements. Importantly, these two types of PNMS are often not correlated with one another (Zijlmans, Riksen-Walraven, & de Weerth, 2015), yet heightened maternal cortisol remains the proposed mechanism behind the relationship between PNMS and child behavior in many studies.

Perhaps most frequently, the association between both types of PNMS and increased vulnerability to later emotional and behavioral problems has been attributed to interference with the development of the Hypothalamic-Pituitary-Adrenal (HPA) axis. The HPA-axis responds to stress by ultimately secreting glucocorticoid hormones, most notably cortisol in humans, into the bloodstream (Gunnar & Quevedo, 2007). Since cortisol can pass through the placenta’s semi-permeable membrane and cross the fetus’s blood-brain barrier, maternal cortisol is capable of potentially influencing the fetus’s early neuroendocrinological development (Gunnar & Quevedo, 2007). Previous studies have indeed found a correlation between psychosocial PNMS and HPA activity during the first year of life (Tollenaar, Beijers, Jansen, Riksen-Walraven, & de Weerth, 2011) as well as cortisol circadian rhythm during the first six years of life (Simons, Beijers, Cillessen, & de Weerth, 2015). Furthermore, dysregulation of the HPA-axis, often exhibited through altered circadian cortisol levels, has been widely associated with emotional and behavioral problems in childhood and adolescence (Gunnar & Vazquez, 2006; van Goozen, Fairchild, Snoek, & Harold, 2007; Shirtcliff & Essex, 2008). However, little is known about whether HPA activity, as indicated by circadian cortisol, mediates the relationship between PNMS and behavioral outcomes in late childhood.

There is also research which suggests that positive parent-infant relationships mitigate the negative behavioral outcomes associated with prenatal stress. Loman and Gunnar (2010)
have proposed an early-life stress model which hypothesizes that caregiving affects stress-response systems, such as the HPA-axis, in a way that influences the development of later emotional regulation. Consistent with this model, parent caregiving behavior of higher quality (e.g. greater sensitivity and responsiveness) has been associated with decreased HPA-axis reactivity (Gunnar & Quevedo, 2007), a lack of cortisol elevation during crying behavior (Gunnar & Quevedo, 2007), better self-regulation in infants (Frick et al., 2017) and toddlers (Ispa, Su-Russell, Palermo, & Carlo, 2017), and resilience to adverse environments (Agnafors et al., 2017). Moreover, postnatal maternal sensitivity was found to be correlated with cortisol reactivity and partially explain the relationship between prenatal maternal anxiety and infant cortisol reactivity (Grant et al., 2009). Therefore, postnatal parental sensitivity throughout the first year might buffer the relationship between PNMS and the development of child behavioral problems.

In addition to the potential buffering by maternal sensitivity, the child’s sex could also moderate the association between PNMS and child development. During pregnancy, there appears to be a sex difference in the fetus’s ability to biologically buffer increased physiological PNMS (i.e. maternal cortisol)—exemplified by the more efficient adaptation to prenatal increases in glucocorticoids in females than males—and the infant’s subsequent HPA-axis development in which males are more vulnerable (Sandman, Glynn, & Davis, 2013; Stinson et al., 2015; Stroud et al., 2016; Rosenfeld, 2015). Consistent with the biological accounts of greater male vulnerability to PNMS early in life, prospective research examining child behavioral outcomes has found that psychosocial PNMS is more strongly associated with hyperactivity and inattention in boys than girls at 4 years of age (O’Connor, Heron, Golding, Beveridge, & Glover, 2002; Van den Bergh & Marcoen, 2004).

However, aside from the prenatal and early postnatal periods, females seem to be either equally or more at risk than males for adverse emotional and behavioral development.
PRENATAL STRESS AND CHILD BEHAVIOR

following PNMS. The sex differences in hyperactivity and inattention as predicted by PNMS which were present at 4 years of age were not found at 7 years of age (O’Connor, Heron, Golding, & Glover, 2003). Additionally, other studies have concluded that girls are more susceptible than boys to adverse emotional outcomes in mid- to late-childhood following physiological PNMS, particularly in terms of internalizing behavior and affective disorders (Davis & Sandman, 2012; Buss et al., 2012 as reported in Sandman, Glynn, & Davis, 2013). Fetal programming of sex differences in psychopathology and disease risk in general has previously been proposed (Goldstein, Handa, & Tobet, 2013; Sandman, Glynn, & Davis, 2013), but since their developmental pathways remain far from being disentangled, current research findings on this topic are ambiguous and conflicting. Through the investigation of sex as a moderator of child outcomes associated with PNMS, its role in the course of emotional and behavioral development might be better understood.

The current research aimed to prospectively examine the relationship between PNMS and child behavioral outcomes from a biopsychosocial perspective, accounting for the potentially mediating role of circadian cortisol and buffering influence of maternal sensitivity. Additionally, this study extends the limited previous research on sex differences as they relate to PNMS by including sex of the child as a moderator. The four hypotheses for the current study are based on previous research and are as follows:

1. Psychosocial and physiological PNMS will predict internalizing and externalizing behavior at 10 years of age.
2. Toddler stress physiology will mediate the relationship between PNMS and child behavioral outcomes.
3. High levels of maternal sensitivity between birth and 1 year of age will act as a buffer, moderating the relationship between PNMS and child behavioral outcomes, and between PNMS and toddler stress physiology.
4. The child’s sex will moderate the links between PNMS and child behavioral outcomes, PNMS and toddler stress physiology, and toddler stress physiology and child behavioral outcomes.

**Method**

**Participants**

A total of 193 Dutch mother-child pairs participated in the Basale Invloeden op de Baby Ontwikkeling (BIBO) study. This ongoing study began when the mothers were in late pregnancy and has followed the children through 10 years of age. The study was approved by the Social Science Ethical Committee at Radboud University and participants gave informed consent prior to data collection. Only healthy mothers who had a singleton pregnancy without any complications were eligible to participate. Furthermore, mothers who were mentally or physically ill, used drugs during pregnancy, had a preterm delivery, or were not proficient in Dutch were excluded, and the majority of mothers were college- or university-educated. Children in this study were born full term and physically healthy, as assessed by birth weight and 5-minute Apgar scores (see Beijers, Jansen, Riksen-Walraven, & de Weerth, 2010 for further exclusion and demographic details). Thus, of the 220 mother-child pairs who originally enrolled in the study, the remaining 193 participants constituted a particularly low-risk sample.

**Procedure**

At 37 weeks gestation, mothers completed four self-report questionnaires regarding psychosocial stress and self-collected saliva samples over the course of two days to measure physiological stress via circadian cortisol. Mother-child interactions were recorded during a bathing session at 5 weeks of age and while playing with toys at 1 year of age to evaluate maternal sensitivity. Mothers collected saliva samples from the 2.5-year-old children over two days to measure toddler circadian cortisol. At 10 years of age, both mothers and children
completed a questionnaire about child behavior problems. Figure 1 provides an overview of the procedure and materials.

**Materials**

*Prenatal Maternal Psychosocial Stress*

Psychosocial PNMS was measured via four maternal self-report questionnaires. These questionnaires included the Pregnancy-Related Anxieties Questionnaire-Revised (PRAQ-R; Van den Bergh, 1990) to assess pregnancy-specific anxiety, the Pregnancy Experience Scale (PES; DiPietro et al., 2004) to measure pregnancy-related daily hassles, the State-Trait Anxiety Inventory (STAI; Van der Ploeg, Defares, & Spielberger, 1981; Spielberger, 1983) to assess state anxiety, and the Alledaagse Problemen Lijst (Vingerhoets et al., 1989) to measure maternal daily hassles.

The PRAQ-R contains 34 items which are categorized into three subscales: fear of giving birth (PRAQ-R 1), fear of having a handicapped child (PRAQ-R 2), and concern about own physical appearance (PRAQ-R 3). Similar to Beijers et al. (2010), only the first two subscales were used, which consisted of seven items in total and included statements such as “I was afraid of giving birth because I had never seen one before” and “Sometimes I thought that our baby would be a sick or ill child.” Responses to each item were rated on a 5-point scale, with a greater score indicating a greater prevalence of pregnancy-related anxiety.

The PES is comprised of 43 pregnancy-specific situations which participants rated their experience with on two 5-point scales: one for how much the situation was a hassle, and the other for how much the situation was uplifting. For example, items included “Talking with your partner about the baby’s name” and “Conversations with your family on issues related to childcare and child rearing.” Final pregnancy-related daily hassle scores were created by dividing the sum of the hassle responses by the sum of the uplift responses.

The state anxiety subscale of the STAI contains 20 items, such as “I feel comfortable”...
and “I am restless.” Each item was rated on a 4-point scale, with a greater score indicating greater levels of current anxiety.

The APL consists of 49 statements of situations, and participants indicated the extent to which each situation bothered them within the past two months on a 4-point scale. For example, statements included “You had a conflict with your partner” and “Property was damaged or stolen.” A final score representing how intensely daily hassles were experienced was created by summing the responses for the degree to which participants were bothered and dividing this value by the frequency of daily hassles.

*Prenatal Maternal Physiological Stress*

The physiological assessment of maternal stress, circadian cortisol, was evaluated by the area under the curve to the ground (AUC_g; Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). Saliva samples were self-collected over the course of two days, at five specific times per day: after awakening (C1), 30 minutes after awakening (C2), 12:00 (C3), 16:00 (C4), and 21:00 (C5). Only samples collected within certain time ranges were acceptable to use in further analyses: 6:00-10:00 and within 15 minutes after awakening for C1, 25-35 minutes after awakening for C2, 11:30-13:30 for C3, 15:30-17:30 for C4, and 20:00-23:00 for C5 (Beijers et al., 2010). The AUC_g was calculated for both days using the C1, C3, C4, and C5 samples to exclude the cortisol awakening response (Simons et al., 2015; Pruessner et al., 2003). Since the AUC_g from day 1 and day 2 were highly correlated \((r=0.704, p<0.001)\) and not significantly different from one another \((t(218)=-0.30, p=0.763)\), missing AUC_g values for either one of the days were imputed and the final AUC_g was averaged across days.

*Maternal Sensitivity*

Both mother-child interactions were video-recorded and then later rated for maternal sensitivity by two trained independent observers. The bathing session at 5 weeks of age was
rated for “maternal sensitivity” on a 9-point scale as established by Ainsworth (Ainsworth, Blehar, Waters, & Wall, 1978). During the play interaction at 1 year of age, the “mother’s supportive presence” was rated on a 7-point scale as defined by Erickson, which is comparable to Ainsworth’s “maternal sensitivity” (Erickson, Sroufe, & Egeland, 1985). There was good interobserver reliability for both of these ratings, with intraclass correlations of 0.94 and 0.95 for Ainsworth’s “maternal sensitivity” and Erickson’s “mother’s supportive presence” respectively.

A single maternal sensitivity score was created for each mother-child dyad by creating a z-score for each maternal sensitivity rating and calculating the mean of these scores since they were significantly correlated ($r=0.15$, $p=0.046$). For mothers missing data from the 1-year observation only ($n=8$), the 5-week rating was manually imputed as their single maternal sensitivity score because the t-test comparing the 5-week and 1-year sensitivity ratings was non-significant. Additionally, maternal sensitivity was turned into a categorical variable with two levels (“high sensitivity” and “low sensitivity,” divided at the median) for the purposes of conducting a moderation analysis involving the latent psychosocial PNMS variable (Rosseel, 2012).

**Toddler Physiological Stress**

The 2.5-year-old’s circadian cortisol was evaluated by the AUC$_g$ (Pruessner et al., 2003) in a similar manner as the prenatal maternal AUC$_g$. Toddler saliva samples were collected by mothers on two consecutive days, at four times per day: between 6:00 and 10:00 (C1), between 10:00 and 12:00 (C2), between 14:00 and 16:00 (C3), and between 18:00 and 21:00 (C4). The AUC$_g$ was calculated separately for day 1 and day 2 using the C1, C2, C3, and C4 samples and then averaged across days (Simons et al., 2015; Pruessner et al., 2003).

**Child Behavioral Outcomes**

The child outcomes, internalizing and externalizing behavior, were evaluated based on
the maternal- and self-reported responses to the Child Strengths and Difficulties Questionnaire (SDQ; Goodman, 1997). The SDQ contains a total of 25 statements to which the respondents indicate how true each statement is on a 3-point scale ranging from 0 to 2. Subscales were made by grouping these statements based on five types of behavior which were equally assessed within the SDQ: emotional symptoms, peer problems, hyperactivity, conduct problems, and prosocial behavior. In this study, the internalizing variable was formed by combining the emotional and peer subscales, and the externalizing variable was created by combining the hyperactivity and conduct subscales (Goodman, Lamping, & Ploubidis, 2010). Therefore, scores for both the internalizing and externalizing variables could range from 0-20.

One example of an internalizing item is “Afraid of a lot of things, is quickly anxious” for the maternal-report questionnaire and “I am afraid of many things, I am quickly anxious” for the self-report version. Externalizing items were for instance, “Restless, overactive, cannot sit still for a long time” for maternal-report and “I am restless, I cannot sit still for a long time” for self-report.

**Statistical Analysis**

A moderated mediation analysis was conducted through structural equation modeling (SEM) in R (R Core Team, 2016). Prenatal maternal psychosocial stress was constructed as a latent variable, while all other measures remained observed variables. Two theoretical models were tested which differed only on the child outcome variables: one used the child self-report of internalizing and externalizing behaviors, and the other used the maternal-report of child internalizing and externalizing behaviors.

Prior to executing the SEM analyses, the univariate distributions and number of outliers for each variable were examined. All variables other than maternal daily hassles (APL), prenatal maternal circadian cortisol, maternal sensitivity, and self-reported externalizing behaviors were significantly skewed, and fear of giving birth (PRAQ-R 1),
pregnancy-related daily hassles (PES), toddler circadian cortisol, and maternal-report of internalizing behaviors were significantly kurtosed. However, despite the lack of normality for these variables, no transformations were applied because the estimator used for subsequent analyses, MLR, is robust against violations of normality (Rosseel, 2012). Outliers (>3 SD) were also identified for the following variables: pregnancy-specific anxiety (PRAQ-R 1: \( n=5 \), PRAQ-R 2: \( n=1 \)), pregnancy-related daily hassles (\( n=4 \)), state anxiety (\( n=3 \)), prenatal maternal circadian cortisol (\( n=3 \)), toddler circadian cortisol (\( n=2 \)), self-reported internalizing behavior (\( n=1 \)), maternal-report of internalizing behavior (\( n=2 \)), and maternal-report of externalizing behavior (\( n=1 \)). Outliers which were greater than 4 SD above or below the mean were truncated at either 3 SD or the SD of the most extreme case, whichever had the greatest absolute value, via Winsorization (Dixon & Yuen, 1974).

Many variables contained at least some missing cases. The physiological PNMS and toddler physiological stress variables had 62 and 86 missing cases respectively due to either incomplete or insufficient collection of saliva samples (for more details on cortisol collection and exclusion criteria, see Simons et al., 2015). There was one mother who completed neither the 5 week nor the 12 month home visit, and was therefore missing a maternal sensitivity score. Finally, 33 children and 37 mothers did not complete the SDQ at 10 years of age.

**Results**

**Descriptive Analyses**

The means and standard deviations of each variable are presented in Table 1. Additionally, Table 2 contains the correlations between the variables, all of which were weak to moderate in magnitude. The prenatal maternal psychosocial stress variables were significantly correlated with one another (\( 0.15 \leq r \leq 0.40 \), \( 0.036 \leq p \leq 0.001 \)), except for pregnancy-specific anxiety (PRAQ-R 1 & 2) and maternal daily hassles (APL). There was also a significant correlation between prenatal maternal state anxiety and maternal-reports of
child internalizing behavior \( (r=0.27, \ p=0.001) \) as well as self-reported externalizing behavior \( (r=0.18, \ p=0.031) \). The child internalizing and externalizing behaviors were significantly correlated based on both self- \( (r=0.35, \ p<0.001) \) and maternal-report \( (r=0.25, \ p=0.002) \). Self-reported externalizing behavior was additionally correlated with maternal-reported internalizing behavior \( (r=0.25, \ p=0.002) \). Furthermore, the self- and maternal-reported internalizing behaviors were significantly correlated with one another \( (r=0.23, \ p=0.004) \), as were the self- and maternal-reported externalizing behaviors \( (r=0.49, \ p<0.001) \).

Table 1

Descriptive Statistics for Winsorized Data by Sex

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Mean (Standard Deviation)</th>
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</thead>
<tbody>
<tr>
<td>PRAQ-R 1</td>
<td>174</td>
<td>5.33 (2.37)</td>
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<tr>
<td>PRAQ-R 2</td>
<td>174</td>
<td>8.52 (2.78)</td>
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<tr>
<td>PES</td>
<td>174</td>
<td>0.33 (0.21)</td>
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<td>STAI</td>
<td>174</td>
<td>32.15 (8.73)</td>
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<tr>
<td>APL</td>
<td>174</td>
<td>1.14 (0.46)</td>
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<tr>
<td>Prenatal AUCg (nmol/L)</td>
<td>131</td>
<td>10,654.64 (2,418.83)</td>
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<tr>
<td>Maternal Sensitivity</td>
<td>192</td>
<td>0.03 (0.77)</td>
</tr>
<tr>
<td>Toddler AUCg (nmol/L)</td>
<td>107</td>
<td>5,162.43 (1373.32)</td>
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<tr>
<td>Internalizing (Child self-report)</td>
<td>160</td>
<td>4.77 (2.84)</td>
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<tr>
<td>Internalizing (Maternal-report)</td>
<td>156</td>
<td>2.80 (2.49)</td>
</tr>
<tr>
<td>Externalizing (Child self-report)</td>
<td>160</td>
<td>6.32 (2.66)</td>
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<tr>
<td>Externalizing (Maternal-report)</td>
<td>156</td>
<td>3.97 (3.16)</td>
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Table 2

*Correlations for Winsorized Data*

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<th>Variable</th>
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<tbody>
<tr>
<td>1. PRAQ-R 1</td>
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<td>2. PRAQ-R 2</td>
<td>0.27***</td>
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<td>3. PES</td>
<td>0.27*** 0.24**</td>
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<td>4. STAI</td>
<td>0.35*** 0.16* 0.40***</td>
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<td>5. APL</td>
<td>0.15* 0.13 0.28*** 0.27***</td>
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<td>6. Prenatal AUCg</td>
<td>0.05 0.08 -0.02 0.08 0.01</td>
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<td>7. Maternal Sensitivity</td>
<td>-0.10 -0.22** -0.03 -0.06 0.02 0.10</td>
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<td>8. Toddler AUCg</td>
<td>-0.13 0.04 0.06 -0.07 0.16 -0.14 0.01</td>
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<tr>
<td>9. Internalizing (C)</td>
<td>0.07 0.22** -0.06 0.03 -0.07 0.14 -0.08 -0.17</td>
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<td>10. Internalizing (M)</td>
<td>0.16* 0.12 0.13 0.27** 0.02 -0.02 -0.03 -0.12 0.23**</td>
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<tr>
<td>11. Externalizing (C)</td>
<td>0.05 0.05 -0.02 0.18* 0.00 0.09 -0.08 0.04 0.35*** 0.25**</td>
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<tr>
<td>12. Externalizing (M)</td>
<td>0.08 0.12 0.00 0.05 0.06 -0.04 -0.05 0.05 0.13 0.25** 0.49***</td>
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<tr>
<td>13. Child’s Sex</td>
<td>0.07 0.10 0.07 -0.02 0.21** 0.05 0.09 -0.01 0.11 -0.18* -0.01 -0.11</td>
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</table>

*p < 0.08, *p < 0.05, **p < 0.01, ***p < 0.001

*Note.* Spearman correlations were used for all variables, and calculations were based on pairwise deletion. The child’s sex was a dichotomous variable coded as males=1 and females=2.

**Preliminary Analyses**

Despite the significant correlations between the self- and maternal-reports of internalizing and externalizing behavior, the means of these variables were significantly different from each other, as illustrated in Figure 2. Both children and their mothers reported significantly more externalizing than internalizing behaviors ($t(316)=5.05, p<0.001$ and $t(293)=3.63, p<0.001$ respectively), and mothers reported significantly lower internalizing and externalizing behaviors than the children self-reported ($t(310)=-6.53, p<0.001$ and $t(302)=-7.12, p<0.001$ respectively). Therefore, the self-reported and maternal-reported behavioral outcomes were not combined into internalizing and externalizing latent variables, but kept as observed variables in separate self-report and maternal-report models.
Main Analyses

The structural equation modeling analysis which was conducted aimed to elucidate the relationships between PNMS, maternal sensitivity, the child’s sex, toddler circadian cortisol, and child behavioral outcomes. The self-report and maternal-report models were specified in R and then fit via the `sem()` function from the lavaan package, using MLR estimates and bias-corrected bootstrapped 95% confidence intervals (Rosseel, 2012; R Core Team, 2016). Full information maximum likelihood (FIML) was used to handle the missingness when running the models.

Before testing the regression pathways, robust goodness of fit indices were examined and model adjustments were made accordingly to ensure that the tested models adequately fit the observed data. Three absolute fit indices—Chi-square, RMSEA (Root Mean Square Error of Approximation), and SRMR (Standard Root Mean Square Residual)—and one incremental fit index—CFI (Comparative Fit Index)—were used to determine whether or not the model fit was sufficient for both the self-report and maternal-report models (Kline,
2005). The cut-off values for these indices were ≥0.05 for the Chi-square, ≤0.05 for the RMSEA and the SRMR, and ≥0.095 for the CFI (Hooper, Coughlan, & Mullen, 2008). Additionally, factor loadings and their corresponding significance values were inspected.

**Self-report Model**

The originally hypothesized model did not adequately fit the observed data according to each of the four robust goodness of fit tests (Chi-square ($\chi^2(18)=37.46, p=.005$); RMSEA (0.076); SRMR (0.072); CFI (0.825)). Therefore, the covariance between fear of giving birth and fear of having a handicapped child (PRAQ-R 1 & 2) was added to the measurement part of the model based on the modification indices. The improved measurement model was found to adequately fit the observed data, as indicated by the Chi-square ($\chi^2(4)=5.17, p=.271$), RMSEA (0.040), SRMR (0.027), and CFI (0.987) tests. However, the improved theoretical model (measurement and structural portions combined) did not converge. The parameter estimates of this model revealed that the factor loadings for psychosocial PNMS were 0.414 for the first pregnancy-related anxiety subscale, 0.320 for the second pregnancy-related anxiety subscale, 0.553 for pregnancy-related daily hassles, 0.655 for state anxiety, and 0.423 for daily hassles. Considering the particularly low factor loading for fear of having a handicapped child (PRAQ-R 2) and lack of model convergence, this indicator was removed from the psychosocial PNMS latent variable in the self-report model.

The subsequent model, with a four-indicator psychosocial PNMS latent variable, also did not immediately fit the data adequately. While the robust Chi-square ($\chi^2(12)=16.48, p=.170$) and RMSEA (0.044) values reached their respective acceptable ranges, the CFI (0.947) and SRMR (0.056) did not. Based on the modification indices, the covariance between the first subscale of the pregnancy-related anxiety questionnaire (PRAQ-R 1) and maternal daily hassles (APL) was added to the model. After this adjustment, the model was found to fit the data with three sufficient goodness of fit indices (Chi-square ($\chi^2(11)=14.45,$
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$p=.209);$ RMSEA (0.041); CFI (0.958)) and one which was nearly sufficient (SRMR (0.055)). All factor loadings for the psychosocial PNMS latent variable were similar to those of the original self-report model and greater than 0.400 (Figure 3).

Figure 3. Standardized regression, covariance, and factor loading estimates for the self-report model. A line with double arrows represents covariance, and significant or marginally significant estimates are indicated by solid lines. Not pictured for the purposes of readability: PRAQ-R 1 and APL covariance ($\beta=-0.12, p=0.204$). The mediation by toddler cortisol and moderation by maternal sensitivity and the child’s sex are also not pictured because these analyses were inconclusive.

This final self-report model was used to test the relationship between PNMS and child internalizing and externalizing behavior. Table 3 contains the estimates, standard errors, and significance values for each of the regressions which were analyzed within the self-report model. Physiological PNMS significantly predicted self-reported internalizing behavior ($B=0.18 \ (0.08), CI [0.02, 0.34]$) and marginally predicted self-reported externalizing behavior ($B=0.17 \ (0.11), CI [-0.03, 0.38]$) at 10 years of age (note: in-text estimates are reported with prenatal maternal AUC$_g$ in µmol/L rather than nmol/L for interpretability). Conversely, psychosocial PNMS was not found to significantly predict either self-reported internalizing or externalizing behavior (Table 3).
Next, mediation by toddler cortisol was examined, but the self-report mediation model only fit the data according to one of the four robust goodness of fit indices; therefore, estimates for the self-report mediation model were not interpreted (see Appendix for more information). Similarly, the multiple group analyses which were conducted to test moderation by maternal sensitivity and the child’s sex within the maternal-report model were also inconclusive. Group comparisons were not made because the model for maternal sensitivity did not converge, and within the model for child’s sex, the psychosocial stress latent variable was found to be significantly different for females and males (χ²(2)=9.92, p=.007; van de Schoot, Lugtig, & Hox, 2012; see Appendix for more information).

**Maternal-report Model**

In the same manner as the self-report model, the original maternal-report model did not sufficiently fit the observed data (Chi-square (χ²(18)=31.72, p=.024); RMSEA (0.065); SRMR (0.067); CFI (0.865)). Also like the self-report model, fear of having a handicapped
child (PRAQ-R 2) was removed from the psychosocial PNMS latent variable in the maternal-report model because of its particularly low factor loading relative to those of the other psychosocial PNMS indicators (0.320 versus 0.414 for PRAQ-R 1, 0.553 for PES, 0.655 for STAI, and 0.423 for APL).

The following model, which used a four-indicator psychosocial PNMS latent variable, fit adequately according to three goodness of fit indices—the robust Chi-square ($\chi^2(12)=12.84$, $p=.381$), RMSEA (0.020), and CFI (0.989)—and approached adequate model fit based on the SRMR (0.053) as well. To improve the model fit, the covariance between prenatal maternal state anxiety and maternal-report of child internalizing behavior was added to the model. The adjusted model was found to fit the data more adequately than the first model (Chi-square ($\chi^2(11)=11.61$, $p=.394$); RMSEA (0.017); CFI (0.992); SRMR (0.051)) and all factor loadings were greater than 0.400; therefore, this model was used for the remaining analyses (Figure 4).

Figure 4. Standardized regression, covariance, and factor loading estimates for the maternal-report model. A line with double arrows represents covariance, and significant estimates are indicated by solid lines. Not pictured for the purposes of readability: STAI and maternal-report of internalizing behavior covariance ($\beta=0.17$, $p=0.250$). The mediation by toddler cortisol and moderation by maternal sensitivity and the child’s sex are also not pictured because these analyses were inconclusive.
The relationship between PNMS and maternal-reports of child internalizing and externalizing behavior was assessed by this final maternal-report model. The estimates, standard errors, and significance values for the regressions tested within the maternal-report model are presented in Table 4. Maternal-reported internalizing behavior was found to be significantly predicted by psychosocial PNMS ($B=0.74$ (0.32), CI [0.11, 1.37]) but not physiological PNMS (Table 4). Furthermore, neither psychosocial nor physiological PNMS significantly predicted maternal-reported externalizing behavior (Table 4).

Table 4

Estimates for the Maternal-report Model

<table>
<thead>
<tr>
<th>Maternal-report Model</th>
<th>B (SE)</th>
<th>β</th>
<th>p</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor Loadings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRAQ-R 1</td>
<td>1.00 (0.00)</td>
<td>0.41</td>
<td>NA</td>
<td>[1.00, 1.00]</td>
</tr>
<tr>
<td>PES</td>
<td>0.11 (0.03)</td>
<td>0.51</td>
<td>&lt;0.001</td>
<td>[0.05, 0.17]</td>
</tr>
<tr>
<td>STAI</td>
<td>6.32 (1.70)</td>
<td>0.71</td>
<td>&lt;0.001</td>
<td>[3.00, 9.65]</td>
</tr>
<tr>
<td>APL</td>
<td>0.19 (0.07)</td>
<td>0.40</td>
<td>0.005</td>
<td>[0.06, 0.32]</td>
</tr>
<tr>
<td>Direct Pathways</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internalizing predicted by psychosocial PNMS</td>
<td>0.74 (0.32)</td>
<td>0.29</td>
<td>0.022</td>
<td>[0.11, 1.37]</td>
</tr>
<tr>
<td>Internalizing predicted by physiological PNMS</td>
<td>0.00 (0.00)</td>
<td>-0.10</td>
<td>0.319</td>
<td>[0.00, 0.00]</td>
</tr>
<tr>
<td>Externalizing predicted by psychosocial PNMS</td>
<td>0.31 (0.39)</td>
<td>0.10</td>
<td>0.430</td>
<td>[-0.46, 1.08]</td>
</tr>
<tr>
<td>Externalizing predicted by physiological PNMS</td>
<td>0.00 (0.00)</td>
<td>-0.03</td>
<td>0.803</td>
<td>[0.00, 0.00]</td>
</tr>
</tbody>
</table>

Note. Mediation and moderation estimates not reported because these analyses were inconclusive.

When testing mediation by toddler cortisol, the maternal-report mediation model did not sufficiently fit the data based on two of the four robust goodness of fit indices, so estimates were not interpreted for the maternal-report mediation model (see Appendix for more information). The analyses testing moderation by maternal sensitivity and the child’s sex within the maternal-report model were inconclusive as well. Group comparisons were
not made because the maternal-report model as a whole was significantly different for the high- and low-sensitivity groups ($\chi^2(22)=37.35, p=.022$) and the psychosocial stress latent variable was significantly different for females and males ($\chi^2(4)=25.76, p=.004$; van de Schoot, Lugtig, & Hox, 2012; see Appendix for more information).

Discussion

The aim of this study was to discern the relationship between PNMS and child internalizing and externalizing behavior, as mediated by toddler cortisol and moderated by maternal sensitivity and the child’s sex. PNMS predicted child internalizing behavior in the current study, but this association was dependent on the reporter of child behavior and the type of PNMS. Psychosocial PNMS was associated with increased child internalizing behavior, but only according to maternal-report. Additionally, physiological PNMS predicted greater amounts of self-reported internalizing behavior. Conclusions about the potential mediation and moderation of these relationships could not be made based on the current analyses.

In general, the association between PNMS and internalizing behavior in late childhood coincides with the findings of previous prospective studies. However, the lack of a relationship between physiological PNMS and maternal-reports of child internalizing behavior in the current study is inconsistent with prior research. Davis & Sandman (2012) and Isaksson et al. (2015) each found that elevated maternal cortisol during pregnancy (physiological PNMS) was associated with increased child internalizing behavior as reported by the mother at 9 years of age. This discrepancy in findings could be explained by the differences in type of cortisol measurements between studies: circadian cortisol ($AUC_{el}$; current study), afternoon cortisol (Davis & Sandman, 2012), and morning cortisol (Isaksson et al., 2015). Notably, the study by Isaksson et al. (2015) also collected a cortisol sample from the mothers in the afternoon but only found an association between prenatal cortisol
and child internalizing behavior for the morning sample.

Conversely, the relationship between psychosocial PNMS and maternal-reports of child internalizing behavior corresponds well with the results of earlier studies. Psychosocial PNMS has been related to child internalizing behavior at the age of 9 (Davis & Sandman, 2012) and was also found to be predictive of greater emotional difficulties beginning at 4 years of age and continuing through 13 years of age (Braithwaite et al., 2013; O’Donnell et al., 2014). The current maternal-report result for psychosocial PNMS is consistent with these previous findings, but its lack of replication within the current study’s self-report model highlights the uncertainty surrounding the interpretation of behavioral outcomes based on maternal-report alone. A mother’s emotional well-being may create a reporter bias, especially when she is reporting on difficulties that her child might be experiencing which are similar to her own (Najman et al., 2001; Leis, Heron, Stuart, & Mendelson, 2013).

Irrespective of the potential maternal bias in assessing child behavior, the finding that psychosocial PNMS is associated with maternal-reports of child internalizing behavior provides valuable insights. First, it indicates that mothers who experienced greater psychosocial stress during pregnancy viewed their children as having more internalizing difficulties than mothers with less psychosocial PNMS did. This could influence the mother-child relationship through parenting style and maternal behavior during play interactions, regardless of whether these emotional difficulties are actually present in the child (Gordon, 1983). Second, the maternal-reports offer a perspective of child internalizing behavior from adult caretakers who have spent extensive amounts of time with the children. Since mothers are aware of their child’s long-term patterns in emotional behavior, it is possible that maternal-report provides an account of child behavior that the child him- or herself would be unable to recognize. Therefore, although maternal-reports might be biased, the mother’s perspective of child behavior is meaningful, especially when combined with the perspectives
of other reporters.

In contrast to the results based on maternal-report, elevated physiological PNMS was related to child self-reported internalizing behavior. Although other prospective studies have found an association between psychosocial PNMS and self-reported internalizing behavior at 14 (Betts, Williams, Najman, & Alati, 2014), 15 (O’Donnell et al., 2014), and 21 years of age (Betts, Williams, Najman, & Alati, 2015), this is the first study to find a relationship between physiological PNMS and self-reported internalizing behavior. Therefore, since (1) the limited self-report research related to psychosocial PNMS to date spans a wide, developmentally unique range of ages and (2) associations between physiological PNMS and self-reported internalizing behavior have not been examined before, conclusions about the association between physiological or psychosocial PNMS and self-reported internalizing behavior cannot be drawn without replication in future studies.

While the general relationship between PNMS and child internalizing behavior was significant based on both self- and maternal-report, the prediction of child externalizing behavior was just marginally significant, and only according to child-self report. This unexpected finding contradicts the results of previously mentioned studies by Braithwaite et al. (2013) and Isaksson et al. (2015), but is consistent with the study by Betts et al. (2014) which found that maternal emotional distress was associated with self-reported internalizing but not externalizing behavior in early adolescence. As suggested by Betts et al. (2014), it is possible that one or more of the variables creating the psychosocial PNMS construct (psychosocial PNMS latent variable in the current study) would have individually predicted externalizing behavior while the combination of psychosocial PNMS variables did not. Alternatively, child emotional difficulties following PNMS might simply be expressed differently across childhood, with externalizing symptoms being more apparent in infancy and early childhood but decreasing in prevalence (or manifesting instead as internalizing
The mediation and moderation analyses were inconclusive due to insufficient model fit and overall model differences between groups. The mediation model might have inadequately fit the data either due to the relatively low sample size for the complexity of the model or because of variations in toddler cortisol levels. For the moderation by maternal sensitivity, the model differences between high- and low-sensitive mothers seemed to be driven by differences in the model as a whole. In contrast, for the moderation by child’s sex, model differences appeared to stem from a distinct formation of the psychosocial PNMS latent variable for females and males. Therefore, future research should disentangle the differences between pregnancy-specific and general prenatal psychosocial stress as they relate to sex-specific postnatal outcomes, such as maternal sensitivity and behavior in late childhood.

The current longitudinal study offers several novel insights due to its prospective design. Since few studies to date have examined behavioral development through late childhood following prenatal stress, the current assessment at 10 years of age is unique in that it assesses behavior late in childhood yet still prior to puberty. Furthermore, the research design aimed to optimize its objectivity by incorporating two types of PNMS as well as two independent reporters of child outcomes. In particular, the relationship found between physiological PNMS and self-reported internalizing behavior was completely independent from any possible maternal reporter bias, making this the first study to our knowledge which has inherently avoided maternal-biased results.

In addition, the relationship found between PNMS and child internalizing behavior in this study’s community sample is generalizable to other low-risk populations, which is both a strength and a limitation. The current population remained a low-risk sample, with both self- and maternal-reported internalizing and externalizing behaviors falling well-
within their expected ranges as established by the SDQ score categorization guidelines (Goodman, 2001). Consequently, increased child internalizing and externalizing behaviors predicted by elevated PNMS are not necessarily indicative of concerning behavioral problems, but instead might represent an increased susceptibility to later psychopathology. Indeed, a previous study conducted in a low-risk population found that psychosocial PNMS was associated with maternal-reported emotional difficulties through 13 years of age and also predicted self-reported internalizing behaviors at 15 years of age (O’Donnell et al., 2014). However, these findings as well as those of the current study might not be translatable to high-risk communities, since the mechanisms behind child development in light of PNMS could be completely different in a more vulnerable population.

The main limitation within this study was that genetic and most postnatal factors were not included in the models. Although the relationship between PNMS and child behavior is evident independent of shared genes (Rice et al., 2010), research suggests that child vulnerability to early-life adversity is moderated by genotype, most notably the brain-derived neurotrophic factor (O’Donnell et al., 2014) and serotonin transport genes (Karg, Burmeister, Shedden, & Sen, 2011). However, genotypic moderation has not been consistently observed across studies (Braithwaite et al., 2013; Risch et al., 2009). It is also possible that postnatal factors, which were not accounted for aside from maternal sensitivity, are associated with behavioral outcomes in late childhood. Importantly, current maternal stress was not controlled for in this study. Nevertheless, other studies which controlled for postnatal maternal stress and a number of additional potential confounders found that PNMS was still a significant predictor of child behavior from mid-late childhood through early adolescence (Davis & Sandman, 2012; Braithwaite et al., 2013; Betts et al., 2014; O’Donnell et al., 2014; Isaksson et al., 2015). Another limitation within this study and the developmental psychology field at large is that little is currently known about the role of
other primary caregivers, especially fathers, as they relate to child emotional and behavioral development.

Future research should incorporate child self-report—or, ideally, a combination of child-, maternal-, paternal-, and teacher-report—into the assessment of emotional and behavioral outcomes for a more complete evaluation of child behavior. Self- and maternal-reports of child behavior could complement one another; however, since they were predicted differently by PNMS in the current study, future studies should be cautious about interpreting findings based on either report alone. Future research should also treat psychosocial and physiological PNMS as independent predictors of child outcomes since they were found to separately predict child behavior. The mechanisms behind their unique prediction of child internalizing behavior are not yet fully understood, so future work should aim to elucidate the biopsychosocial underpinnings as well. Furthermore, these two types of PNMS should be examined concurrently in order to conclude whether or not psychosocial and physiological PNMS have distinct associations with child behavior since these studies are conflicting and scarce.

Although there were differences between psychosocial and physiological PNMS as they related to child- and maternal-reports of internalizing behavior, PNMS was found to be predictive of child internalizing behavior at 10 years of age. This lasting association between prenatal stress and child behavior suggests that PNMS warrants greater attention from midwives, doctors, and other professionals caring for pregnant women. The current study also adds to the considerable amount of empirical research linking adverse prenatal environments with unfavorable behavioral adaptation in children, which collectively affirm the importance of supporting parents during pregnancy. Results from the limited number of prenatal interventions thus far have found promising improvements in the parents and their children, as well as societal cost benefits (Glover, 2014). Considering the long-term association that has
been found between prenatal stress and child behavior, early investments in families could have a lasting positive impact on child development.
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Appendix

Statistical Procedure for Mediation and Moderation Analyses

Mediation by Toddler Stress Physiology

The self-report mediation model fit the data according to the robust Chi-square value ($\chi^2(14)=21.30, p=.094$), but all other goodness of fit indices were inadequate (RMSEA (0.053); SRMR (0.062); CFI (0.915)). All theoretically plausible model modifications were added based on the modification indices, but none of these were found to sufficiently improve the self-report mediation model fit. Therefore, estimates for this model were not interpreted or reported in Table 3.

Similarly, the maternal-report mediation model only fit the data based on two of the robust goodness of fit indices ($\chi^2(14)=18.69, p=.177$; RMSEA (0.042)) but not the others (SRMR (0.059); CFI (0.942)). After adding the relevant modifications as specified by the modification indices, the model still did not adequately fit the data, so estimates were not interpreted for the maternal-report model either.

Moderation by Maternal Sensitivity

A multiple group analysis was conducted to test moderation by maternal sensitivity through estimating separate models for both high- and low-sensitive mothers. The self-report model did not converge, but the maternal-report model was found to be significantly different for the high- and low-sensitivity groups ($\chi^2(22)=37.35, p=.022$). This difference was not resolved by adding any of the relevant modification indices for both groups. When examining measurement invariance more closely to evaluate whether the psychosocial PNMS latent variable does indeed measure the same construct across sensitivity groups, the high- and low-sensitivity self-report models were found to have metric, scalar, and strict invariance (meaning that the factor loadings, intercepts, and residual variances for the psychosocial PNMS latent variable were not significantly different between groups).
However, despite the sufficient measurement invariance within the latent variable, comparisons between the two groups’ estimates were not made because of the lack of configural invariance (i.e. the model fit the data differently) for the entire model between high and low maternal sensitivity groups (van de Schoot, Lugtig, & Hox, 2012).

**Moderation by Child’s Sex**

Moderation by the child’s sex was also examined through a multiple group analysis in which models were estimated separately for female and male children. The self- and maternal-report models had configural invariance, as indicated by the nonsignificant difference between each of their female and male models (self-report: $\chi^2(22)=32.01, p=.077$; maternal-report: $\chi^2(22)=31.65, p=.084$). The factor loadings for the psychosocial PNMS latent variable appeared to be insufficient for the female group within both the self- and maternal-report model though, with pregnancy-related daily hassles (self-report: $\beta=0.518, p=0.195$; maternal-report: $\beta=0.517, p=0.310$) and general maternal daily hassles no longer significantly loading (self-report: $\beta=0.344, p=0.301$; maternal-report: $\beta=0.309, p=0.436$).

Thus, measurement invariance within the psychosocial PNMS latent variable was assessed for the self- and maternal-report models to determine whether there were sex differences in the construction of the latent variable. The female and male models were found to have metric invariance (factor loadings were not different between the sexes), but not configural or scalar invariance (the latent variable as a whole and its intercepts were different between the sexes), for both the self- and maternal-report models. Therefore, the lack of measurement invariance within the psychosocial PNMS latent variable for females and males in both the self- and maternal-report models likely contributed to the abnormalities in the overall models. Consequently, moderation by the sex of the child was not tested further (van de Schoot, Lugtig, & Hox, 2012).