JNeurosci
THE JOURNAL OF NEUROSCIENCE

Research Articles | Development/Plasticity/Repair

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https://doi.org/10.1523/JNEUROSCI.2363-23.2024

Received: 15 December 2023
Revised: 31 March 2024
Accepted: 3 April 2024

Title: Mapping early brain-body interactions: associations of fetal heart rate variation with newborn brainstem, hypothalamic, and dorsal anterior cingulate cortex functional connectivity

Abbreviated title: Fetal heart rate and newborn brain associations

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Number of pages: 16 pages (text, without references), 8 pages (tables and figures)

Number of figures, tables, multimedia, and 3D models (separately): 2 tables, 6 figures

Number of words for abstract, introduction and discussion (separately): 231, 664, 1091

Conflict of Interest Statement

The authors declare no competing financial interests.

Acknowledgments

This work was supported by the National Institute of Mental Health (NIMH) R01MH093677 (C.M. & B.S.P.), the Eunice Kennedy Shriver National Institute for Child Health and Human Development K23HD092589 (M.S.), the NIMH K24MH127381 (M.S.) and R01MH126133 (M.S. & D.S.), and the National Center for Advancing Translational Sciences TL1TR001875 (A.P. & C.H.) grants. We would like to thank Kristiana Barbato, Alida Davis, Erica Lambeth, and Ashley Rainford for their support with data collection and Grace Liu for her support with data management. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Keywords: autonomic nervous system, heart rate, heart rate variability, infant, brain, fMRI, HR, HRV, hypothalamus, brainstem, dACC, language, memory, motor control

Abstract

The autonomic nervous system (ANS) regulates the body's physiology, including cardiovascular function. As the ANS develops during the second to third trimester, fetal heart rate variability (HRV) increases while fetal heart rate (HR) decreases. In this way, fetal HR and HRV provide an index of fetal autonomic nervous system development and future neurobehavioral regulation. Fetal HR and HRV have been associated with child language ability and psychomotor development behavior in toddlerhood. However, their associations with post-birth autonomic brain systems, such as the brainstem, hypothalamus, and dorsal anterior cingulate cortex (dACC), have yet to be investigated even though brain pathways involved in autonomic regulation are well established in older individuals. We assessed whether fetal HR and HRV were associated with the brainstem, hypothalamic and dACC functional connectivity in newborns. Data were obtained from 60 pregnant individuals (ages 14-42) at 24-27 and 34-37 weeks gestation using a fetal actocardiograph to generate fetal HR and HRV. During natural sleep, their infants (38 males and 22 females) underwent a fMRI scan between 40-46 weeks of postmenstrual age. Our findings relate fetal heart indices to brainstem, hypothalamic, and dACC connectivity and reveal connections with widespread brain regions that may support behavioral and emotional regulation. We demonstrated the basic physiologic association between fetal HR indices and lower and higher order brain regions involved in regulatory processes. This work provides the foundation for future behavioral or physiological regulation research in fetuses and infants.

Significance statement

Fetal heart rate indices are quantifiable, developmental markers of the fetal autonomic nervous system. Variations in their trajectories can signal compromised neurodevelopmental outcomes. We assessed associations between fetal heart rate indices and early infant brain development to identify unique or common associations corresponding to autonomic nervous system maturation patterns. We found associations between fetal heart rate indices and infant brainstem, hypothalamic, and dACC connectivity—areas that support autonomic and behavioral regulatory functions. The study demonstrates that these associations between ANS and brain regions involved in autonomic regulation exist early in life. These findings are a first step to understanding how these brain connections form the basis of future regulatory development.

1. Introduction

The autonomic nervous system (ANS) is a component of the peripheral nervous system that regulates the body's physiology. It cooperatively modulates the heart rate through the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). The brain pathways involved in autonomic regulation are well-established in human adults. For example, the hypothalamus connects the lower-order (including the medulla oblongata) and higher-order (including the dorsal anterior cingulate—dACC) nervous systems to interpret environmental stimuli and regulate cardiovascular function (Ulrich-Lai and Herman, 2009).

The ANS regulatory capacity begins as early as eight weeks gestation. ANS activity is a marker of developing fetal brain functions and modulates cardiovascular responses (David et al., 2007; Chouchou and Desseilles, 2014). Fetal ANS development can be non-invasively assessed with fetal heart rate (HR) and fetal heart rate variability (HRV) (Spann et al., 2014; Spann et al., 2015; de la Cruz et al., 2019). Internal and external stimuli cause autonomic adjustments to maintain homeostasis, resulting in natural HR and HRV variations (Oliveira et al., 2019). Fetal HR is mainly controlled by the SNS early in gestation and the PNS later in gestation (Hofmeyr et al., 2014). During the transition from the late second into the third trimester, the development of fetal HR is driven by the increase of the parasympathetic influence and the changes in autonomic control from the medulla to higher cortical regions (David et al., 2007; DiPietro et al., 2015). This shift is reflected in the decline of mean fetal HR during rest (Dipietro et al., 2001; DiPietro et al., 2015; Heuser, 2020; Cerritelli et al., 2021). By 30 weeks gestation, ANS modulation involves input from the dACC and medial prefrontal cortex (mPFC) (Robinson et al., 1966; Horiuchi et al., 2006).

Fetal HR and HRV are also associated with risk for poor neurodevelopmental outcomes (Hofmeyr et al., 2014; Karmakar et al., 2015; Howland et al., 2020). Fetal HR and HRV correlate with later higher motor control and language development scores (DiPietro et al., 2007) and early temperament and emotion regulation scores (Feldman, 2006; Werner et al., 2007; Dipietro et al.,

2018; Howland et al., 2020; Pingeton et al., 2021). Additionally, prenatal exposures to maternal hyperglycemia and environmental toxins can alter fetal ANS development (DiPietro et al., 1999; Monk et al., 2000; DiPietro et al., 2002; Monk et al., 2004; Zisser et al., 2006; DiPietro et al., 2013). Overall, this suggests fetal ANS activity, as measured by HR indices, is an important indicator for developmental outcomes.

Functional neuroimaging studies with healthy adults connect cortico-limbic activity and autonomic regulation (de la Cruz et al., 2019). Strong age-dependent associations exist between HRV and functional connectivity of the posterior cingulate cortex and the medial prefrontal cortex (mPFC) (Kumral et al., 2019). Connectivity between brain regions involved in ANS regulation exists as early as 24-27 weeks of gestation (Thomason et al., 2015; Borsani et al., 2019). Further, functional networks are largely observable in the neonatal period (Doria et al., 2010; Gao et al., 2015; Gao et al., 2017). For example, dACC shows strong connectivity to the insula in the neonatal period (Spann et al., 2018). Nevertheless, studies associating early brain functioning with ANS regulation are lacking. In the single published study, higher fetal HRV assessed at 34–37 weeks gestation was positively associated with greater infant connectivity between the dACC and mPFC (Spann et al., 2018).

This study investigated the associations between fetal HR indices during the second and third trimesters and newborn brain connectivity. We acquired fetal HR data during the second and third trimesters to measure fetal ANS development. We assessed brainstem, hypothalamus, and dACC functional connectivity using resting-state fMRI data acquired at 40-46 weeks postmenstrual age (PMA). Our primary hypothesis was that third trimester fetal HR indices would associate significantly with newborn functional brain connectivity in the seed areas involved with the ANS. Our secondary hypothesis was that second trimester fetal HR and HRV would yield similar associations. The novelty of this research precluded specific hypotheses about the direction of these effects.

2. Materials and Methods

2.1. Participants

Pregnant individuals, aged 14-42, were recruited in the second trimester (13-28 weeks) through the Departments of Obstetrics and Gynecology at Columbia University Irving Medical Center (CUIMC), Weill Cornell Medical College, and flyers posted in the CUIMC vicinity. All pregnant participants had no major health problems during recruitment and received routine prenatal care. Adult participants provided informed consent. If they were under 18, they completed an assent form, and their parent signed a consent form. The New York State Psychiatric Institute Institutional Review Board approved the procedures. Participants were excluded from the studies if they acknowledged using recreational drugs, tobacco, or alcohol, taking medications that affect cardiovascular function, or not speaking English fluently.

2.2. Fetal assessment

To maximize reproducibility, pregnant individuals participated in a standardized, validated protocol (Besinger and Johnson, 1989; DiPietro et al., 1999; DiPietro et al., 2004). They were asked to refrain from eating 1.5 hours before the visit. During data collection, individuals were awake to avoid acute increases or decreases in fetal HR or movement that would affect data collection. Finally, HR indices were collected after 20 weeks of gestation when they are more stable.

Fetal HR was acquired while the participants were in a semi-recumbent position for 20 minutes, using a Toitu MT 325 fetal actocardiograph (Toitu Co.,Ltd, Tokyo, Japan) during the 24-27th (second trimester) and 34-37th (third trimester) weeks of gestation. The Toitu detects fetal HR via a single transabdominal Doppler transducer. The signal is processed through a series of filters. These filters remove the frequency components of the Doppler signal that are associated with fetal HR (Besinger and Johnson, 1989; DiPietro et al., 1999; DiPietro et al., 2004). Fetal HR output

was digitized at 50 Hz using a 16 bit A/D card (National Instruments 16XE50). Fetal HR below 80 beats per minute (bpm) or above 200 bpm were removed. Custom MATLAB programs (http://www.mathworks.com) were used to calculate mean fetal HR and the standard deviation of fetal HR (i.e., HRV). A detailed algorithm description has previously been published (Doyle et al., 2015; Spann et al., 2015; Spann et al., 2018).

2.3. Infant imaging

2.3.1 Infant MRI preparation and data acquisition

Sixty infants (38 males and 22 females) were scanned within the first weeks of postmenstrual life (PMA ≤ 46 weeks). After they were fed and swaddled, they were given time to fall asleep naturally. We used foam ear plugs, wax, and ear shields (Natus Medical) to dampen the scanner noise. The infants' heart rate and oxygen saturation were monitored continually during the scan (InVivo Research, Biopac). Images were obtained using a 3 Tesla Signa MRI scanner (General Electric).

There are two different sets of parameters using during scanning, earlier subjects used a different sequence than the later subjects. The images for the earlier subjects (n=38) were acquired using a 3 Tesla General Electric Signa MRI scanner with an eight-channel head coil. A 2D, multiple shot, fast spin echo sequence was employed to obtain high-resolution anatomical T2-weighted images, with PROPELLER (Periodically Rotated Overlapping Parallel Lines with Enhanced Reconstruction) used to decrease motion artifacts in the reconstructed MR images (Pipe, 1999): repetition time (TR) = 10,000 ms; echo time (TE) = 130 ms; echo train length (ETL) = 32; matrix size = 192 × 192; field of view (FOV) = 190 × 190 mm; phase FOV = 100%; slice thickness = 1.0 mm; number of excitations (NEX) = 2. The spatial resolution of the T2-weighted images was 1 mm^3. Functional images were acquired using a standard echoplanar imaging sequence: TR = 2200 ms; TE = 30 ms; matrix size = 64 × 64; FOV = 190 × 190 mm; phase FOV

= 100%; slice thickness = 5.0 mm, contiguous; number of slices = 24; bandwidth = 7812.5 Hz; voxel size = 2.969 x 2.969 x 5. Due to the infant waking, the number of runs acquired were different for each participant. A median of 6 runs of 102 volumes (3 min 44.4s each) were collected per infant. The images for the newer subjects (n=10) were acquired using a 3 Tesla General Electric Signa Premier with a 48-channel head coil and the anatomical T2-weighted images were acquired with: TR=3202 ms; TE=60 ms; matrix size=256x256; FOV=256x256 mm; phase FOV=100%; ETL=140; slice thickness=0.9 mm. Functional images for the new subjects were acquired using a standard echoplanar imaging sequence: TR=2000 ms; TE=30 ms; matrix size=64 x 64; FOV=190 x 190 mm; phase FOV = 100%; slice thickness = 3.0mm; number of slices = 34; bandwidth=7812.5 Hz; voxel size = 2.969 x 2.969 x 3. The functional sequences have built-in discarded volumes to allow the tissue to reach a steady state. The number of runs varied per participant and a median of 3 runs of 90 volumes were obtained for each infant. When combining all participants, we removed the last 12 volumes from the fMRI data from the earlier subjects to match the number of volumes from the newer subjects.

2.3.2. Pre-processing

Anatomical images were skull stripped using FSL (https://fsl.fmrib.ox.ac.uk/fsl/). If, after visual inspection, any non-brain tissue remained, it was removed manually. Unless otherwise specified, all further analyses were performed using BioImage Suite (Joshi et al., 2011). Anatomical images were non-linear registered to a custom, age-appropriate template (Spann et al., 2018) using a validated algorithm (Scheinost et al., 2017). After the anatomical scans were registered to the template, functional images were rigidly aligned to the anatomical images. All transformation pairs were calculated independently and combined into a single transform, warping the single participant results into common space. This single transformation allows the individual participant images to be transformed to common space with only one transformation, thereby reducing interpolation error.

We performed motion correction on the functional data with SPM12 (https://www.fil.ion.ucl.ac.uk/spm/). The frame-to-frame motion was calculated across all the functional volumes. Data were further cleaned as previously described (Kwon et al., 2014). Linear and quadratic drifts, mean cerebrospinal fluid signal, mean white matter signal, mean gray matter signal, and a 24-parameter motion model (6 motion parameters, 6 temporal derivatives, and their squares) were regressed from the data. A Gaussian filter with an approximate cutoff frequency of 0.12 Hz was used to smooth the functional data temporarily.

Because motion and the amount of data available for analysis can affect functional connectivity measures (Van Dijk et al., 2012; Noble et al., 2017), we used a strict inclusion criterion that participants had at least 2 runs of data with an average frame-to-frame motion of <0.15 mm. We used the average of 2 runs per subject. Only one infant was removed using these criteria. If more than two resting state runs were available, we included the ones with the lowest average frame-to-frame motion in the analysis.

2.3.3. Seed connectivity

The seed regions of interest were defined as the bilateral medulla, hypothalamus, and dorsal anterior cingulate cortex (dACC). The seeds were manually defined on the reference brain (Fig. 1). The approximate MNI coordinates are: dACC (-1,24,26), medulla (-4,-36,-35), and hypothalamus (-2,-3,-3). The temporal signal noise ratios for each seed are 170.71±109.35 for the dACC, 49.37±32.84 for the medulla, and 65.36±34.86 for the hypothalamus. In each participant, the time course of the reference region was computed by averaging the time course of every voxel within the seed region. This time course was correlated with the time course for every voxel in gray matter to create a map of r-values representing seed-to-whole-brain connectivity. Using the Fisher's transform, we transformed these r-values to z-values, generating one map for each seed and representing the strength of correlation with the seed for each participant.

241 *2.4. Statistical analyses*

242 Our primary analysis assessed the association of mean resting fetal HR and HRV during
243 the third trimester with measures of connectivity of the seed areas to the whole brain. The second-
244 trimester associations were also assessed. Our sample during the second trimester was smaller
245 (n=33) than in the third trimester (n=48); therefore, these results are presented as secondary.
246 Finally, for exploratory analyses, we associated the change from second to third trimester fetal
247 HR indices and seed connectivity (n=27). The imaging data were analyzed using voxel-wise linear
248 models controlling for biological sex, motion, maternal age, scanner/sequence, and PMA.
249 Significant imaging clusters were shown at $p<0.05$, corrected for multiple statistical comparisons.
250 We corrected for multiple comparisons across gray matter using cluster-level correction estimated
251 via AFNI's 3dClustSim (version 16.3.05, https://afni.nimh.nih.gov/) with 10,000 iterations,
252 smoothness estimated with the -ACF option, an initial cluster forming threshold of $p=0.001$, and
253 the gray matter mask applied in preprocessing.

254

3. Results

3.1. Demographics

Of the 60 participants, our final sample size consisted of 48 neonates with usable fetal HR in the third trimesters and high-quality fMRI data and of 33 neonates with usable fetal HR in the second trimesters and high-quality fMRI data. The average age of the pregnant women was 21 (20.98 ± 5.5) years; the majority were Hispanic (80%). Neonates were scanned at an average 43 (42.97 ± 2.04) weeks PMA; the majority were male (63%). The infants were healthy and were born without delivery complications at gestational age >37 weeks. The mean frame-to-frame motion was 0.05 mm and was not correlated with our main outcomes (fetal HR and HRV; r's<0.05).

3.2. Primary Analyses

3.2.1. Associations of mean resting fetal HR in the third trimester with bilateral medulla, dACC, and hypothalamic connectivity in neonates (n=48).

Higher mean fetal HR was positively associated with the connectivity between the medulla and the bilateral precentral and postcentral gyrus and the right inferior parietal lobe (IPL; Fig. 2). Higher mean fetal HR displayed a positive association with the connectivity between the hypothalamus and the left and right anterior part of the middle frontal gyrus (MFG) (Fig. 2). Higher mean fetal HR was inversely associated with connectivity between the dACC and left cerebellum. Additionally, higher mean fetal HR was associated with positive connectivity between the dACC and a cluster that extends in the IPL and the superior temporal gyrus (STG; Fig. 2). The location and size of all significant clusters are summarized in Table 1.

3.2.2. Associations of fetal HRV in the third trimester with bilateral medulla, dACC, and hypothalamic connectivity in neonates (n=48).

Higher fetal HRV was positively associated with the connectivity between the medulla and the left precuneus and paracentral lobule (Fig. 3). Higher fetal HRV was inversely associated with the connectivity between the hypothalamus and the left middle temporal gyrus (Fig. 3). Higher fetal HRV was positively associated with connectivity between the bilateral dACC and the left superior frontal gyrus and between the bilateral dACC and the right lateral occipital gyrus (Fig. 3). The location and size of all significant clusters are summarized in Table 1.

3.3. Secondary analyses

3.3.1. Associations of mean resting fetal HR in the second trimester with bilateral medulla, hypothalamic, and dACC connectivity in neonates (n=33).

Higher mean fetal HR was inversely associated with the medulla-left MFG connectivity and positively with medulla-right STG and IPL connectivity (Fig. 4). Higher mean fetal HR was positively associated with the connectivity between the hypothalamus and left subcortex and between the hypothalamus and left precuneus/paracentral lobule (Fig. 4). Higher mean fetal HR was inversely associated with the connectivity between the dACC and the right cerebellum, between the dACC and the right basal ganglia, and between the dACC and left insula (Fig. 4). Higher mean fetal HR was associated with the connectivity between the dACC and the bilateral visual cortex and between the dACC and the right lateral occipital gyrus (Fig. 4). The location and size of all significant clusters are summarized in Table 2.

3.3.2. Associations of fetal HRV in the second trimester with bilateral medulla, hypothalamic, and dACC connectivity in neonates (n=33).

Higher fetal HRV was positively associated with the connectivity between the medulla and right cerebellum, between the medulla and the left fusiform gyrus/cerebellum, and between the medulla and right SPL (Fig. 5). Higher fetal HRV was positively associated with connectivity between hypothalamus and right and left subcortex (Fig. 5). Higher fetal HRV was positively

associated with connectivity between dACC and middle cingulate cortex (Fig. 5). The location and size of all significant clusters are summarized in Table 2.

3.4. Exploratory analyses

3.4.1. Associations of the change in mean fetal HR from the second to third trimester with bilateral medulla, hypothalamic, and dACC connectivity in newborns (n=27).

Higher change in fetal HR was positively associated with the connectivity between the medulla and the left precentral and postcentral gyrus (Fig. 6). Higher change in fetal HR was inversely associated with the connectivity between the medulla and the cerebellum (Fig. 6). No associations with the hypothalamus were observed. Higher change in fetal HR was positively associated with the connectivity between the dACC and the right inferior frontal gyrus (Fig. 6). The location and size of all significant clusters are summarized in Table 3.

3.4.2. Associations of the change in fetal HRV from the second to third trimester with bilateral medulla, hypothalamic, and dACC connectivity in neonates (n=27).

Higher change in fetal HRV was positively associated with the connectivity between the medulla and the right inferior occipital gyrus (Fig. 7). Higher change in fetal HRV was inversely associated with the connectivity between the medulla and the left hippocampus and between the medulla and the right cerebellum (Fig. 7). Higher change in fetal HRV was positively associated with the connectivity between the hypothalamus and the bilateral precuneus and paracentral lobule and between the hypothalamus and the left precentral gyrus (Fig. 7). Higher change in fetal HRV was inversely associated with the connectivity between the hypothalamus and left middle temporal gyrus (Fig. 7). Higher change in fetal HRV was positively associated with the connectivity between the dACC and the left middle frontal gyrus (Fig. 7). The location and size of all significant clusters are summarized in Table 3.

4. Discussion

This study investigated the associations between fetal HR indices and functional connectivity of brain regions involved in autonomic regulation, including the medulla, hypothalamus, and dACC (Critchley et al., 2003). Our main findings are significant associations ($p<0.05$, corrected) within both trimesters for fetal HR and HRV with these brain regions. Our results suggest that a diverse network of brain regions engage with core regulatory regions and are thereby associated with autonomic regulation at this early age. They complement results from prior neuroimaging studies with adults, which demonstrated that multiple, widespread brain regions extending from the neocortex to the brainstem are involved in ANS regulation (Ulrich-Lai and Herman, 2009; Beissner et al., 2013; Macey et al., 2015; de la Cruz et al., 2019; Matusik et al., 2023).

Though links have been shown in non-human primates and adults (Candia-Rivera, 2022), this study demonstrates that these associations between ANS and brain regions involved in autonomic regulation exist early in life. These findings align with the maturation of autonomic regulation. The medulla oblongata is a primary regulator of fetal heart rate, but autonomic regulation shifts to include higher-order cortical regions around the end of the second trimester (Jongen et al., 2017; Mulkey and Plessis, 2018; Heuser, 2020). For example, ANS regulation involves input from the dACC and mPFC by 30 weeks gestation, which is characterized by a decline in mean fetal HR during rest (Robinson et al., 1966; Dipietro et al., 2001; Horiuchi et al., 2006; DiPietro et al., 2015; Heuser, 2020; Cerritelli et al., 2021). This progression is reflected in our fetal HR findings. For example, in our exploratory analyses, positive associations are observed in cortical areas, whereas inverse associations are observed in the subcortex and cerebellum, consistent with the shift to higher-order cortical regions in the third trimester.

Our findings aid the understanding of the ANS in behavioral and emotional regulation, as well. These functions are important in identifying risks for compromised development of self-regulation. Indeed, the motivation and ability to regulate internal physiological states serve as a foundation for other social-emotional regulation (Thompson and Levitt, 2010). Fetal HR and HRV associated with brain regions involved in behavioral and emotional regulation in early infancy. For example, the post-central gyrus, hypothalamus, and temporal lobe play roles in sensory and emotion processing (Fanselow and Dong, 2010; Potegal, 2012; Wong and Gallate, 2012; Kropf et al., 2019). The cerebellum also plays a critical role in social and emotional functions during infancy (Koziol et al., 2014; Beuriat et al., 2022). The cerebellum and the pre- and post-central gyrus modulate various sensory and motor functions that promote appropriate infant regulation to facilitate learning and environmental engagement (Diamond, 2000; Williams et al., 2020). Sensory or emotional stimuli influence ANS regulation and higher-order brain regions involved in behavioral and emotional regulation. Fetal HR indices correlate with behavioral (Dipietro et al., 2018; Howland et al., 2020) and emotional regulation in infancy (Feldman, 2006; Pingeton et al., 2021) and sensorimotor development at two years (DiPietro et al., 2007). This study adds to the previous literature by showing that the brain correlates of ANS regulation measured during the fetal period align with previous findings.

Fetal HR and HRV are markers of neurodevelopmental outcomes (Hofmeyr et al., 2014; Karmakar et al., 2015; Howland et al., 2020). Prior work demonstrated their associations with motor control, language development (DiPietro et al., 2007), and temperament (Feldman, 2006; Werner et al., 2007; Dipietro et al., 2018; Howland et al., 2020; Pingeton et al., 2021). Many brain regions connected to the regulatory seed regions detected in our analyses involve similar abilities (e.g., language, speech, sensory and motor processing). For example, the strong associations between fetal HR and HRV and connectivity between language processing regions are also novel including the superior temporal and precentral gyri. Evidence has suggested that language

networks are already present during the third trimester of gestation (Ghio et al., 2021; Scheinost et al., 2022). There are also indications that infants are ready to learn language from birth (Berent et al., 2021). Fetal HR and HRV correlated with language ability at 2.5 years of age (DiPietro et al., 2007). However, the mechanistic link between fetal HR indices and developmental outcomes is unknown. Functional connectivity in the neonatal period may represent such a mediating pathway. Neonatal connectivity predicts short (Scheinost et al., 2020) and long-term behavioral outcomes (Sun et al., 2023). Fetal MR indices likely influence neonatal connectivity, which in turn, influence later behavior. Nevertheless, this indirect mediation path has yet to be tested and remains future research.

There are several strengths to this study. We acquired data prospectively beginning in the second trimester of pregnancy and continuing into infancy. Including both trimesters is a strength as it allows us to track changes in fetal HR indices and their associations with infant brain networks across pregnancy rather than provide a snapshot of one timepoint. Our study also has several limitations. Two of the three seeds used in these analyses are subcortical and brainstem structures. These seeds had lower temporal signal noise ratios (tSNR) than the dACC seed (Figure 1). The sample size is small at n=48. However, this size is consistent with other infant studies (Korom et al., 2021) and neuroimaging studies more broadly (Szucs and Ioannidis, 2020). Our data did not facilitate time course analyses. Richer indices of heart variability may show different associations than we observed. Our maternal sample was young (mean age of 21). Thus, the observed associations may not generalize to other pregnant populations. Similarly, a majority of the sample is male. Investigations into the role of sex in the current study necessitate a larger sample size for greater statistical power. Despite the longitudinal nature of our study, we did not collect neuroimaging and heart rate data simultaneously. Relatedly, we also did not have longitudinal neuroimaging data. Future work should include fetal fMRI collected at the same gestational age as the heart rate data. Further, longitudinal studies should include data on the perinatal transition to understand how birth changes any observed associations (Scheinost et al.,

2022). Additionally, we did not collect behavioral data during the neonatal period to correlate with the connectivity measures. The role of the observed results and later behavior is unclear. Finally, while we used a standardized protocol to minimize external influences on fetal vigilance state, the fetal vigilance state (Suwanrath and Suntharasaj, 2010) is unknown. State differences could have affected the heart rate indices. However, such discrimination of states would be challenging before 32 weeks of gestation when only periods of fetal activity and quiescence can be distinguished (Brändle et al., 2015).

5. Conclusion

Our findings show that neonatal brain regions—involved in autonomic regulation during postnatal development—have significant associations with fetal HR and HRV during the second and third trimester of gestation. They add to adult studies linking functional neuroimaging to autonomic regulation by showing association earlier in life. Future studies should aim to investigate how these brain connections mediate future development of autonomic and behavioral regulation abilities in childhood.

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605 **Table 1.** Results of the correlations between bilateral medulla, hypothalamus, and
606 dACC voxel-wise neonate functional connectivity and third trimester mean resting fetal
607 HR and HRV (n=48).

Seed (bilateral)	Region	Volume (# voxels)	Association type
	FETAL MEAN HEART RATE		
MEDULLA			
	Pre/postcentral gyrus (L)	496	Positive
	Pre/postcentral gyrus (R+L)	339	Positive
	Inferior parietal lobe (R)	239	Positive
HYPOTHALAMUS			
	Middle frontal gyrus (L)	327	Positive
	Middle frontal gyrus (R)	284	Positive
dACC			
	Cerebellum (L)	480	Negative
	Inferior parietal lobule & Superior temporal gyrus (L)	273	Positive
	FETAL MEAN HEART RATE VARIABILITY		
MEDULLA			
	Precuneus & paracentral lobule (L)	331	Positive
HYPOTHALAMUS			
	Middle temporal gyrus (L)	323	Negative
dACC			
	Lateral occipital gyrus (R)	412	Positive
	Superior frontal gyrus (L)	328	Positive

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Table 2. Results of the correlations between bilateral medulla, hypothalamus and dACC voxel-wise neonate functional connectivity and second trimester mean resting fetal HR and HRV (n=33).

Seed (bilateral)	Region	Volume (# voxels)	Association type
	FETAL MEAN HEART RATE		
MEDULLA			
	Middle frontal gyrus (L)	437	Negative
	Superior temporal gyrus & inferior parietal lobule (R)	317	Positive
HYPOTHALAMUS			
	Subcortex (L)	324	Positive
	Precuneus & paracentral lobule (L)	215	Positive
dACC			
	Insula (L)	587	Negative
	Cerebellum lobules V-VI & Crus I (R)	567	Negative
	Visual cortex (R-L)	382	Positive
	Lateral occipital gyrus (R)	251	Positive
	Basal ganglia (R)	226	Negative
	FETAL HEART RATE VARIABILITY		
MEDULLA			
	Cerebellum lobules V-VI & fusiform gyrus (L)	964	Positive
	Cerebellum lobules V-VI (R)	208	Positive
	Superior parietal lobule (R)	204	Positive
HYPOTHALAMUS			
	Subcortex (R)	768	Positive
	Subcortex (L)	320	Positive
dACC			
	Middle cingulate cortex	226	Negative

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622 **Table 3.** Results of the correlations between bilateral medulla, hypothalamus, and
623 dACC neonate functional connectivity and the change from second to third trimester
624 mean resting fetal HR and HRV (n=27).

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Seed (bilateral)	Region	Volume (# voxels)	Association type
	CHANGE IN FETAL MEAN HEART RATE		
MEDULLA			
	Pre/postcentral gyrus (L)	1144	Positive
	Cerebellum (R+L)	1043	Negative
dACC			
	Inferior frontal gyrus (R)	376	Positive
	CHANGE IN FETAL HEART RATE VARIABILITY		
MEDULLA			
	Cerebellum (R)	379	Negative
	Hippocampus (L)	337	Negative
	Inferior occipital gyrus (R)	203	Positive
HYPOTHALAMUS			
	Precentral gyrus (L)	418	Positive
	Precuneus and paracentral lobule (R)	267	Positive
	Middle temporal gyrus (L)	256	Negative
	Precuneus and paracentral lobule (L)	218	Positive
dACC			
	Middle frontal gyrus (L)	480	Positive

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Figure 1. Sagittal views of the three regions of interest used for seed analysis that were manually defined on a custom, neonatal template (Spann et al., 2018). The bilateral medulla is shown in blue. The hypothalamus is shown in green. The dorsal anterior cingulate cortex (dACC) is shown in red. The approximate MNI coordinates are: dACC (-1,24,26), medulla (-4, -36,-35), and hypothalamus (-2,-3,-3). The temporal signal noise ratios for each seed are 49.37±32.84 for the medulla, 65.36±34.86 for the hypothalamus, and 170.71±109.35 for the dACC.

Figure 2. Associations between mean fetal heart rate (HR) during in the third trimester and newborn bilateral medulla, hypothalamus and dACC connectivity (n=48). (*top*) Higher mean fetal HR was positively associated with newborn connectivity between bilateral medulla and bilateral precentral, postcentral gyrus and the right inferior parietal lobe. (*middle*) Higher levels of mean fetal HR were positively associated with newborn connectivity between bilateral hypothalamus and the left and right middle frontal gyrus (left panel). (*bottom*) Higher levels of mean fetal HR were negatively associated with newborn connectivity between bilateral dACC and the left cerebellum. Higher levels of mean fetal HR positively associated with bilateral dACC and left inferior parietal lobule and superior temporal gyrus connectivity.

Figure 3. Associations between fetal heart rate variability (HRV) during in the third trimester and newborn bilateral medulla, hypothalamus, and dACC connectivity (n=48). (*top*) Higher fetal HRV was positively associated with the connectivity between the medulla and the left precuneus and paracentral lobule. (*middle*) Higher fetal HRV was inversely associated with the connectivity between the hypothalamus and the left middle temporal gyrus. (*bottom*) Higher fetal HRV was positively associated with connectivity between the bilateral dACC and the left superior frontal gyrus and between the bilateral dACC and the right lateral occipital gyrus.

Figure 4. Associations between the mean fetal HR during in the second trimester and newborn bilateral medulla, hypothalamus and dACC connectivity (n=33). *(top)* Higher mean fetal HR was inversely associated with the medulla-left MFG connectivity and positively with medulla-right STG and IPL connectivity. *(middle)* Higher mean fetal HR was positively associated with the connectivity between the hypothalamus and left subcortex and between the hypothalamus and left precuneus/paracentral lobule. *(bottom)* Higher mean fetal HR was inversely associated with the connectivity between the dACC and the right cerebellum, between the dACC and the right basal ganglia, and between the dACC and left insula. Higher mean fetal HR was associated with the connectivity between the dACC and the bilateral visual cortex and between the dACC and the right lateral occipital gyrus.

Figure 5. Associations between fetal HRV during in the second trimester and newborn bilateral medulla, hypothalamus, and dACC connectivity (n=33). *(top)* Higher fetal HRV was positively associated with the connectivity between the medulla and right cerebellum, between the medulla and the left fusiform gyrus/cerebellum, and between the medulla and right SPL. *(middle)* Higher fetal HRV was positively associated with connectivity between hypothalamus and right and left subcortex. *(bottom)* Higher fetal HRV was positively associated with connectivity between dACC and middle cingulate cortex.

Figure 6. Associations of the change in mean fetal HR from the second to third trimester with bilateral medulla, hypothalamic, and dACC connectivity in neonates (n=27). *(top)* Higher change in fetal HR was positively associated with the connectivity between the medulla and the left precentral and postcentral gyrus and inversely associated with the connectivity between the medulla and the cerebellum. *(bottom)* Higher change in fetal HR was positively associated with the connectivity between the dACC and the right inferior frontal gyrus.

Figure 7. Associations of the change in fetal HRV from the second to third trimester with bilateral medulla, hypothalamic, and dACC connectivity in neonates (n=27). *(top)* Higher change in fetal HRV was positively associated with the connectivity between the medulla and the right inferior occipital gyrus and was inversely associated with the connectivity between the medulla and the left hippocampus and between the medulla and right cerebellum. *(middle)* Higher change in fetal HRV was positively associated with the connectivity between the hypothalamus and the bilateral precuneus and paracentral lobule and between the hypothalamus and the left precentral gyrus. Higher change in fetal HRV was inversely associated with the connectivity between the hypothalamus and middle temporal gyrus. *(bottom)* Higher change in fetal HRV was positively associated with the connectivity between the dACC and the left middle frontal gyrus.