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Prenatal origins of temperament: Fetal cardiac development & infant surgency, negative affectivity, and regulation/orienting

B.C. Pingeton [a,*], S.H. Goodman [a], C. Monk [b,c]

[a] Department of Psychology, Emory University, United States
[b] Department of Obstetrics and Gynecology and Psychiatry, Columbia University Irving Medical Center, United States
[c] New York State Psychiatric Institute, United States

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ABSTRACT

Temperament, *i.e.* individual differences in reactivity and self-regulation, emerges early in infancy; might temperament originate during fetal development? Mixed findings and methodological issues in the literature examining this consideration limit our understanding of the continuity between these fetal indices and infant temperament. The primary aims of the current study were to improve on published studies by (a) using standardized and well-accepted fetal cardiac (actocardiograph) and infant temperament measures (the Infant Behavior Questionnaire—Revised; IBQ-R) (b) expanding fetal assessments to include coupling (the cross correlation of heart rate with movement), and (c) examining a diverse sample to determine if findings of associations between fetal neurobehavior and infant temperament generalize beyond cohorts that are demographically well-resourced and predominantly white. Building on theory and empirical findings, we hypothesized that (1) FHR would be positively associated with Surgency and Negative Affectivity, (2) FHRV would be positively associated with Surgency, and Regulation/Orienting and inversely associated with Negative Affectivity, and (3) fetal coupling would be positively associated with Regulation/Orienting and Surgency and inversely associated with Negative Affectivity. We collected 20 min of fetal data (m gestational age = 34.42 weeks) and mothers completed the IBQ-R (n = 90 women; 60 % non-Caucasian race; 63 % Latina ethnicity). We found that FHR was positively associated with Negative Affectivity but not associated with Surgency (or Regulation/Orienting). FHRV was inversely associated with Surgency but not associated with Negative Affectivity or Regulation/Orienting. Coupling was positively associated with Regulation/Orienting and Surgency but not associated with Negative Affectivity. Our findings, from a more diverse sample and with established measures, provide further evidence that individual differences in reactivity and regulation can be identified in the *in-utero* period and show theory-based continuity to specific infant temperament constructs.

1. Introduction

Temperament, defined as constitutionally based individual differences in reactivity and self-regulation, is identified in early infancy, is relatively stable, and predicts behavioral, emotional, and social functioning over the course of development, including into adulthood (Rothbart, 1986; Sanson et al., 2004; Zentner & Bates, 2008). The term "constitutional" in the definition of temperament

* Corresponding author.
E-mail address: bpinget@emory.edu (B.C. Pingeton).

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references biological origins, albeit with expected influences of maturation and experience (Rothbart & Derryberry, 1981). Given this theory and evidence for the importance of infants' temperament, researchers have turned to questions about the origins of temperament. Specifically, if temperament is to some degree constitutional, might infant temperament originate during fetal development? Dating back to the 1938 Fels Institute study, researchers have speculated that temperament may have its origins in patterns of fetal heart rate (FHR), fetal heart rate variability (FHRV), and fetal movement (FM) (as reviewed in: DiPietro et al., 2015).

An important consideration in the study of fetal antecedents of infant temperament is the approach to measurement of FHR, FHRV, and FM and infant temperament. Regarding these fetal measures, investigators have generally measured either baseline or reactivity to an experimentally imposed stressor. Although reactivity has merits in the study of physiological antecedents of temperament (given that reactivity is a core feature of the definition of temperament), evidence is stronger for the longitudinal stability of baseline fetal cardiac measures relative to stressor-elicited fetal cardiac response (DiPietro et al., 2000; DiPietro, Hodgson, Costigan, & Johnson, 1996; Lewis et al., 1970). This stability is critical given the purported constitutional nature of temperament; thus, we focus on baseline measures.

Regarding temperament, one of the most widely studied models of infant, and later childhood, temperament is the tri-factor model developed by Rothbart and colleagues (Rothbart & Bates, 2007). Rothbart and colleagues find empirical support for three factors — Surgency (comprised of items related to positive affectivity and extraversion), Negative Affectivity (NA), and Regulation/Orienting (sometimes called Effortful Control) — in infancy through toddlerhood (Putnam et al., 2006), childhood (Rothbart et al., 2001), and adolescence (Capaldi & Rothbart, 1992). For infants, the tri-factor model is measured by caregiver report on the Infant Behavior Questionnaire—Revised (IBQ-R). The IBQ-R asks caregivers to report the frequency of specific, observable behaviors related to temperament factors, which capture the infant's typical behavior across situations and contexts. Values are generated along a continuum of temperament factors and constructs, reflecting contemporary conceptualizations of temperament that emphasize the continuous nature of temperamental dimensions (*e.g.* Clark, 2005). Rothbart's temperament instruments are widely used, psychometrically sound, and converge with other methods of temperament assessment. Moreover, mothers' report on their infants' temperament offers many advantages over other approaches (Gartstein et al., 2012) and recent studies offer assurances that mothers' psychopathology does not bias their reports of their children's temperament (Olino et al., 2020). Temperament is moderately stable from infancy to preschool (Casalin et al., 2012; Putnam et al., 2008), although factors such as birth order, gender, and method of assessment (*e.g.* laboratory or parent-report) may impact the degree of stability in a child's early years (Bornstein et al., 2015; Rothbart et al., 2000).

Beyond measurement, an important consideration in evaluating the extant literature regarding fetal origins of infant temperament is sample characteristics. The extent to which findings about the origins of infant temperament generalize to diverse samples is predicated on the extent to which racially, ethnically, culturally, and socioeconomically diverse samples are employed in the study of the fetal origins of temperament. Unfortunately, reflecting a problem endemic to psychological research writ large (see: Rad et al., 2018; Roberts et al., 2020), studies in this literature have employed almost exclusively samples that are majority white, educated, and economically advantaged. Several studies have found differences between low- and middle-income families in both fetal development and infant temperament (*e.g.* Conger et al., 2010; Fernandes et al., 2018; Lantz et al., 2005; Rothwell & Han, 2010). Regarding the association between fetal characteristics and infant temperament, study sample homogeneity constrains our ability to generalize findings to diverse samples.

1.1. Fetal heart rate and infant temperament

Autonomic nervous system (ANS) processes regulate heart rate. Specifically, the ANS supports the regulatory and reactivity behaviors that are central to Rothbart's definition of temperament (Derryberry & Rothbart, 1988). Despite this, there are few studies testing the prospective relationship between fetal heart rate (FHR) and infant temperament. Using Kagan's laboratory assessment of temperament, both Werner et al. (2007) and Snidman et al. (1995) failed to show an association between baseline FHR and temperament (n's = 50 and 66, respectively). Findings from mother-reported temperament measures have been mixed. Using the Infants' Characteristics Questionnaire (ICQ; Bates et al., 1979), which has several shortcomings (Gartstein et al., 2012), DiPietro, Hodgson, Costigan, and Johnson (1996) found that FHR at 36-weeks' gestation (n = 31) was associated with infant "difficult" temperament factors, with more consistent support at six relative to three months of age across the four temperament factors. Werner et al. (2007) relied on the original Infant Behavior Questionnaire (Rothbart, 1981) and derived composite positive- and negative-reactivity variables. The positive-reactivity composite consisted of the Smiling and Activity subscales (*e.g.* frequency of smiling and laughter; movement of arms and legs) and the negative-reactivity composite consisted of the Fear and Distress to Limitations subscales (*e.g.* infant distress in novel situations; fussing/crying/showing distress while waiting for food). Werner et al. (2007) found ($n = 50$) that baseline FHR was positively associated with positive reactivity but not with negative reactivity. In a recent study of (n =149) dyads, using factors from the revised Infant Behavior Questionnaire (IBQ-R; Gartstein & Rothbart, 2003), Howland et al. (2020) found a small, non-significant positive association between FHR and the Negative Affectivity and a small, non-significant inverse association between FHR and Regulation/Orienting. Drawing conclusions from this divergent set of findings is difficult due to concerns about many of the methods of temperament assessment used in these studies. Moreover, studies relied on samples that were small and lacking in sociodemographic diversity (exceptions to the latter being Werner et al. and Howland et al.) We aimed to contribute to this literature by addressing these methodological concerns, testing association between FHR and temperament characteristics.

Overall, the current study addressed some concerns about measurement of the two key constructs and sought to increase knowledge of the extent to which FHR and infant temperament are associated in economically and ethnically diverse samples. Given the methodological shortcomings and mixed findings of the published studies that examined FHR and infant temperament, we based our hypotheses instead on two sets of findings: (a) continuity of heart rate from fetal periods through childhood (DiPietro et al., 2007) and (b) the involvement of heart rate in the ANS. On these bases, we hypothesized (Hypothesis 1) that FHR will be prospectively positively related to infants' positive and negative affectivity (Surgency and NA), speculating that a higher FHR would be associated with more frequent and stronger emotive behaviors in infancy. As a test of the specificity of this hypothesis, we also examined the association between FHR and Regulation/Orienting, expecting that they would not be associated. Further, to specify the temperament subscales that might account for the predicted associations between FHR and the broad infant temperament factors of Surgency and NA, we planned to test the association between FHR and subscales if either or both of the predicted associations were significant.

1.2. Fetal heart rate variability (FHRV) and infant temperament

Heart rate variability is thought to index regulation, *i.e.* maintaining homeostasis in response to changes in the environment (Beauchaine & Thayer, 2015; Porges, 2007). Measures of heart rate variability are believed to measure parasympathetic coordination between the brain and cardiovascular system, via the vagus nerve (Beauchaine, 2001). Heart rate variability and vagal tone in infants, children, and adults is associated with a broad array of measures of adaptive functioning and adjustment, such as social competence, secure attachment, memory, and executive functioning (Beauchaine & Thayer, 2015; Graziano & Derefinko, 2013). Individual differences in RSA or FHRV are moderately stable from the 3rd trimester to toddlerhood (DiPietro et al., 2007) and from infancy to early childhood (Bornstein & Suess, 2000). FHRV may reflect origins of infant emotion regulation and ability to control attention, *i.e.*, all three IBQ-R temperament factors.

A few studies tested these predictions. In a small sample ($n = 31$), DiPietro, Hodgson, Costigan, Hilton et al. (1996) and DiPietro, Hodgson, Costigan, Johnson (1996) failed to find FHRV at 36-weeks gestational age to predict any of the four ICQ derived infant temperament factors. Greater FHRV significantly predicted higher infant activity level, albeit at three and not six months of age, and based on a single question with no established psychometric properties. In two additional studies, researchers failed to show an association between FHRV and infant temperament measures, with temperament measured as lab based behavioral inhibition ($n = 49$; Werner et al., 2007), with the IBQ ($n = 49$; Werner et al., 2007) and with the IBQ-R ($n = 149$; Howland et al., 2020). Overall, taking into consideration the concerns about the approaches to measuring temperament, the typically small sample sizes, albeit in more diverse samples, these studies limit the ability to draw conclusions about FHRV being associated with infant temperament.

The current study aimed to correct for some of the methodological concerns that characterize most of the literature reporting on associations between baseline FHRV and infant reactivity and regulation. Specifying hypotheses for the relationship between FHRV and temperament was challenging due to a lack of extant empirical support. That is, Howland et al.'s (2020) failure to find significant associations between FHRV and infant temperament contradicted the well-replicated associations between HRV and adaptive functioning, including in infancy, and the continuity of HRV. We were persuaded by robust theoretical support for the relationship between FHRV and adaptive functioning, and thus we hypothesized (Hypothesis 2) that FHRV would be positively associated with Surgency, and Regulation/Orienting and inversely associated with Negative Affectivity.

1.3. Fetal coupling and infant temperament

A third aspect of fetal functioning, coupling of fetal heart rate and movement, also has conceptual and empirical links with infant temperament. Fetal coupling is an index of fetal heart rate change in response to fetal movement, whether measured in terms of frequency (the number of coupling instances) or latency (the amount of time between a change in heart rate and movement). Given observations that, across gestation, coupling frequency increases and latency shortens, coupling is interpreted to signal CNS maturation (DiPietro, Hodgson, Costigan, Hilton et al., 1996). Coupling requires neural control of the cardiac and somatic systems and has been associated with development of the parasympathetic nervous system (PNS) (DiPietro et al., 2001; DiPietro et al., 2010).

Despite this strong basis for expecting fetal coupling to predict infant temperament domains associated with regulation and the PNS, we found no published studies reporting on the association between coupling and infant temperament. Thus, a further aim of the current study was to examine the relationship between fetal coupling and infant temperament to inform our understanding of possible prenatal origins of parasympathetic control (indexed by fetal coupling) of emotion regulation (indexed by infant temperament). Given coupling's hypothesized function in both emotion regulation and neurological development, we hypothesized (Hypothesis 3) that coupling would be positively associated with Regulation/Orienting and Surgency and inversely associated with NA. As with our other fetal variables, to specify the temperament subscales that might account for the predicted associations between coupling and the three broad infant temperament factors, we planned to test the association between coupling and subscales if any of the predicted associations between coupling and a temperament factor were significant.

1.4. The current study

Overall, with our hypotheses, we aimed to improve on methodological shortcomings and weak or mixed findings in the published literature to further the understanding of fetal origins of temperament. We hypothesized that fetal heart rate (Hypothesis 1) will be prospectively positively related with infants' positive and negative affectivity (Surgency and NA), that FHRV (Hypothesis 2) would be positively associated with Surgency and Regulation/Orienting and inversely associated with NA, and that coupling (Hypothesis 3) would be positively associated with regulation (Regulation/Orienting) and Surgency and inversely associated with NA. Given our measurement and sampling concerns with most previous studies, we tested our hypotheses in a diverse sample using well-established and psychometrically sound approaches to measuring fetal variables and infant temperament. We collected data in the 3rd trimester due to the stability of fetal heart variables by this developmental window (DiPietro et al., 2000). We measured temperament at four months, close to the earliest age that infant temperament can be measured reliably.

2. Method

2.1. Participants

Data were derived from a longitudinal study of prenatal/infant development conducted in the Department of Psychiatry, Division of Behavioral Medicine at Columbia University Irving Medical Center (CUIMC). Women were recruited through the the Department of Obstetrics and Gynecology at CUIMC for a study assessing the effects of prenatal stress on epigenetic markers in placental tissue. Pregnant women were enrolled between eight to 26 weeks gestation. Participants were healthy, pregnant women, aged 18–45 years, carrying a singleton pregnancy, who reported that they did not smoke, drink, or take recreational or psychotropic drugs during their pregnancy, who could read and write English fluently, and did not have a diagnosis of Bipolar Disorder. The New York State Psychiatric Institutional Review Board approved all study procedures and all women provided informed consent.

Of the $n = 187$ women enrolled, $n = 90$ women and children participated at infant age 4-months, which was presented to the women as an optional component of their participation. The average gestational age at the time of the fetal session was 34.42 weeks (SD = 1.33). There were no statistically significant differences between the included and excluded participants in terms of age, gravidity, parity, race, ethnicity, Medicaid status, marriage status, or infant sex.

2.2. Fetal data collection

Fetal data collection was as described in previous work (Doyle et al., 2015; Gustafsson et al., 2018). Although several epochs of fetal data were collected, we relied on a 20-minute baseline period for hypothesis testing. Data were obtained using a Toitu MT 325 fetal actocardiograph (Toitu Co., Ltd, Tokyo, Japan), which detects FM and FHR via a single transabdominal Doppler transducer. The fetal data were collected from the Toitu's output port, digitized at 50 Hz using a 16–bit A/D card (National Instruments 16XE50) and analyzed offline.

FHR was calculated as the mean value for the baseline period. FHR in typically developing fetuses in the third trimester ranges from 120 beats per minute (bpm) to 160 bpm, with a mean of 136–139 bpm (Pildner von Steinburg et al., 2013). Per standard procedures, FHRV was computed by calculating the standard deviation of the FHR. In typically developing fetuses, FHRV increases across gestation (Van Leeuwen et al., 1999). In a large sample, DiPietro et al. (2015) found an average FHRV of 5.80 ($SD = 1.56$) in the third trimester. Coupling, operationalized here as the frequency of instances of fetal heart rate and fetal movement synchrony divided by the total number of movements (described in detail in Doyle et al. (2015)) was computed in overlapping four minute segments; artifacts were removed and the average of these segments was computed for the time period. DiPietro et al.'s (2015) large cohort found a mean coupling index of .32 (.10) in the third trimester.

2.3. Infant temperament

When the infants were on average postnatal age 18.10 weeks, $SD = 2.05$, mothers completed the 191-item parent report instrument, Infant Behavior Questionnaire—Revised (IBQ-R) (Gartstein & Rothbart, 2003). The IBQ-R instructs parents to rate their infant's behavior during the past week in a variety of domains on a seven-point scale, from one (Never) to seven (Always). The questionnaire yields scores on 14 scales, with ten to 18 items per scale; scale scores are a mean, with higher scores indicating more of the measured temperament characteristic (items can be scored on a range from 1 to 7).

Based on factor analytic work (Gartstein & Rothbart, 2003), scales cluster into three overarching factor scores: Surgency/Extraversion, Negative Affectivity (NA), and Orienting/Regulatory Capacity. Surgency/Extraversion is a measure of positive emotionality. Six subscales comprise this factor: Approach, Vocal Reactivity, High Intensity Pleasure, Smiling and Laughter, Activity Level, and Perceptual Sensitivity. NA is a measure of negative emotionality. Four subscales comprise this factor: Sadness, Distress to Limitations, Fear, and (reverse scored) Falling Reactivity/Soothability. Regulation/Orienting is a measure of effortful control. Four subscales comprise the factor: Low Intensity Pleasure, Cuddliness/Affiliation, Duration of Orienting, and Soothability.

The IBQ-R's reliability and validity have been demonstrated through internal consistency, mono-method discriminant validity, a similar factor structure between the IBQ-R and other methods of temperament assessment, convergence with laboratory observation, and modest inter-rater reliability between caregivers (Gartstein & Rothbart, 2003; Parade & Leerkes, 2008). Paralleling other studies (*e.g.* Dias et al., 2021; Gartstein & Rothbart, 2003), internal consistency (Cronbach's alpha) for our sample was good for all factors ($\alpha_{Surgency} = .94$, $\alpha_{NA} = .86$, $\alpha_{Regulation/Orienting} = .88$) and acceptable for all subscales (α's ranged from .72 to .88).

2.4. Mothers' demographic characteristics and birth outcomes

Women self-reported their demographic characteristics during the first study session. Women gave permission for access to their electronic medical records on labor and delivery, from which we abstracted gestational age (GA) at delivery and infant sex.

2.4.1. Approach to data analyses

We calculated descriptive statistics using the mean and standard deviation for continuous variables and percentage for categorical variables. To address missing data, we used fully conditional specification multiple imputation with five iterations using all study variables in SPSS Statistics Software (version 25). To test for the potential need to include covariates in hypothesis testing, we tested the association between study variables and the timing of the fetal session (in weeks GA), GA at the time of birth, mothers' age at delivery and infant sex.

We planned our approach to data analyses of the three hypotheses to minimize the number of analyses we conducted. Thus, we used two stages of data analyses to test our three hypotheses. First, we planned to test the association between fetal markers and temperament factors, either by running three multivariate regressions, one for each of the three DV infant temperament factors (Surgency, NA, and Regulation/Orienting), or, if we did not need to include covariates based on preliminary analyses, using bivariate correlations. Second, if a hypothesized association between a fetal variable and temperament factor was significant, we planned to run a second set of multivariate regressions or correlations on the temperament subscales rather than the factor scores. These analyses were meant to specify the narrower temperament subscales that accounted for associations between fetal variables and broad infant temperament factors for the purposes of conceptual understanding and interpretation.

3. Results

3.1. Descriptive statistics and preliminary analyses

3.1.1. Participant characteristics

See Table 1 for a description of participant characteristics. Demographic data reflect that the sample was ethnically (60 % non-Caucasian race; 63 % Latina ethnicity) and socio-demographically diverse. About half of the women (49 %) had less than a bachelor's degree and about half (46 %) of the women used Medicaid for health insurance.

3.1.2. Missing data

Overall, missing data for our predictor variables was low and we had no suspicion of data missing not at random. For FHR and FHRV, we imputed $n = 12$ values; for coupling, we imputed $n = 17$ values. Most fetal variables were missing due to a renovation of the laboratory, which resulted in equipment being unavailable towards the end of data collection. All results are from pooled data unless otherwise indicated. See Supplementary Table 1 for the non-imputed correlations between study variables.

3.1.3. Examination of potential covariates

See Table 2 for descriptive statistics of fetal variables and infant temperament measures.

There were no significant associations between the gestational variables (GA at fetal session, GA at birth, and mothers' age at delivery) and any study variables (all r's $< .22$). Regarding sex differences, only two out of 20 fetal or temperament variables showed significant sex differences. The first was coupling (non-imputed means: $m_{male} = .67$, $m_{female} = .63$) and the second was the temperament subscale of Smiling/Laughing (non-imputed means: $m_{male} = .5.00$ $m_{female} = 4.40$). Based on these findings, we did not include any covariates.

3.2. Hypothesis testing: fetal-temperament associations

Since we found that there were no relevant covariates and no associations between our fetal variables, we analyzed the correlations between study variables (rather than using a series of multiple regressions), since doing so reduced the number of statistical tests and

Table 1
Demographic Characteristics.

Variable	*M* or %	*N*
Maternal Characteristics		
Age (*M*)	30.20 (6.81)	90
Caucasian (%)	40	90
Latina (%)	63	90
Married (%)	47	90
Born in USA (%)	67	90
SES Characteristics		
< Bachelor's Degree (%)	49	90
Medicaid (%)	46	87
Income < $25,000 (%)	32	90
Income >$100,000 (%)	23	90
Pregnancy Characteristics		
Gravidity (*M*)	2.02 (2.28)	89
Parity (*M*)	.89 (1.09)	89
GA at birth (*M*)	39.29 (1.21)	90
Male Infants (%)	50	90

Table 2
Means and Standard Deviations of Study Variables.

	M	*SD*	*N*
Fetal Variables			
FHR (bpm)	139.41	8.55	78
FHRV	7.64	2.71	78
Coupling	.65	.09	73
Temperament Factors			
Surgency	4.27	.91	90
NA	3.12	.60	90
Regulation/Orienting	4.32	.74	90
Temperament Subscales			
Activity	4.30	.89	90
Distress	3.40	.75	90
Fear	2.33	.88	87
Duration of Orienting	4.20	1.26	90
Smiling/Laughter	4.70	1.23	90
High IP	5.37	1.33	90
Low IP	5.21	1.09	90
Soothability	3.64	.83	87
Falling Reactivity	3.87	.58	90
Cuddliness	4.20	.78	90
Perceptual Sensitivity	3.22	1.35	90
Sadness	2.85	1.05	90
Approach	3.96	1.17	87
Vocal Reactivity	4.11	1.20	90

Note: IP = Intensity Pleasure. FHRV = SD (FHR); Coupling = the frequency of instances of fetal heart rate and fetal movement synchrony divided by the total number of movements. *Temperament subscale and factor scores are the average of items that load onto each subscale or factor (items are rated on a scale of 1–7, with higher scores signifying more endorsement of a behavior).*

was the most parsimonious statistical design. See Table 3 for the correlations between fetal variables and temperament factors (and subscales, when analyzed).

Hypothesis 1. FHR will be positively related to Surgency and NA.

FHR was not statistically significantly associated with Surgency. FHR was positively associated with NA and FHR was not significantly associated with Regulation/Orienting.

Given the support for our hypothesized association between FHR and NA, we examined the associations between FHR and the four subscales that comprise the NA factor. FHR was significantly associated with two of the four subscales; FHR was positively associated with Distress to Limitations and Sadness. FHR was not significantly associated with Falling Reactivity or Fear.

Hypothesis 2. FHRV will be positively associated with Surgency and Regulation/Orienting and inversely associated with NA

Table 3
Correlations for hypothesis testing.

	FHR	FHRV	Coupling
FHR	1.00		
FHRV	.14	1.00	
Coupling	−.05	−.05	1.00
Surgency	.09	−.23*	.24*
Activity Level	–	−.15	.20*
Approach	–	−.21*	.12
High IP	–	−.15	.22*
Perceptual Sensitivity	–	−.18	.23*
Smiling/Laughter	–	−.22*	.18
Vocal Reactivity	–	−.16	.14
NA	.26*	.02	.07
Distress to Limitations	.28*	–	–
Fear	.13	–	–
Sadness	.31*	–	–
Falling Reactivity	−.03	–	–
Regulation/Orienting	−.03	−.23	.33**
Duration of Orienting	–	–	.25*
Low IP	–	–	.32**
Soothability	–	–	.21
Cuddliness	–	–	.16

Note: IP = Intensity Pleasure.

FHRV was inversely associated with Surgency. FHRV was not significantly associated with Regulation/Orienting or NA.

Given our findings that FHRV and Surgency were significantly (inversely) associated, we examined the associations between FHRV and the subscales that comprise the Surgency factor. FHRV was inversely associated with the Approach and Smiling/Laughter subscales, and not significantly associated with any of the other subscales which comprise the Surgency factor.

Hypothesis 3. Coupling will be positively associated with Regulation/Orienting and Surgency and inversely associated with NA

Coupling was positively associated with Regulation/Orienting and Surgency. Coupling was not significantly associated with NA.

Based on significant associations between coupling and both Surgency and Regulation/Orienting, we reported the associations between coupling and the subscales that comprise those two temperament factors. In terms of the Surgency factor's six subscales, coupling was significantly positively correlated with three, Activity Level, High Intensity Pleasure, and Perceptual Sensitivity, and not significantly associated with the other three. In terms of the Regulation/Orienting factor's four subscales, coupling was statistically significantly associated with two. Coupling was positively associated with Duration of Orienting and Low Intensity Pleasure.

4. Discussion

In response to increasing interest in the fetal origins of development, we contributed to this line of research with a prospective, longitudinal study design, testing to what extent fetal heart indices predict infant temperament, a highly meaningful child outcome. We found that three baseline fetal indicators—fetal heart rate (FHR), fetal heart rate variability (FHRV), and coupling—predicted some facets of infant temperament, measured with a psychometrically sound approach in a diverse sample. As we hypothesized, FHR was positively associated with NA and not associated with Regulation/Orienting. Contrary to our hypotheses FHR was not associated with Surgency. Contrary to our hypotheses, FHRV was inversely associated with Surgency and FHRV was not associated with NA or Regulation/Orienting. Finally, as hypothesized, coupling was positively associated with Regulation/Orienting and Surgency. Contrary to our hypothesis, coupling was not associated with NA. Overall, our findings are consistent with the idea that fetal physiological processes of reactivity and regulation precede and underlie the psychological construct of temperament as observed in infants.

After analyses at the level of the three IBQ-R factors, we conducted sensitivity analyses to determine which subscales were responsible for the association between fetal variables and temperament factors and to assess whether maternal psychological distress was associated with temperament characteristics. For FHR, we found a positive association between FHR and the Distress to Limitations and Sadness subscales of the NA factor. For FHRV, we found an inverse association between FHRV and the Approach and Smiling/Laughter subscales of the Surgency factor. Finally, for coupling, we found a positive association between coupling and the Duration of Orienting and Low Intensity Pleasure from the Regulation/Orienting factor and subscales between coupling and the Activity Level, High Intensity Pleasure, and Perceptual Sensitivity subscales from the Surgency factor. To rule out potential bias in infant temperament ratings being influenced by maternal depression, anxiety, and/or perceived stress, we tested the association between mothers' depression, anxiety, and perceived stress levels at the 4-month postnatal visit and all our infant temperament factors and subscales. We found no statistically significant associations between concurrent maternal anxiety, depression, or perceived stress and infant temperament (for a description of measures and results see Supplemental Tables 3 and 4), suggesting that our results are not confounded by maternal psychological distress.

Our findings help to clarify the literature, relative to the disparate findings noted in the introduction. In terms of FHR, our finding of a positive association with NA and no significant association with Surgency converge with Howland et al. (2020), though they also found an association between FHR and Regulation/Orienting, which we did not. However, their positive association between FHR and NA and Surgency failed to reach statistical significance, *i.e.* they reported these associations as statistical trends. DiPietro, Hodgson, Costigan, and Johnson (1996) found that FHR was positively associated with Activity Level albeit, as already noted, their operationalization of Activity Level was a single question with no psychometric data reported. In our findings, FHR was not associated with Surgency, which is the factor that Activity Level loads onto. More broadly, given the methodological shortcomings and mixed findings of the published studies that examined FHR and infant temperament, we had based our hypotheses on two sets of findings: (a) continuity of heart rate from fetal periods through childhood (DiPietro et al., 2007) and (b) the involvement of heart rate in the ANS. In light of these sets of findings, overall, our findings on FHR and infant temperament suggest that negative affectivity in infants may be at least partially a continuation of autonomic processes that originate during the fetal period.

In terms of FHRV, our findings notably add to the literature given that neither Howland et al. (2020) nor Werner et al. (2007) found significant associations between FHRV and infant temperament, albeit with the methodological limitations in the latter study. Based on the strength of knowledge of associations between HRV and adaptive functioning, we hypothesized that FHRV would be positively associated with Surgency and Regulation/Orienting and inversely associated with Negative Affectivity. Our findings that FHRV was inversely associated with Surgency and not associated with NA or Regulation/Orienting were incongruent with our hypothesis and combined with the lack of findings in the published literature, should be interpreted cautiously. However, if replicated in future work, this finding suggests that the parasympathetic regulation indexed via FHRV may be associated with infants' later regulation of positive arousal.

Finally, a major contribution of this study is the inclusion of fetal coupling as a predictor variable of later infant temperament. Given coupling's hypothesized function in both emotion regulation and neurological development, we hypothesized that coupling would be positively associated with Regulation/Orienting and Surgency and inversely associated with NA. Our findings that coupling was positively associated with Surgency and Regulation/Orienting suggest that fetal coupling indexes aspects of offspring's constitutional regulatory capabilities that are associated with infant's reactivity to positive stimuli and their ability to modulate their attention and inhibit certain behavioral responses.

From a developmental perspective, it is important to consider how our findings might generalize to studies of older children. That is, to what extent might fetal variables predict temperament in middle childhood or adolescence? In a study of fetal variables and temperament in 7−14-year-old children ($n = 333$), DiPietro et al. (2018) found that lower resting FHR and FHRV and faster coupling latency predicted children's higher levels of behavioral inhibition (operationalized using Fear and Shyness subscales on Rothbart's childhood temperament scales), but not Surgency. Further research is needed to understand longitudinal patterns in associations between fetal measures and temperament beyond infancy and to test models of likely transactional associations between child and environmental predictors of those developmental pathways. Further, future studies might consider taking a person-centered approach to assessing the fetal origins of temperament, to evaluate whether specific profiles of fetal variables are associated with characteristic clusters of temperament factors across time.

We found associations between fetal cardiac measures and infant temperament characteristics; however, our effect sizes were only small to moderate. Since we do not see fetal heart processes as being either defining of, or prodromal to temperament, it is important to ask: what else contributes to individual differences in reactivity and regulation, and under what circumstances might the continuity between fetal cardiac measures and temperament be disrupted? There are several other factors that may impact the development of temperament and the continuity between fetal cardiac measures and temperament. Among these factors are parenting styles (Dalimonte-Merckling & Brophy-Herb, 2019), genetics (Papageorgiou & Ronald, 2017), and the intrauterine environment (Gartstein & Skinner, 2018). Our study situates FHR, FHRV, and coupling as factors preceding temperament in some offspring; a critical next step, however, will be testing if the association between fetal cardiac variables and temperament is moderated by other biological (*e.g.* epigenetic) or environmental (*e.g.* parenting) factors.

Our results point to several other important future directions. First, investigators should continue to prioritize sampling diverse populations to test the generalizability of findings of the fetal origins of infant temperament. This is essential since researchers have shown that fetal characteristics can systematically vary between cultural contexts (DiPietro et al., 2004). Additional studies sampling diverse cohorts are necessary for bettering our understanding of why there are some discrepancies between our study and others' results (reviewed above). Are these differences a function of sample characteristics, measurement, or other factors? As it stands, with the majority of studies consisting of predominantly Caucasian and economically advantaged subjects, our ability to interpret different patterns of findings is curtailed. Second, these findings highlight the need to continue to conduct research using psychometrically sound measures based on well-validated theories of temperament and that allow researchers to assess temperament across time.

A third future direction from our work is based on research derived from the Research Domain Criteria (RDoC) constructs. There is overlap between aspects of temperament and factors from several of the RDoC systems. For example, researchers' have increasingly focused on the relationship between dispositional levels of fear (an aspect of NA) and an evoked physiological startle response (*e.g.* Kastner-Dorn et al., 2018). Although we found that baseline FHR was not associated with infant fear (a subscale of NA), FHR was positively associated with the NA factor and with two other NA subscales: infant sadness and distress to limitations. Future studies might consider if constructs such as infant sadness and distress to limitations precede a startle response-fear association in later development, and if, as in our sample, baseline fetal measures predict this meaningful and clinically relevant outcome.

Our study has several notable strengths. As mentioned, this study represents a major diversification of the samples relative to all but a few of the published studies regarding the fetal origins of infant temperament. Also as noted, we used well-validated and psychometrically sound measures of both fetal cardiac development and infant temperament. Finally, this study is also, to our knowledge, the first to test the association between fetal coupling and infant temperament. We found that FHRV and coupling shared little common variance and they predicted different temperament variables, suggesting that coupling may uniquely index physiological regulation and prenatal precursors of temperament as compared to FHRV. Another strength of our study is that to reduce the number of statistical tests, we restricted examination of associations between fetal variables and temperament subscales to findings of statistically significant associations between fetal variables and the associated temperament factor.

There are also limitations that should be noted when interpreting our results. First, we used baseline measures of fetal cardiac variables. Baseline measures are the most longitudinally stable, an important consideration given the putative constitutionality of temperament; however, since temperament is defined partially by reactivity, using an evoked physiological fetal variable may be worth consideration for future study designs. Reliable and safe experimental manipulations using human fetuses are an important next step in the field of individual differences research generally and fetal origins of behavior more specifically (Reid & Dunn, 2021). Second, although our study took an important step in the direction of sampling a more diverse population, future studies should continue to sample from other underrepresented populations. Third, our assessment of temperament was done via a single, albeit psychometrically sound, parent-report measure; future studies should consider collecting infant temperament data from multiple caregivers or multiple temperament assessment tools. Finally, our sample size, while larger than several of the previous studies, was modest ($n = 90$).

Despite these limitations, our study found support for prenatal antecedents to infant temperament in a diverse sample using well-validated measures. These findings contribute to our understanding of how emotional development begins before birth. Specifically, our findings support the longstanding supposition of fetal origins of temperament.

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NSF.

CRediT authorship contribution statement

B.C. Pingeton: Conceptualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **S.H. Goodman:** Conceptualization, Resources, Writing - review & editing, Supervision. **C. Monk:** Investigation, Resources, Data curation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.infbeh.2021.101643.

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