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Regular Article

From prenatal maternal anxiety and respiratory sinus arrhythmia to toddler internalizing problems: The role of infant negative affectivity

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Abstract

Prenatal maternal anxiety is considered a risk factor for the development of child internalizing problems. However, little is known about potential mechanisms that account for these associations. The current study examined whether prenatal maternal anxiety was indirectly associated with toddler internalizing problems via prenatal maternal physiology and infant negative affectivity. We examined these associations in a longitudinal study of 162 expectant mothers from their third trimester until 18 months postpartum. Path analyses showed that higher prenatal anxiety was associated with higher infant negative affectivity at 7 months, which in turn was associated with higher toddler internalizing problems at 18 months. Prenatal anxiety was not indirectly associated with child outcomes via baseline or task-evoked respiratory sinus arrhythmia (RSA) in response to an infant cry while pregnant. However, pregnant women with greater decreases in task-evoked RSA had toddlers with greater internalizing problems, which was mediated by infant negative affectivity at 7 months. Findings suggest that prenatal anxiety and RSA reactivity to an infant cry may be independent risk factors for the development of infant negative affectivity, which in turn increases risk for toddler internalizing problems. These findings contribute to a growing literature on mechanisms that underlie intergenerational transmission of internalizing problems.

Keywords: internalizing problems; negative affectivity; prenatal anxiety; respiratory sinus arrhythmia

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Introduction

Elevated internalizing problems emerge as early as toddlerhood, with some children exhibiting stable, high internalizing symptoms that persist into adolescence (e.g., Sterba et al., 2007). Understanding developmental factors that contribute to early internalizing psychopathology may be informative for determining optimal intervention targets aimed at reducing the likelihood of maladaptive socioemotional outcomes. Parental anxiety is one risk factor associated with the development of childhood internalizing behaviors (depression and anxiety) (e.g., Ahmadzadeh et al., 2019; Barker et al., 2011; Field, 2017). Both theoretical and empirical work on internalizing problems across generations highlight multiple mechanisms underlying these associations; these include heritability of internalizing problems, prenatal programming of neurobiological systems in the child, and environmental transmission (e.g., Eley et al., 2015; Goodman & Gotlib, 1999; Perlman et al., 2022). These same mechanistic processes may also shape temperament, which can be associated with higher child internalizing problems.

During pregnancy, at least one in five pregnant people receive a diagnosis of a mood and/or anxiety disorder (Araji et al., 2020). Given these high prevalence rates, it is important to consider downstream consequences of prenatal anxiety symptoms that may impact the emergence of child internalizing problems. Anxiety symptoms during pregnancy are associated with behavioral and emotional problems in children (e.g., O'Connor et al., 2003). These findings suggest that the psychological states of expectant mothers during pregnancy may play a programming role, though the pathways by which maternal mental health associates with toddler internalizing problems are not well understood. The present study examined whether prenatal maternal anxiety symptoms may be indirectly associated with child internalizing problems via risk factors such as prenatal maternal physiology and infant temperamental negative affect.

Prenatal maternal anxiety and stress physiology

Prenatal maternal anxiety symptoms may play an important role in shaping malleable biological systems of a developing fetus. Fetal programming and adaptive calibration models take an evolutionary approach that highlight the importance of the prenatal environment for offspring adaptation (Del Giudice, 2012; Del

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Giudice et al., 2011). These models suggest that the prenatal environment provides important information about the external world, and the fetus develops accordingly to prepare for survival. Therefore, it is important to consider possible mechanisms linking prenatal maternal anxiety to later infant and toddler outcomes.

While there is a high comorbidity between anxiety and depressive symptoms during pregnancy, the tripartite model (Clark & Watson, 1991) proposes that physiological hyperarousal (i.e., excessive increase in physiological responsivity to stimuli) is a specific characteristic of anxiety disorders. It is possible that maternal anxiety symptoms during pregnancy may influence the developing fetus through its effects on maternal stress physiology. Maternal autonomic nervous system responses may play a mediating role between maternal anxiety symptoms and fetal systems underlying arousal and reactivity, as maternal autonomic nervous system activity during pregnancy plays a key role in neurovascular signaling to regulate blood pressure and blood flow (Fu & Levine, 2009). Additionally, there is some evidence that the vagus nerve regulates contractions of the uterine muscle and affects uterine blood flow in animals (e.g., Ortega-Villalobos et al., 1990). Taken together, these may be mechanisms by which autonomic nervous system activity could impact the developing fetus and signal stress in the environment.

One measure of parasympathetic nervous system activity is respiratory sinus arrhythmia (i.e., high-frequency heart rate variability; RSA). RSA is a psychophysiological measure of arousal regulation and is one index of a person's capacity to self-regulate in order to engage with environmental demands (Beauchaine, 2001). Baseline RSA captures parasympathetic nervous system activity at rest. Under optimal stimulus conditions, baseline RSA can provide insight into a person's parasympathetic functioning and their capacity to regulate in the face of environmental stressors. RSA reactivity, or change in RSA from baseline to task, can provide information on physiological regulation during a stressor. Generally, a moderate decrease in RSA is considered an adaptive response as the body prepares itself to react to a stressor while an excessive decrease in RSA to a stressor may reflect rapid and exaggerated shifts in mood/affect (Beauchaine, 2001).

RSA is often conceptualized as a transdiagnostic biomarker of psychopathology (Beauchaine & Thayer, 2015). Therefore, it is not surprising that some associations emerge between RSA and anxiety symptoms specifically. In adult, nonpregnant clinical samples, individuals diagnosed with an anxiety disorder exhibit differential patterns of autonomic reactivity marked by "heightened" (i.e., excessive or contextually disproportionate) decreases in RSA, perhaps reflecting processes such as hypervigilance for threat (e.g., Campbell & Wisco, 2021; Thayer et al., 1996). However, findings are not always consistent, likely due to differences in sampling, the types of tasks that researchers use, and heterogeneity of anxiety disorders. Several studies fail to find differences in task-based RSA between individuals with and without an anxiety disorder (e.g., Fisher & Newman, 2013; Hammel et al., 2011). Additionally, there were no associations between anxiety symptoms and either baseline RSA or RSA reactivity in nonclinical samples (Jönsson, 2007). Given mixed findings in the extant literature between trait anxiety and RSA in adults (e.g., Campbell & Wisco, 2021 for review), more research is needed to better understand these associations. Even less work has been conducted during pregnancy, though there is some evidence that there are associations between anxiety symptoms and RSA during the prenatal period. Trait anxiety during pregnancy was associated with lower, resting state high-frequency heart rate variability (i.e., RSA; Mizuno et al., 2017), consistent with studies that lower baseline RSA is often associated with psychopathology. Less is known about associations between prenatal anxiety symptoms and RSA reactivity to a stressor.

One specific stressor that may capture physiological processes in expectant mothers is an infant cry stimulus. Responses during the infant cry task may reflect important individual differences in adult affect, physiology, and behavior (e.g., Barr et al., 2014). For example, psychophysiological responses elicited by stimuli involving infant cries have been associated with prenatal emotion dysregulation, such that expectant mothers with higher levels of emotion dysregulation exhibited more blunted RSA responsivity to an infant cry task (Lin et al., 2019). Additionally, expectant mothers' responses to an infant cry paradigm during pregnancy have been linked with both infant outcomes (e.g., newborn neurobehavior; Ostlund et al., 2019) as well as postpartum caregiving and measures of maternal sensitivity (e.g., Leerkes et al., 2022; Ablow et al., 2013). Specifically, physiological arousal during pregnancy is associated with higher postpartum sensitivity and may reflect an adaptive response during this developmental period (Leerkes et al., 2022). This body of work establishes the infant cry task as an important paradigm to consider in relation to mechanisms underlying parental affect and infant outcomes. Given that prenatal trait anxiety may be linked with differences in physiological responses to an infant cry task, which in turn may be associated with infant outcomes, it is important to test these associations as a possible mechanistic pathway.

In light of mixed findings, more work is needed to understand links between prenatal autonomic reactivity and infant outcomes. A current gap in the literature involves understanding how prenatal maternal anxiety and maternal RSA reactivity are associated during pregnancy, as well as how measures of prenatal maternal RSA may be associated with child socioemotional outcomes. Understanding these links between trait anxiety and both baseline RSA and RSA reactivity during pregnancy can inform our understanding of how the maternal stress response system may underlie processes involved in fetal programming of offspring outcomes.

Infant negative affectivity

Temperament traits are biologically based individual differences in domains such as reactivity, sociability, activity, and emotionality (Rothbart & Bates, 2006; Shiner et al., 2012). Studies have established associations between child temperament and later internalizing psychopathology, with a large literature focusing on temperamental negative affectivity (Klein et al., 2012). Negative affectivity is characterized by individual differences in sadness, discomfort, frustration, fear, and difficulty with soothing (Rothbart & Bates, 2006). Infants and children with high negative affect may have higher stress during negative experiences due to increased reactivity and difficulties regulating negative emotions, which in turn increases risk for the development of internalizing problems (Klein et al., 2012).

Emerging evidence suggests infant temperament may be partially shaped by the prenatal environment, including expectant mothers' anxiety symptoms during pregnancy (Werner et al., 2007; Foss et al., 2022). This idea is consistent with prenatal programming perspectives, as temperament traits are often considered a marker of susceptibility to later environmental risk factors (Gallagher, 2002; Hartman & Belsky, 2018). Higher levels of maternal anxiety symptoms *in utero* may signal to the developing fetus a need to develop heightened awareness of the environment

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through biological processes such as stress hormones and physiological changes. Fetal programming scholars propose that a fetus adapts to the anticipated postnatal environment based on maternal cues, leading to different child phenotypes (Lester et al., 2014; Schlotz & Phillips, 2009). If a pregnant woman has more maternal anxiety symptoms, it may be indicative to the fetus that they may be born into a stressful environment. Thus, the development of postnatal plasticity in infants may be a beneficial and adaptive trait to meet the demands of a stressful environment (Hartman & Belsky, 2018). A systematic review of 23 studies found consistent positive associations between prenatal maternal anxiety symptoms and infant negative affectivity and reactivity assessed during the first two years of life, with effect sizes ranging from low to moderate (Korja et al., 2017). However, one gap that remains is understanding how prenatal maternal autonomic physiology may also be associated with the development of infant negative affectivity. A better understanding may shed light on whether maternal stress physiology during the third trimester may play a programming role in the development of infant temperament.

Additionally, prenatal maternal anxiety symptoms may be indirectly associated with internalizing problems via infant negative affectivity. For example, maternal self-reported prenatal stress, anxiety, and depressive symptoms early in pregnancy were directly associated with toddler negative affectivity at age 3, which in turn was associated with internalizing problems at age 5 (Hentges et al., 2019). These findings provide some evidence that there may be developmental cascades from pregnancy to early childhood that set the stage for children's risk for internalizing psychopathology. However, less work has examined the mechanisms underlying these indirect associations between maternal anxiety symptoms and infant outcomes in relation to maternal physiological processes during pregnancy. More multimethod research is needed to better understand these pathways from the prenatal period to infancy and toddlerhood to capture earlier indices of risk for intervention and prevention.

The present study

Although there is some evidence that infant negative affectivity is associated with both prenatal anxiety symptoms and later internalizing behaviors, there has been less work directly examining infant negative affectivity as a possible pathway by which exposure to maternal prenatal anxiety symptoms may be associated with toddler internalizing problems. In addition, to our knowledge, no studies to date have examined whether maternal prenatal physiology might be a possible mechanism in the association between prenatal maternal anxiety symptoms and child outcomes. The present study aimed to address these gaps in the literature by examining whether prenatal maternal anxiety symptoms are indirectly associated with toddler internalizing problems at age 18 months via prenatal maternal RSA and infant negative affectivity at 7 months. Based on prior findings in the extant literature, we hypothesized that higher levels of prenatal maternal anxiety would be associated with lower levels of maternal baseline RSA and larger decreases in RSA from baseline to an infant cry task, which in turn would be associated with higher infant negative affectivity at seven months and higher toddler internalizing problems at 18 months. Additionally, we also accounted for associations with 7-month postpartum maternal anxiety symptoms to examine the robustness of associations between our prenatal constructs and child outcomes. We hypothesized that while there would be positive associations between prenatal and 7-month maternal anxiety symptoms, associations between prenatal anxiety symptoms, prenatal RSA, and infant outcomes would remain significant even after accounting for the effects of postnatal maternal anxiety symptoms. Given our hypotheses that prenatal maternal physiology may be a specific pathway by which maternal prenatal anxiety is associated with infant negative affectivity and toddler internalizing problems, we also conducted post-hoc tests examining these associations with maternal prenatal depressive symptoms. We hypothesized that while prenatal maternal depressive symptoms may be associated with infant negative affect and toddler internalizing problems, we would not find associations between prenatal maternal depressive symptoms and prenatal maternal physiological measures.

Method

Participants

Participants consisted of 162 mother–infant dyads. All study procedures were approved by the Institutional Review Boards of a large public research university and research hospital. Pregnant individuals were recruited during their third trimester of pregnancy at their prenatal care appointments or via email/phone. Pregnant individuals were between the ages of 18 and 40 ($M = 28.96$ years, $SD = 5.14$). Eligibility was determined by scores on the Difficulties in Emotion Dysregulation Scale (DERS; Gratz & Roemer, 2004). Because most individuals report low to moderate levels of emotion dysregulation, we oversampled for high levels of emotion dysregulation to achieve a more uniform distribution (for recruitment and sampling details, see authors blinded for review, 2019). Additionally, expectant mothers were excluded from participation in the study if they were expecting more than one child or were diagnosed with gestational diabetes or preeclampsia to reduce variability in obstetric and birth complications within the study sample. All participants in the study provided a written informed consent before every assessment. Table 1 contains information on sample demographics for the present study.

Procedures

Third trimester

Eligible participants completed questionnaires online prior to a laboratory visit. The laboratory visit occurred between 26 and 40 weeks gestation ($M = 33.58$ weeks, $SD = 2.99$) and consisted of a battery of tasks and interviews. Of note, data were collected for two participants prior to 28 weeks of gestation (i.e., during late second trimester) – post-hoc analyses revealed no significant differences when these two participants were excluded from analyses, so we opted to keep them in the final sample and model. Relevant for the current study, RSA was collected during a 10-minute resting period (baseline), a Trier Social Stress Task (not analyzed here), and then an infant cry task (described below).

Infant cry task

Participants watched a series of 1-minute video clips comprising an infant cry task. The series began with a neutral seascape baseline, which was a view of the ocean with waves washing in and out. The second clip participants viewed was an *infant play* clip, in which a girl infant was seen playing with a toy with a female adult (only the adult's arms and part of her body were visible). Next, participants viewed the *infant cry* clip, in which the same girl infant was seen sitting and crying by herself. Lastly, the participants viewed a

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Table 1. Sample demographics ($N = 162$)

	n (%)
Maternal Race and Ethnicity	
Hispanic	**43 (26.5%)**
American Indian or Alaskan Native	1 (0.6%)
White	40 (24.7%)
Multiracial	2 (1.2%)
Non-Hispanic	**119 (73.5%)**
American Indian or Alaskan Native	4 (2.5%)
Asian	15 (9.3%)
Hawaiian or Pacific Islander	2 (1.2%)
Black or African American	2 (1.2%)
White	88 (54.4%)
Multiracial, non-Hispanic/Latina	8 (4.9%)
Maternal Education at the Prenatal Visit	
Less than 12th grade	4 (2.5%)
High school graduate or equivalent	21 (13.0%)
Some college or technical school	51 (31.5%)
College graduate	51 (31.5%)
Any postgraduate school	32 (19.7%)
Did not report	3 (1.8%)
Household Income at the Prenatal Visit	
Under $9,000	7 (4.3%)
$9,000 - $14,000	9 (5.6%)
$15,000 - $19,999	7 (4.3%)
$20,000 - $24,999	3 (1.9%)
$25,000 - $29,999	7 (4.3%)
$30,000 - $39,999	16 (9.9%)
$40,000 - $49,999	9 (5.6%)
$50,000 - $79,999	48 (29.6%)
$80,000 - $99,999	17 (10.5%)
$100,000 or more	24 (14.8%)
Did not report	15 (9.3%)
Maternal Relationship Status at the Prenatal Visit	
Married	124 (76.5%)
Single and never married	27 (16.7%)
Separated or divorced	9 (5.6%)
Widowed	0 (0%)
Did not report	2 (1.2%)
Infant Biological Sex	
Male	77 (47.5%)
Female	85 (52.5%)
Parity	
1	77 (47.5%)
2	43 (26.6%)
3	20 (12.3%)
4+	17 (10.5%)
Did not report	5 (3.1%)

seascape recovery, which was the same ocean scene as the *seascape baseline*.

7-month postpartum

Mothers were invited to participate in a follow-up visit, which included completing questionnaires online prior to a laboratory visit. Temperament and self-report trait anxiety measures were collected at this timepoint from questionnaires.

18-month postpartum

Mothers were once again invited to participate in a follow-up visit, which included completing questionnaires online prior to a laboratory visit. Parent-report measures of child socioemotional functioning (including internalizing problems) at this timepoint were used.

Measures

State-Trait Anxiety Inventory (STAI; Spielberger et al., 1983)

The State-Trait Anxiety Inventory (STAI) is a 40-item survey assessing emotional, physiological, and cognitive symptoms of anxiety (e.g., "*I feel nervous and restless*") on a 4-point scale from 1 (*Almost Never*) to 4 (*Almost Always*). The STAI is divided into two sections that assess state and trait anxiety. For the current study, the scores that reflect trait (general) anxiety were used. These items asked participants to report how they generally feel. The STAI has demonstrated both good internal consistency and reliability in other studies (Spielberger et al., 1983). In our data, the internal consistency for the STAI trait scores was $\alpha = .93$. Observed scores in our study ranged from 20 to 77, and 43% of our participants scored above the clinical cutoff of the STAI (i.e., above 40).

The Center for Epidemiological Studies–Depression Scale (CES-D; Radloff 1977)

The Center for Epidemiological Studies–Depression Scale (CES-D) assesses depressive symptoms like poor appetite, loneliness, and sleep issues. Respondents rate 20 items on a scale from 0 (rarely or none of the time) to 3 (most or all of the time), with total scores ranging from 0 to 60. Scores above 16 indicate a risk for clinical depression (Lewinsohn et al., 1997). For our study, we used CES-D scores as a continuous variable, with observed values between 0 and 50, with good internal consistency ($\alpha = .92$).

Respiratory sinus arrhythmia (RSA)

Electrocardiogram data were collected using BioLab acquisition software (version 3.1) and wireless MindWare mobile devices sampled at 500 Hz (MindWare Technologies, Ltd., Gahanna, OH). Data were captured via a three-lead spot electrode pattern with the negative lead on expectant mothers' right clavicle, the positive lead on the bottom left rib, and the ground lead on the bottom right rib. RSA was scored in 60-s epochs (consistent with guidelines by Berntson et al., 1997) by trained research assistants using MindWare's heart rate variability analysis software. To calculate RSA, heart rate variability analysis software automatically identified R peaks within each QRS complex and checked whether resulting interbeat intervals were within the expected deviation based on surrounding data and expected ranges for an interbeat interval series (Berntson et al., 1990). All data points flagged by the software as potentially aberrant were reviewed and, where appropriate, corrected by trained research assistants. Files with ambiguous or noisy data were referred to the senior author for review. In some cases, partial or whole tasks were unusable due to electrode nonadherence to the skin, research assistant error, or

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excessive movement artifacts. After the signal was cleaned, the heart period time series was detrended, and a Fast Fourier Transform was used to identify high-frequency variation. As respiration rates of pregnant individuals are higher than typical adults, we used the frequencies between .12 and .60 Hz to identify high-frequency variation.

Baseline RSA was computed by averaging all the epochs during the 10-minute resting task. We utilized this measure of baseline RSA because it was collected prior to participants completing other stress-inducing tasks during the laboratory visit and may better reflect participants' ANS functioning at rest. We had usable RSA data during the 10-minute resting task from 98% of participants; of the 98%, 1% of participants provided partially usable RSA data (i.e., at least 2 epochs were recorded during the resting task). Additionally, 2% of participants had unusable RSA data due to equipment malfunction.

We computed a reactivity score for the infant cry task by subtracting RSA levels during the *seascape baseline* video from RSA levels during the *infant cry* video. In this way, negative scores reflected RSA decreases in response to the potentially stressful video. Because participants completed another stress-inducing task prior to the infant cry task, the *seascape baseline* was used to compute reactivity instead of the 10-minute baseline to reduce extraneous noise in the data that may be due to changes in RSA from other stress-inducing tasks that occurred between the 10-minute baseline and the infant cry task. We opted to characterize reactivity using a difference score instead of a residual change score as residual scores (i.e., change less than or greater than expected based on sample trend line) can be more difficult to interpret (for review, see Burt & Obradovic, 2013). We had usable RSA data during the infant cry task from 96% of participants; 4% of participants did not have usable data due to equipment malfunction or excessive movement artifacts.

Infant Behavior Questionnaire (IBQ-R; Gartstein & Rothbart, 2003)

The Infant Behavior Questionnaire (IBQ-R) is a 191-item survey designed to assess general patterns of behavior associated with temperament in infancy (3–12 months). At the 7-month time-point, Mothers rated how often they observed a behavior in the past week. Each item describes an infant behavior (e.g., *During feeding, how often did the baby lie or sit quietly?*) using a 7-point scale from 1 (*Never*) to 7 (*Always*). Parents are also given a "not applicable" response option for use when the infant has not been observed in the situation described. Each item loads onto one of 14 subscales: Activity Level, Distress to Limitations, Fear, Duration of Orienting, Smile/Laughter, High-intensity Pleasure, Low-intensity Pleasure, Soothability, Falling Reactivity (Rate of Recovery from Distress), Cuddliness, Perceptual Sensitivity, Sadness, Approach, and Vocal Reactivity. The IBQ-R has demonstrated good internal consistency, reliability, and validity, including correlations with laboratory observations (Gartstein & Marmion, 2008; Goldsmith & Campos, 1990; Parade & Leerkes, 2008). Consistent with other research (Gartstein & Rothbart, 2003), items from the sadness ($\alpha = .66$), distress to limitations ($\alpha = .70$), fear ($\alpha = .81$), and falling reactivity ($\alpha = .77$) subscales were used to compute a Negative Affectivity score, which had an internal consistency of $\alpha = .83$.

Infant-Toddler Social and Emotional Assessment (ITSEA; Carter et al., 2003)

The Infant-Toddler Social and Emotional Assessment (ITSEA) is a 166-item measure designed to assess social and emotional problems and competencies in children aged 12–35 months. Each item assesses different behaviors (e.g., *"Seem nervous, tense, or fearful"*) and is answered on a 3-point scale from 0 (*Not True*) to 2 (*Very True/Often*). The ITSEA contains 17 subscales that capture four broad domains: externalizing behavior problems, internalizing behavior problems, dysregulation, and competence. For the current study, scores on the internalizing domain were used, and this domain had an internal consistency of $\alpha = .79$.

Analytic plan

Missing data

There were 12 patterns of missing data, and Little's MCAR test indicated the data likely were missing completely at random, $\chi^2 = 43.3$, $p = .70$. Thus, full information maximum likelihood (FIML) was used to account for missing data in subsequent analyses. FIML estimators in multiple regression models with missing data are shown to produce less biased parameter estimates, especially compared to listwise deletion, pairwise deletion and mean imputation (Enders, 2001; Graham, 2012).

Path analyses

We ran path analyses using the *lavaan* package (Rosseel, 2012) in R Studio version 4.1.1 to examine the associations between prenatal anxiety symptoms, prenatal physiology, infant negative affectivity, and toddler internalizing problems. We ran two separate path models: the first model included baseline RSA as the measure of prenatal physiology, and the second model included RSA reactivity to the infant cry task as the measure of prenatal physiology. We also ran path models that accounted for 7-month postpartum maternal anxiety symptoms. Lastly, we ran path analyses to examine associations between prenatal depressive symptoms, prenatal physiology, infant negative affectivity, 7-month maternal depressive symptoms, and toddler internalizing problems. All regression coefficients reported are standardized. Our estimates and standard errors were computed using bias-corrected bootstrapping with 1000 draws. The *lavaan* package uses FIML to account for unbiased estimates of data when data is missing at random. Model fit was evaluated using χ^2, the comparative fit index (CFI), the Tucker–Lewis index (TLI), standardized root mean square residual (SRMR), and the root mean square error of approximation (RMSEA). Good model fit is indicated by $p < .05$, CFI $\geq .95$, TLI $\geq .95$, SRMR $\leq .08$, RMSEA $\leq .06$ (Hu & Bentler, 1999).

Covariates

We included prenatal psychotropic medication use (including stimulants, antidepressants, antipsychotics, anti-anxiety, or mood stabilizers) as a covariate in all analyses given prior studies that establish associations between prenatal psychotropic medication use on infant neurodevelopment (e.g., Gentile, 2010), as well as potential associations between psychotropic medication use and electrocardiogram data (e.g., Kemp et al., 2014, O'Regan et al., 2015). In our sample, participants that indicated psychotropic medication use had lower baseline RSA compared to participants without psychotropic medication use ($F = 11.51$, $p < .01$). Additionally, consistent with sex differences in the extant literature, parents of girls endorsed significantly more internalizing problems than parents of boys in our sample ($F = 8.47$, $p < .01$). Thus, we also included child sex as a covariate in all analyses.

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Table 2. Means, standard deviations, and correlations with confidence intervals

Variable	*N*	*M*	1	2	3	4	5	6	7
1. Prenatal Trait Anxiety	161	39.72 (13.18)							
2. Prenatal Baseline RSA	160	5.31 (1.14)	−.20*						
			[−.34, −.04]						
3. Prenatal Seascape Baseline RSA	157	6.08 (1.25)	−.18*	.78**					
			[−.33, −.02]	[.71, .84]					
4. Prenatal Infant Cry RSA	159	5.49 (1.20)	−.10	.80**	.78**				
			[−.26, .05]	[.74, .85]	[.71, .84]				
5. Prenatal Infant Cry RSA Reactivity	156	−0.60 (0.80)	.08	−.04	−.39**	.27**			
			[−.08, .23]	[−.19, .12]	[−.52, −.25]	[.12, .41]			
6. 7−month Infant Negative Affectivity	120	3.28 (0.75)	.20*	.07	.10	.03	−.16		
			[.02, .37]	[−.11, .25]	[−.08, .28]	[−.15, .21]	[−.34, .02]		
7. 7−month Maternal Trait Anxiety	125	38.33 (11.68)	.69**	−.19*	−.19*	−.11	.09	.19*	
			[.58, .77]	[−.36, −.01]	[−.36, −.01]	[−.28, .07]	[−.09, .27]	[.00, .35]	
8. 18−month Toddler Internalizing Problems	111	0.52 (0.20)	.18	.11	.21*	.18	−.07	.44**	.21*
			[−.01, .36]	[−.08, .29]	[.02, .39]	[−.01, .35]	[−.25, .13]	[.26, .59]	[.01, .39]

Note. M and *SD* are used to represent mean and standard deviation, respectively. Values in square brackets indicate the 95% confidence interval for each correlation. *indicates $p < .05$. **indicates $p < .01$.

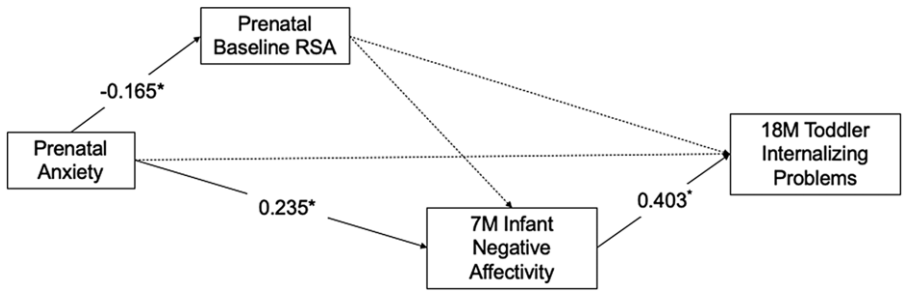

Figure 1. Path model depicting associations between prenatal anxiety symptoms, prenatal baseline RSA, 7-month infant negative affectivity and 18-month toddler internalizing problems. Solid lines indicate significant associations ($p < .05$); dashed lines indicate nonsignificant associations.

Results

Descriptive statistics

Table 2 contains the descriptive statistics and correlations among key variables for the current study. Of note, prenatal anxiety symptoms were negatively correlated with prenatal baseline RSA ($r = -.16$, $p = .04$) but not RSA reactivity. Prenatal anxiety symptoms also were positively correlated with infant negative affect ($r = .20$, $p = .03$). Toddler internalizing scores at 18 months were correlated with both prenatal seascape baseline RSA scores ($r = .19$, $p = .04$) and infant negative affect ($r = .44$, $p < .01$).

Path models

Figure 1 depicts the first path model examining associations between prenatal anxiety symptoms, prenatal baseline RSA, 7-month infant negative affectivity and 18-month internalizing problems ($\chi^2 = 6.18$, $p = 0.52$, CFI = 1.00, TLI = 1.00, RMSEA < .01, SRMR = .04). Prenatal anxiety symptoms were positively associated with 7-month infant negative affect ($\beta = 0.235$, $p = .01$), which in turn was positively associated with 18-month toddler internalizing problems ($\beta = 0.403$, $p < .01$). The indirect path from prenatal anxiety symptoms to 18-month toddler internalizing problems via 7-month infant negative affectivity was significant ($\beta = 0.095$, $p = .03$, CI [0.011, 0.179]). Although prenatal anxiety symptoms were negatively associated with prenatal baseline RSA ($\beta = -0.165$, $p = .04$), prenatal baseline RSA was not significantly associated with 7-month infant negative affectivity or 18-month toddler internalizing problems.

Figure 2 depicts the path model examining associations between prenatal anxiety symptoms, prenatal RSA reactivity to the infant cry task, 7-month infant negative affectivity, and 18-month toddler internalizing problems ($\chi^2 = 6.97$, $p = .43$, CFI = 1.00, TLI = 1.00, RMSEA < .01, SRMR = .04). Once again, prenatal anxiety symptoms were positively associated with 7-month infant negative affectivity ($\beta = 0.230$, $p = .01$), which were positively associated with 18-month toddler internalizing problems ($\beta = 0.415$, $p < .01$). The indirect path from prenatal anxiety symptoms to 18-month toddler internalizing problems via 7-month infant negative affectivity was significant ($\beta = 0.100$,

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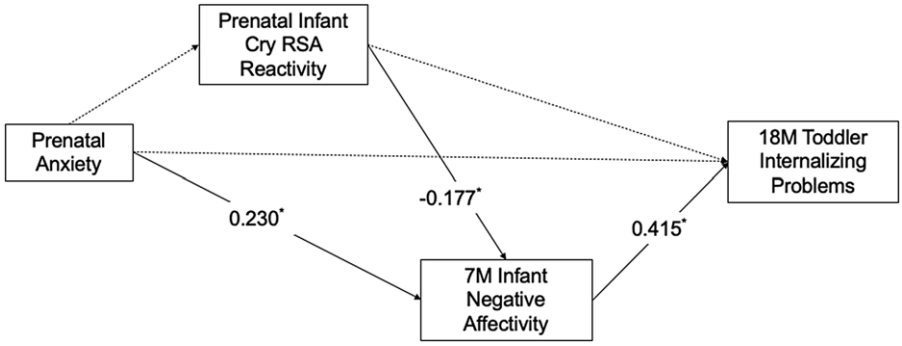

Figure 2. Path model depicting associations between prenatal anxiety symptoms, prenatal RSA reactivity to an infant cry task, 7-month infant negative affectivity and 18-month toddler internalizing problems. Solid lines indicate significant associations ($p < .05$); dashed lines indicate nonsignificant associations.

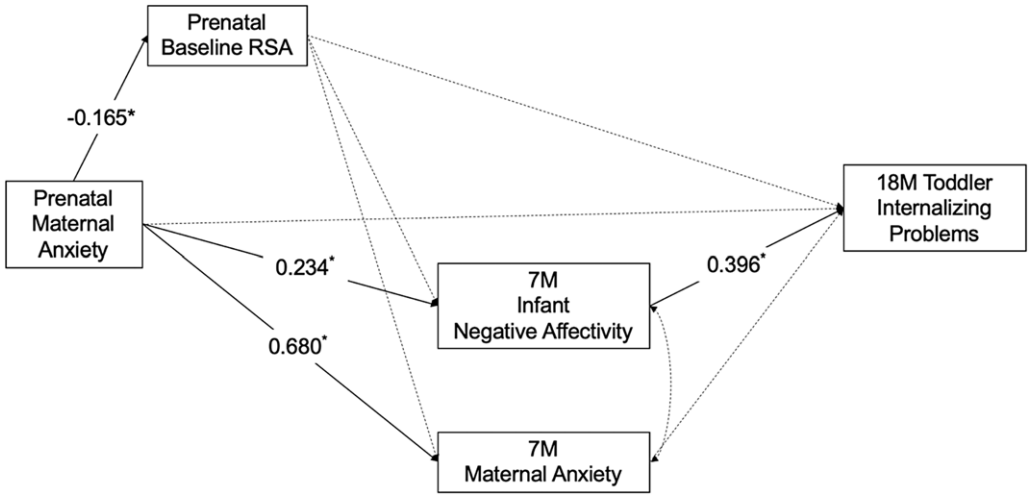

Figure 3. Path model depicting associations between prenatal anxiety symptoms, prenatal baseline RSA, 7-month infant negative affectivity and 18-month toddler internalizing problems while accounting for associations with 7-month maternal anxiety symptoms. Solid lines indicate significant associations ($p < .05$); dashed lines indicate nonsignificant associations.

$p = .04$, CI [0.006, 0.185]). Although prenatal anxiety symptoms were not significantly associated with prenatal RSA reactivity, prenatal RSA reactivity was negatively associated with 7-month infant negative affectivity scores ($\beta = -0.177$, $p = .01$). Greater prenatal RSA decreases from baseline to task were associated with higher levels of infant negative affectivity. There was also a significant indirect path from prenatal RSA reactivity scores to 18-month toddler internalizing problems via 7-month infant negative affectivity scores ($\beta = -0.074$, $p = .03$, CI [−0.137, −0.010]).

7-month postpartum anxiety scores

We accounted for 7-month postpartum anxiety symptoms in our path models - expectedly, prenatal anxiety symptoms were significantly and positively associated with 7-month maternal anxiety symptoms in both models. Figure 3 depicts the path model examining associations between prenatal anxiety symptoms, prenatal baseline RSA, 7-month infant negative affect, 7-month maternal anxiety symptoms, and 18-month internalizing problems ($\chi^2 = 7.94$, $p = .54$, CFI = 1.00, TLI = 1.00, RMSEA < .01, SRMR = .04). Findings were largely consistent with the path model with prenatal anxiety symptoms and baseline RSA (i.e., Figure 1), even after accounting for 7-month postpartum maternal anxiety symptoms. Consistent with prior models, the indirect path from prenatal anxiety symptoms to toddler internalizing problems via infant negative affectivity remained significant ($\beta = 0.093$, $p = .05$, CI [0.007, 0.178]).

Figure 4 depicts the path model examining associations between prenatal anxiety symptoms, prenatal RSA reactivity to the infant cry task, 7-month infant negative affect, 7-month maternal anxiety symptoms, and 18-month internalizing problems ($\chi^2 = 8.65$, $p = .47$, CFI = 1.00, TLI = 1.00, RMSEA < .01, SRMR = .05). The findings were once again consistent with the path model with prenatal anxiety symptoms and RSA reactivity (i.e., Figure 2) even after accounting for 7-month maternal anxiety symptoms. The indirect path from prenatal anxiety symptoms to toddler internalizing problems via infant negative affectivity was significant ($\beta = 0.094$, $p = .04$, CI [0.007, 0.180]). The indirect path from prenatal RSA reactivity to toddler internalizing problems via infant negative affectivity was also significant ($\beta = -0.073$, $p = .03$, CI [−0.136, −0.009]).

Post-hoc models with prenatal and 7-month depressive symptoms

Findings from path models examining associations between prenatal maternal depressive symptoms, prenatal maternal RSA, infant negative affect, and toddler internalizing problems were consistent with associations with prenatal maternal anxiety symptoms. Figure 5 ($\chi^2 = 9.24$, $p = .42$, CFI = 1.00, TLI = 0.99, RMSEA = .01, SRMR = .05) shows that there is an indirect effect from maternal depressive symptoms to toddler internalizing problems via infant negative affectivity ($\beta = 0.106$, $p = .03$, CI [0.01, 0.20]). Additionally, we also found that prenatal maternal depressive symptoms were negatively associated with baseline RSA ($\beta = -0.209$, $p = .10$, CI [−0.355, −0.063]).

Figure 6 ($\chi^2 = 10.03$, $p = .35$, CFI = 0.99, TLI = 0.97, RMSEA = .03, SRMR = .05) shows that there were no significant

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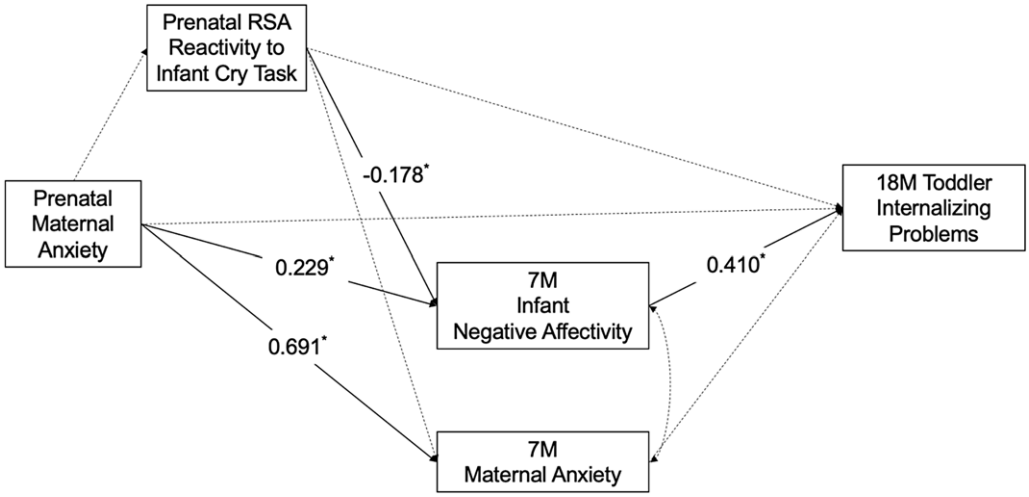

Figure 4. Path model depicting associations between prenatal anxiety symptoms, prenatal RSA reactivity to an infant cry task, 7-month infant negative affectivity and 18-month toddler internalizing problems while accounting for associations with 7-month maternal anxiety symptoms. Solid lines indicate significant associations ($p < .05$); dashed lines indicate nonsignificant associations.

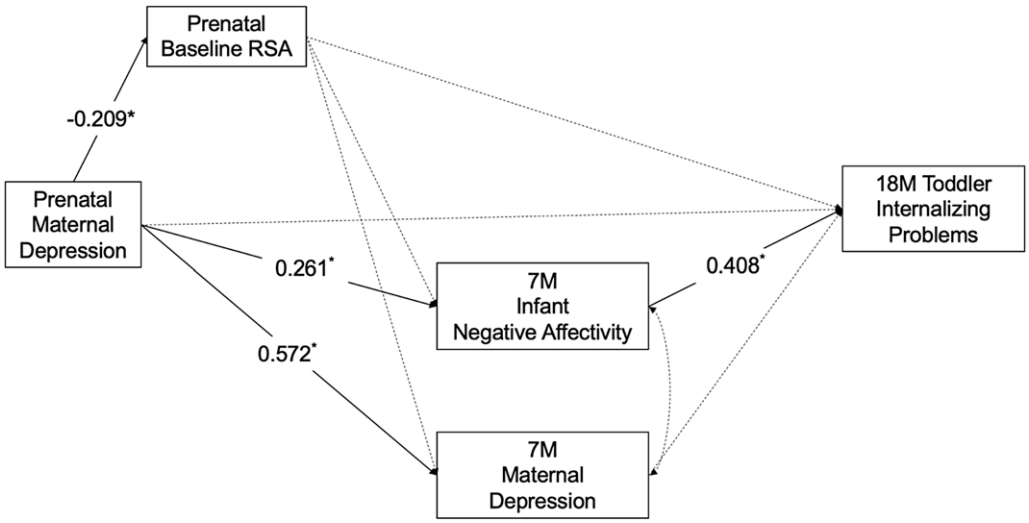

Figure 5. Path model depicting associations between prenatal depressive symptoms, prenatal baseline RSA, 7-month infant negative affectivity and 18-month toddler internalizing problems while accounting for associations with 7-month maternal depressive symptoms. Solid lines indicate significant associations ($p < .05$); dashed lines indicate nonsignificant associations.

associations between prenatal maternal depressive symptoms and RSA reactivity to the infant cry task ($\beta = -0.119$, $p = .01$, CI [−0.027, 0.265]). Similar to the model with anxiety symptoms, there were also significant indirect paths from prenatal depressive symptoms to toddler internalizing problems via infant negative affectivity ($\beta = 0.107$, $p = .02$, CI [0.015, 0.198]), as well as from prenatal RSA reactivity to toddler internalizing problems via infant negative affectivity ($\beta = -0.083$, $p = .02$, CI [−0.151, −0.015]).

Discussion

The present study aimed to better understand how risk factors such as expectant mothers' stress physiology during pregnancy and infant temperament may explain associations between prenatal anxiety symptoms and toddler internalizing problems. We find evidence of two pathways from prenatal risk factors of anxiety symptoms and stress reactivity to toddler internalizing problems via infant temperamental negative affect. Although we did not find evidence that RSA explained the link between prenatal anxiety symptoms and child outcomes, our results suggest that pregnant individuals' trait anxiety and parasympathetic nervous system functioning may have unique consequences for child outcomes.

The role of negative affectivity in intergenerational transmission of internalizing risk

Our findings suggest that there are joint contributions of prenatal maternal anxiety symptoms and prenatal maternal RSA reactivity to the development of infant negative affectivity, which in turn lead to the development of internalizing problems at 18 months. Associations between maternal anxiety symptoms and infant negative affectivity likely reflect a combination of genetic influences, prenatal programming, as well as postnatal environmental mechanisms. Although maternal postnatal anxiety

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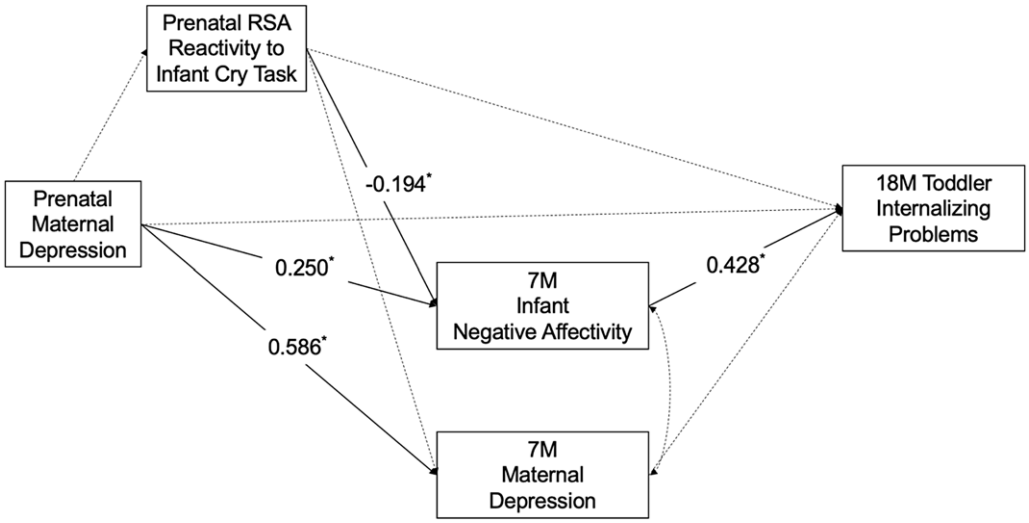

Figure 6. Path model depicting associations between prenatal depressive symptoms, prenatal RSA reactivity, 7-month infant negative affectivity and 18-month toddler internalizing problems while accounting for associations with 7-month maternal depressive symptoms. Solid lines indicate significant associations ($p < .05$); dashed lines indicate nonsignificant associations.

symptoms may also have cascading effects on infant negative affectivity through processes such as their responses to threat (Perlman et al., 2022), we accounted for 7-month postpartum maternal anxiety symptoms in some of our models. Therefore, it is likely that there are prenatal processes contributing to associations between maternal anxiety symptoms and infant negative affectivity. Our results provide some evidence that higher levels of anxiety during the prenatal period may play a programming role in infant negative affectivity. Infant negative affectivity is a marker of increased sensitivity to the environment as it often moderates associations between environmental risk factors and children's internalizing problems later in development (Gallagher, 2002; Stright et al., 2008).

It is important to note that maternal anxiety symptoms, maternal physiological reactivity, and child negative affectivity may be due to shared experiences of environmental stressors. Maternal anxiety, in particular, may be an adaptive response that can lead to survival strategies in dangerous environments and situations (Gutiérrez-García & Contreras, 2013). Given that mechanisms linking environmental influences on fetal development are often due to exposures via the birthing parent (e.g., Hogg et al., 2012), it is important to consider how prenatal maternal anxiety and stress may be the mechanism by which child negative affectivity develops. Hartman and Belsky (2018) suggest that higher prenatal maternal stress could be a response to an unpredictable or chaotic environment. Dysregulated physiological responses may serve as cues to the fetus that the postnatal environment may likewise be unpredictable. In this context, infants who are more susceptible to the postnatal environment may likewise also show more adaptation to the postnatal environment. While observational studies are limited in making conclusions around prenatal programming processes due to possible genetic associations between parent anxiety symptoms and child negative affectivity, there are animal studies that provide some evidence that prenatal stress programs offspring sensitivity to context (Hartman & Belsky, 2018). Thus, it is plausible that higher levels of maternal anxiety symptoms *in utero* may signal to the developing fetus that there is a need to develop an increased sensitivity to a stressful environment, leading to increased negative affectivity.

Additionally, one of the novel findings in our study is that expectant mothers' parasympathetic stress reactivity during the third trimester associated with infant negative affectivity assessed at seven months. Little work has examined how prenatal autonomic stress responses associate with infant and toddler outcomes, though some emerging work has examined how prenatal autonomic responses to an infant cry task are associated with infants' behavioral avoidance during a still-face task (Speck et al., 2023). Results from the present study add to the literature and show that a greater decrease in RSA from a baseline to an infant cry stimulus was associated with higher levels of infant negative affectivity. It is possible that decreases in RSA in response to stressors during the third trimester may signal to the fetus that the postnatal environment is unpredictable through possible mechanisms such as increased blood flow and/or pressure. Thus, the infant may subsequently develop higher levels of negative affectivity as a result of being more attentive to stressful stimuli in the postnatal environment. If this interpretation is accurate, our finding may provide preliminary evidence that maternal stress reactivity could play a role in programming negative affectivity as early as the prenatal period, even after accounting for trait levels of anxiety.

Postnatal maternal psychopathology and the caregiving environment may also contribute to risk for internalizing behavior in toddlers. For example, mothers whose RSA decreased to the infant cry video may be more responsive and sensitive to infant cues (Del Vecchio et al., 2009; Leerkes et al., 2012). Specifically, Ablow and colleagues (2013) found that decreases in RSA across the infant cry task during pregnancy were linked to greater sensitivity when mothers responded to their own distressed infant postpartum. There is also some evidence that maternal sensitivity does not mediate associations between prenatal RSA and infant behaviors (Speck et al., 2023). Given that prenatal decreases in RSA are associated with both greater maternal sensitivity as well as higher negative affectivity, it is plausible that there may be other parenting behaviors that may link greater decreases in RSA with higher infant negative affectivity. Future work should examine whether parents' postnatal behavioral responsivity to their infants may play a role in infants' development of negative affectivity

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(e.g., by conveying that infant negative affectivity will be met with prompt parental responses). If this is the case, the association between prenatal RSA responsivity and infant negative affectivity may be behaviorally mediated during the early postpartum period. While we accounted for 7-month anxiety symptoms in our models, it is also important to note the high positive association between prenatal and 7-month anxiety symptoms. Studies have found associations between higher levels of maternal anxiety and parenting behaviors associated with higher levels of child anxiety such as overprotection (e.g., Buss et al., 2021). It is possible that mothers who experience elevated levels of anxiety symptoms may engage in parenting behaviors that elicit more negative affectivity in their infants.

Prenatal anxiety symptoms are not associated with child outcomes via prenatal RSA

Our findings also add to the extant literature by clarifying the link between expectant mothers' anxiety symptoms and RSA during the third trimester. Expectant mothers with higher levels of trait anxiety had lower levels of baseline RSA, suggesting that expectant mothers with higher levels trait anxiety had poorer capacity for regulation. This is consistent with both theoretical perspectives and empirical findings that highlight lower baseline RSA as a transdiagnostic marker of poorer emotion regulation and psychopathology (Beauchaine & Thayer, 2015). One of the strengths of the study is our oversampling of individuals with both lower and higher levels of emotion dysregulation, which underscores the relevance of our findings for individuals across a spectrum of emotion regulation capacities and anxiety levels.

Nonetheless, we did not find a significant association between prenatal anxiety symptoms and RSA reactivity to an infant cry task. This was contrary to our expectations given prior findings showing associations between anxiety symptoms and RSA reactivity to emotional stressors (Campbell & Wisco, 2021), though many of these studies were conducted in nonpregnant samples. Additionally, this result was not consistent with findings from our own sample that self-reported prenatal emotion dysregulation, more broadly, is associated with prenatal RSA reactivity (Lin et al., 2019). Given that emotion dysregulation is a transdiagnostic marker of psychopathology and is positively associated with anxiety, we expected that we would find similar associations with RSA reactivity. It may be that there are other emotional stressors that better capture differences in anxious expectant mothers' stress physiology. In developmental samples, there is work demonstrating that associations between RSA reactivity and internalizing symptoms are emotion-specific, such that fear- and sadness-eliciting tasks are associated with internalizing symptoms (Fortunato et al., 2013). Though the infant cry task is designed to be subjectively aversive and upsetting (Zeskind & Marshall, 1988) and physiologically arousing (Wiesenfeld et al., 1985), it may be that an infant cry task does not elicit negative emotions associated with trait anxiety. Post-hoc descriptive analyses in our sample using participants' self-reported ratings of emotional states during the infant cry task show a range in how stressed and anxious participants were, and some participants reported increases in stress while others reported decreases. There are findings that show associations between attachment and physiological responses to an infant cry task (Ablow et al., 2013), suggesting that attachment may better explain differences in how pregnant people are responding to an infant cry stimulus. Alternatively, another avenue for future research on associations between prenatal anxiety symptoms and prenatal physiological regulation is examining the role of recovery from the infant cry stimulus. It may be that recovery from a stressor, or an individual's ability to adapt to components of the emotional experience when the context has changed, may be a better indicator of physiological processes underlying trait anxiety during pregnancy.

Contrary to our hypotheses, we did not find an indirect path from prenatal anxiety symptoms to child outcomes via measures of prenatal RSA, suggesting that maternal anxiety symptoms are not associated with the specific child outcomes assessed at 7 and 18 months via prenatal RSA. One possible explanation for this finding is that parasympathetic measures may not best capture links between parent and child internalizing symptoms. Specifically, there is some work suggesting that trait anxiety during the third trimester of pregnancy is associated with sympathetic functioning as opposed to parasympathetic activity (Kimmel et al., 2021). Additionally, it may be that fetal programming occurs primarily through hormonal changes (e.g., increased glucocorticoids during stressful experiences) as part of hypothalamic–pituitary–adrenal axis activity (Kim et al., 2015) as opposed to changes in autonomic nervous system functioning. Although it is important to note that the different branches of the nervous system do not work in isolation, future research should consider sympathetic functioning and the HPA axis activity as possible mechanisms underlying how expectant mothers' anxiety during pregnancy may be associated with child outcomes. An additional avenue for future work also includes examining how prenatal anxiety may be a predictor of different child outcomes (e.g., child parasympathetic functioning) that are indicative of internalizing risk via prenatal parasympathetic functioning.

Another possibility for the lack of indirect association from prenatal anxiety symptoms to infant negative affectivity via RSA may be that RSA serves as a moderator instead of a mediator. For example, studies have found that maternal resting RSA moderates associations between maternal traits and parenting behaviors in mothers of preschoolers (e.g., Root et al., 2016). Another future direction for disentangling these associations may include testing whether prenatal RSA may moderate associations between prenatal maternal traits (such as anxiety symptoms) and child outcomes. Lastly, it is important to note that associations between our constructs of interest were consistent in our models testing prenatal and 7-month anxiety symptoms as well as post-hoc models testing associations with prenatal and 7-month depressive symptoms. While we hypothesized that there may be anxiety-specific physiological processes, especially in relation to physiological arousal and stress responsivity, we did not find any evidence for this. One possibility is due to the high comorbidity and correlation between anxiety and depressive symptoms during the third trimester within our sample ($r = .70$, $p < .01$), making it difficult to test disorder specificity. Future work should continue to interrogate mechanisms underlying the contributions of maternal psychopathology to child risk to better understand specific targets for intervention during the perinatal period.

Limitations and future directions

It is important to consider the current study's limitations to inform future directions. First, we only included information about prenatal anxiety symptoms and physiology at one timepoint during the third trimester. There is some evidence that women with an anxiety diagnosis do not show the same decrease in RSA

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from the first to third trimester as nonclinically diagnosed women during pregnancy (Braeken et al., 2015). Additionally, fluctuations in stress levels during pregnancy are associated with infant negative affect (MacNeill et al., 2023). The timing, type, and chronicity of prenatal exposures (such as exposure to prenatal anxiety symptoms) could alter fetal development in different ways. While studies have often found high correlations among repeated measures of anxiety symptoms across different trimesters (e.g., Davis et al., 2007), there are also studies that have identified different trajectories of prenatal anxiety symptoms (Irwin et al., 2020). However, there is some support for the importance of physiological processes during the third trimester and infant temperament. Davis et al. (2007) found that prenatal cortisol during the third trimester, but not earlier, was predictive of infant negative reactivity. Our findings may reflect a similar association emerging between physiology during the third trimester and infant negative affectivity. Future research should examine how patterns of anxiety symptoms and physiology across the prenatal period may be associated with both infant negative affectivity and toddler internalizing problems to elucidate the importance of timing of stress exposure during pregnancy.

It Is also important to note that our measures of prenatal anxiety symptoms, infant negative affectivity, and toddler internalizing problems were all based on maternal reports. Thus, future research would benefit from using observational measures and/or reports of child outcomes using other informants to account for common method variance. Additionally, researchers have found evidence of associations between prenatal anxiety symptoms and postpartum parenting stress (Huizink et al., 2017), suggesting that postpartum parenting stress may also be a possible pathway by which prenatal maternal anxiety symptoms are associated with infant and toddler outcomes related to toddler internalizing problems. Future work should also consider how expectant mothers' prenatal anxiety symptoms, or other forms of psychopathology, may be associated with postpartum parenting behaviors to increase risk for their children's development of internalizing problems.

Another future direction involves considering nonlinear associations between maternal anxiety symptoms, physiology, and child outcomes, and examining change in RSA during longer stressors. One way to do this involves utilizing alternate modeling approaches to characterize physiological reactivity: for example, researchers could model dynamic fluctuations and nonlinear change in physiological regulation on a smaller timescale with larger sample sizes. Relatedly, while the one-minute assessment of the infant cry task is standard (Ablow et al., 2013), future work could consider less intense cry bouts that last longer than 60 s, so as to not be too burdensome to participants. Our findings may also have some limitations in generalization given our sample demographics. Future work should examine these associations in more diverse samples or in relation to related contextual processes (e.g., considering how racism/discrimination may contribute to prenatal anxiety symptoms or stress). Lastly, our study suggests that infant negative affect may be an important pathway from maternal psychopathology to future child socio-emotional outcomes. These results warrant further exploration with other aspects of maternal psychopathology.

Conclusions

Results from the present study filled two major gaps in the literature: (1) how anxiety symptoms and RSA (i.e., para-sympathetic functioning) are associated during the prenatal period, and (2) how prenatal risk factors are indirectly associated with toddler internalizing problems via infant negative affect. We found evidence for two pathways by which prenatal risk was associated with toddler internalizing problems via infant negative affect. Our findings further the extant literature on possible prenatal mechanisms underlying the intergenerational transmission of internalizing problems and highlight possible targets for early prevention and intervention efforts such as reducing prenatal anxiety symptoms and stress.

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