

A Method for Measuring Dynamic  
Respiratory Sinus Arrhythmia (RSA) in Infants and Mothers

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## Abstract

The measurement of respiratory sinus arrhythmia (RSA) in infants, children and adults is critical to the study of physiological regulation, and more recently, interpersonal physiological covariation, but it has been impeded by methods that limit its resolution to 30 sec or more. Recent analytical developments have suggested methods for studying dynamic RSA in adults, and we have extended this work to the study of infants and mothers. In the current paper, we describe a new analytical strategy for estimating RSA time series for infants and adults. Our new method provides a means for studying physiological synchrony in infant-mother dyads that offers some important advantages relative to existing methods that use inter-beat-intervals (e.g. Feldman et al., 2011). In the middle sections of this paper, we offer a brief tutorial on calculating RSA continuously with a sliding window and review the empirical evidence for determining the optimal window size. In order to confirm the reliability of our results, we briefly discuss testing synchrony by randomly shuffling the dyads to control for spurious correlations, and also by using a bootstrapping technique for calculating confidence intervals in the cross-correlation function. One important implication that emerges from applying this method is that it is possible to measure both positive and negative physiological synchrony and that these categorical measures are differentially predictive of future outcomes.

# A Method for Measuring Dynamic Respiratory Sinus Arrhythmia (RSA) in Infants and Mothers

## 1. Introduction

Self-regulation is foundational to the healthy development of human infants as they learn to control their state, attention, emotion, and behavior (Belsky, Friedman, & Hsieh, 2001; Gross, 2013; Moore & Calkins, 2004; Porges, 1996; Richards, 1985; Rothbart, Poser, & Boylan, 1990). In the past two decades, there has been growing interest in the study of physiological regulation as a complement to these behavioral studies (e.g. Conradt & Ablow, 2010; Moore & Calkins, 2004; Perry, Calkins, & Bell, 2016). Much of this research has focused on parasympathetic functioning as a physiological substrate of emotional reactivity and regulation (Beauchaine, 2015; Berntson, Quigley, & Lozano, 2007; Blandon, Calkins, Keane, & Brien, 2010). A frequently used measure for studying the regulation of the parasympathetic nervous system (PNS) is respiratory sinus arrhythmia (RSA).

RSA measures cardiac vagal tone which is derived from heart rate variability at the frequency of breathing and serves as an indicator of the parasympathetic influence on heart rate (Porges, 1985; 1991). During periods of calm and quiescence the autonomic nervous system (ANS) via the vagus nerve services the needs of the internal viscera to enhance growth and restoration, but during environmental challenges the ANS responds to acute demands by increasing metabolic output via vagal withdrawal and sympathetic excitation.

It is important to appreciate that heart rate and RSA are related but are different measures of cardiac activity. Vagal stimulation of the sinoatrial (S-A) node delays the onset of the heart beat resulting in heart rate deceleration, whereas vagal withdrawal (i.e., delay of neural transmission) reduces the time between heart beats resulting in heart rate acceleration (Porges,

Doussard-Roosevelt, & Maiti, 1994). Heart rate reflects the degree of physiological arousal experienced by the individual. By contrast, RSA reflects the regulation of this arousal by controlling the ‘vagal brake’ which is responsible for slowing down heart rate and metabolic output indexed as increased variability in the frequency bands of respiration. In addition, heart rate or heart period (i.e., inter-beat-interval), as it is more typically measured, is a composite output reflecting autonomic input from the myelinated vagal fibers, the sympathetic nervous system, and the unmyelinated vagal fibers. It is thus difficult to establish whether one or more of these neural systems are responsible for changes in heart rate as a function of a specific event. By contrast, RSA is primarily a measure of the ventral vagal complex (i.e., the myelinated vagal fibers), and thus changes in RSA reflect changes in the activation of this specific neural circuit.

This distinction between heart rate and cardiac vagal tone becomes especially critical when studying interactional synchrony between infants and parents. Early on young infants demand a great deal of co-regulation from their parents which they receive through face-to-face interactions, close physical contact, and vocal and affective turn-taking (e.g. Brazelton, Koslowski, & Main, 1975; Papousek, 1995; Stern, 1974). These behaviors are all examples of interpersonal synchrony that involve a dynamic and continuous matching of behavioral, physiological, and neural responses that are reciprocally coordinated between parents and their infants (Feldman, 2017). Although evidence for behavioral synchrony has existed for decades, there are a number of technological challenges to studying physiological synchrony. One approach utilized by Feldman and colleagues (Feldman et al., 2011) measured the coordination of heart-rate rhythms (inter-beat-intervals or IBIs) within one second of each other. A problem with this measure is that there are significant differences in the frequencies of heart rates between infants and adults. Additionally, the composite nature of heart rate means it contains

rhythms (e.g., RSA) that operate at different frequencies. These differences result in very large phase lags between the two signals that are highly variable, and thus significantly limits the period of time during which synchrony can be measured. By contrast, while the frequency of respiratory oscillations are different for infants and parents, the *rate* of RSA *magnitude* changes are similar, and thus it is possible to measure synchrony for extended periods of time. The ability to measure RSA synchrony between infants and adults enables researchers to investigate bidirectional influences of the parasympathetic system in a social context, which is especially important in studying how the co-regulation of caregivers contributes to the early development of self-regulation (e.g., Kopp, 1989; Taipale, 2016). The advent of more dynamic models and estimates of RSA will allow for richer descriptions and explanations of the complex interactions between behavior and physiology across infants and adults.

In order to avoid any misunderstanding, the rate of change in RSA should not be confused with the respiratory frequencies used to determine the portion of heart rate variability involved in calculating RSA. These respiratory frequencies differ for adults and infants and range between .12 and .40 Hz for adults and .30 and 1.30 for infants (Porges, 1985). One significant impediment to implementing the standard measure of RSA as an index of dynamic changes in self-regulation is that the periodicity of RSA is fairly slow ranging from a little less than once per second to once every 8 sec. The Nyquist sampling theorem recommends a minimum of two complete cycles when analyzing the variability or power in a time series. As a consequence, RSA is typically derived as an aggregate measure from periods of time that range between 30 and 120 seconds (e.g. Ham & Tronick, 2006; Moore et al., 2009; Waters, West, & Mendes, 2014). This is problematic because an average RSA estimate across these relatively long periods will likely lose out on critical information about the individuals' reactivity and

dynamic regulation of emotion (Miller et al., 2013). Given that the timescale of most social behaviors, such as mutual gaze or smiling or orienting, is only a few seconds (Kaye & Fogel, 1980; Van Egeren, Barratt, & Roach, 2001), estimates of RSA that aggregate responses over 30 sec or longer are likely averaging out critical information about the multidirectional relationships between physiological and behavioral regulation, and how these processes are constrained by and also influence the social interactions between infants and their caregivers.

As a solution to this problem, we developed a continuous measure of RSA that can be sampled multiple times per second. In the current paper, we provide a step-by-step description of how to estimate continuous RSA for infants and their adult caregivers, and also, demonstrate how this measure offers new insights into the dyadic variability and interpersonal co-regulatory dynamics of infants and their mothers during a face-to-face play session.

Recent work has led to the development of novel analytic tools for estimating second-to-second RSA dynamics in adult populations. Gates, Gatzke-Kopp, Sandsten, & Blandon, (2015) used a combination of spectral analysis and a sliding window to measure RSA at 1 Hz in order to study the physiological linkage of husbands and wives during a family play time session. One limitation of this technique is that spectral analysis assumes stationarity (i.e., mean and standard deviation is constant across the entire time series), but this condition is rarely, if ever, maintained with human responses. Fisher, Reeves, & Chi (2016) developed a measure of Dynamic RSA (dRSA), which provides second-to-second variation in RSA by modeling the relationships among RSA, inter-beat intervals, and respiration rate using vector autoregression. It is difficult, however, to obtain direct measures of respiration in young infants (for an exception see McFarland, Fortin, & Polka, 2020). Both analytic techniques provide researchers with useful ways to index RSA dynamics and physiological synchrony in adult populations. Neither of these

techniques is sufficient, however, for addressing the unique challenges created when measuring RSA synchrony *across* different age groups.

The contribution of the current paper is to describe a novel analytic technique that provides developmental researchers with a time-based continuous measure of RSA that is commensurate across developmental populations. Our first goal is to describe this method for estimating the RSA time series continuously for infants and adults. Although there are alternative methods for estimating RSA (e.g., frequency-based methods), we limit our discussion to the Porges & Bohrer (1990) time-based method that is frequently used in the study of infants and toddlers. One of the principal advantages of this method is that it includes a band-pass polynomial filter that controls for the stationarity of the time series and removes the linear dependencies (i.e., autocovariance function) in the signal. After describing our method, we discuss some properties of time-based estimates of RSA, and how to use this measure for studying physiological synchrony. This will include the significance testing of cross-correlation coefficients when measuring physiological synchrony. Specifically, we will discuss both phase shuffling and bootstrapping techniques to control for spurious effects. Lastly, we will compare the results of measuring dynamic RSA and inter-beat intervals (IBIs) to demonstrate that they are very different measures.

### **1.1 Transforming RSA from a Discrete to a Continuous Measure**

Our analytical contribution extends the method described in Porges and Bohrer (1990) to estimate RSA using a time-based approach. The Porges & Bohrer (1990) method for estimating RSA involves four main steps:

1. Electrocardiograms are recorded at a high frequency (e.g., 1 KHz) and then visually inspected to remove artifacts (e.g., movements, ectopic beats, etc.).
2. Inter-beat-intervals are estimated and filtered for periodic and aperiodic components.
3. A bandpass filter is applied to the time series to isolate variance in the frequency range of spontaneous breathing.
4. RSA is calculated from the filtered time series by calculating the natural log of the variance in predetermined epoch lengths (e.g., 30 sec).

Although the estimation of a continuous measure of RSA only includes a few additional steps from the methodology outlined above, there are important parametric decisions that require justification. The main addition to the traditional methodology for time-based estimation of RSA is calculating the natural log of the variance of the component signal in the frequency range of spontaneous breathing within a *sliding window*. Figure 1 summarizes the steps for transforming the signal from IBIs to a continuous RSA signal.



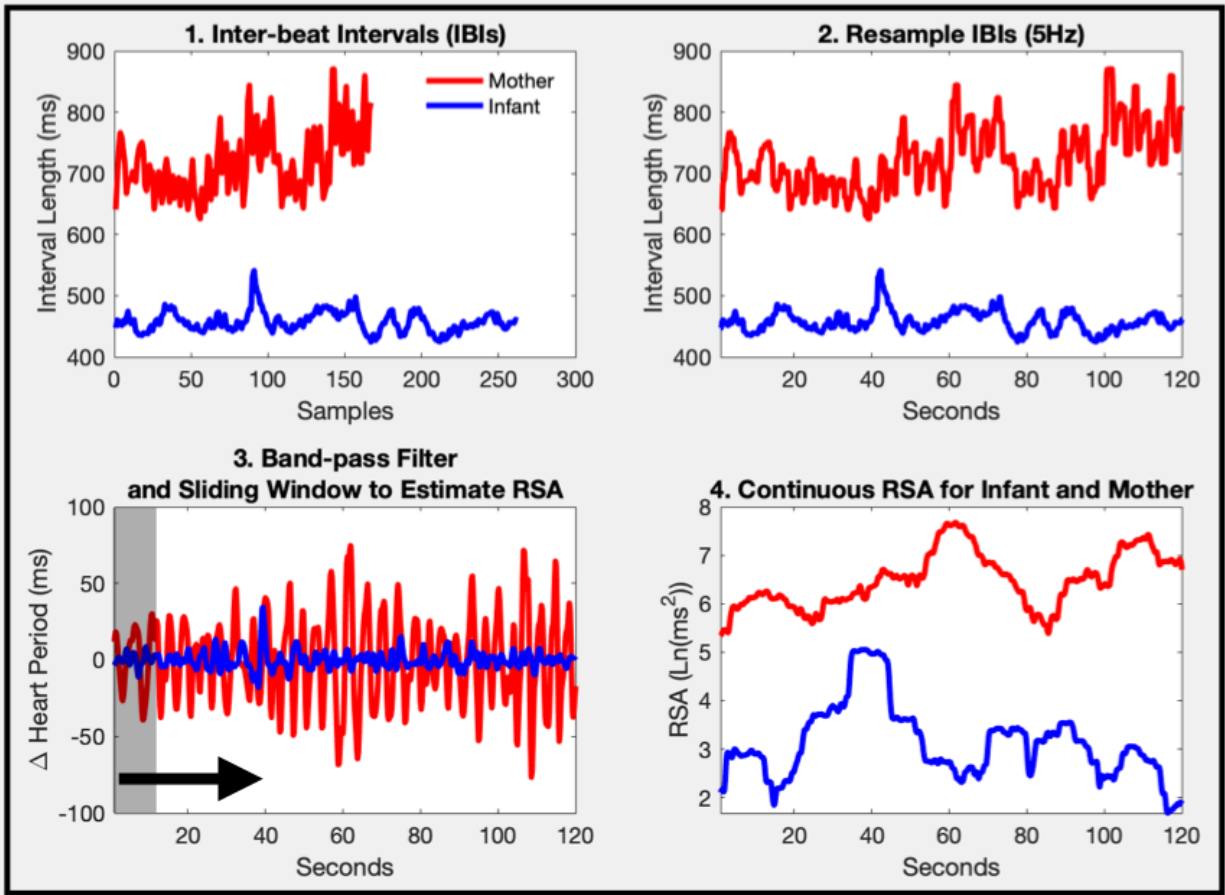


Figure 1: RSA magnitude extracted from mother and infant during the social play phase is computed in 4-steps: (1) infants' and mothers' IBIs are plotted in sequential order (note the different lengths because infant IBIs are shorter relative to mother IBIs), (2) down-sampling the IBIs to 5Hz, (3) applying band-pass filters corresponding to respiratory frequencies and applying a sliding window for estimating RSA (the width of the shaded vertical column represents the duration of the window which is incremented every 200 ms), and (4) plotting continuous RSA signals.

Step 1 depicts the IBIs for both infants and mothers over a period of 60 sec. In Step 2 the IBIs are down-sampled to 5 Hz. Step 3 depicts how RSA is calculated within a specific window size (e.g., 15 sec). After each calculation, the window is advanced in time by 200 ms (resolution is determined by the 5 Hz sampling rate) and then RSA is recalculated over the new 15 sec window, which corresponds to the size of the sliding window. There is one free parameter for this step: sliding window size. In the Results section of this paper, a parameter space of sliding window sizes is explored to determine the optimum timescale at which it is acceptable to estimate RSA when constructing a continuous measure of RSA. Step 4 depicts the RSA signal for an infant and mother based on the repeated RSA calculations that continue until the sliding window reaches the end of the time series.

## **1.2 Hypotheses**

Although the main goal of the current paper is to communicate a novel method for estimating RSA time series, there are two hypotheses associated with selecting the optimal sliding window size. First, increases in window size will lead to lower variability in the time series, but this improvement will asymptote before a window size of 30 sec. Second, properties of window sizes that include at least two respiratory cycles will have stronger positive correlations compared to shorter window sizes (e.g., 5 sec).

## **2. Methods**

We begin by describing the procedure we follow in estimating RSA continuously with a sliding window and also to explore the parameter space of the sliding window. As will become clear, the reliability of the RSA measure will vary across the parameterization of sliding window sizes selected for RSA estimation, and as such will provide informed suggestions for which window size lengths are best to use. The ECG data used for demonstrating our method was

drawn from a study testing 4- to 6-month-old infants with the Face-to-Face Still Face (FFSF) paradigm (Abney et al., under review). We focused on the data collected from both infants and mothers ( $n = 114$  dyads) during the Social Play phase of the experiment lasting 120 sec.

## **2.2 Participants**

114 mothers and their 4- to 6-month-old infants ( $M=4.94$  months,  $SD=0.94$ , 58 males) participated in the study. Families were part of a community sample recruited in a Midwestern college town. Demographic data were collected at the toddler visit in the longitudinal study. Overall, the sample reflects the local county demographics, which according to the most recent census is 83.4% White, 7.0% Asian, 3.6% Black, 3.5% Hispanic and 2.5% Multi-racial or other (US Census Bureau, 2018). Mothers were highly educated and ranged in age from 23- to 42-years-old ( $M = 31.36$ ,  $SD = 4.22$ ). All infants were born within 37-41 weeks gestational age. All mothers consented to participate with their infants and all study procedures were approved by the Indiana University Institutional Review Board.

## **2.3 Procedure**

Upon arriving at the lab, the family was greeted by an experimenter who placed the infant in a seat to get acclimated before testing began. Mothers were seated in a chair facing the infant approximately 30 cm away. The experimenter explained all procedures and obtained informed consent from the mother. Then, infant and mother were each fitted with three disposable Ag/AgCl ECG electrodes in a triangle pattern on their chests to measure their electrocardiographic responses continuously throughout the study. To minimize their attracting attention, the electrode leads were hidden under the infant's clothing and seat cover. The mother wore a wireless electrocardiogram (ECG) transmitter (Biopac Systems Inc., Goleta, CA) that did not restrict her movement. All recording was conducted using the Biopac MP150 system (Biopac

Systems Inc., Goleta, CA). Following set-up, the experimenter sat behind a curtain for the duration of the study and monitored the dyad via webcam.

At the beginning of the study, the baby was placed in an infant seat and tethered around the waist to ensure that she could not climb out. The mother sat in a chair facing her infant. The FFSF paradigm consisted of three sequential phases that were verbally cued by the experimenter. In the Social Play phase, mothers were instructed to interact normally with their infants for two minutes. To minimize interference with heart rate recordings, mothers were told to not touch their infants (Feldman, Singer, & Zagoory, 2010). During the Still Face phase, mothers became unresponsive and posed a neutral expression staring at their infant's forehead while keeping their face and body still for up to two minutes. Afterwards, mothers attempted to repair their relationship with their child and resume normal social interactions during a two-minute Reunion phase. If at any time infants became too distressed (i.e., hard crying for 10 seconds), the session was terminated early at the discretion of the mother or experimenter. In the current paper, we only use data collected during the Play phase.

#### **2.4 Physiological data processing**

Both infants' and mothers' electrocardiograms were recorded at a sampling rate of 1 KHz. Trained research assistants detected and removed artifacts due to movement, ectopic beats, and periodic bradycardias. Editing was completed offline using CardioEdit software (Brain Body Center, University of Illinois at Chicago). As previously mentioned, the Porges & Bohrer (1990) technique for RSA magnitude estimation includes parsing this component signal into discrete epochs (lasting between 10 to 120 sec), then calculating the natural log of the variance in each epoch. As such, RSA is time-averaged across epochs ranging between 10 and 120 sec, but this is not sufficient for capturing how the PNS modulates physiological arousal at

a time-scale commensurate with dynamic changes in emotion, such as facial and vocal expressions.

In order to create a more dynamic measure of RSA, we extended the Porges & Bohrer (1990) technique to include a sliding window (Gates et al., 2015). This technique was used to extract a continuous (updated every 200 ms) estimate of cardiac vagal tone for both infants and mothers. If, for example, the sliding window was 10 sec long, then the first epoch would include the mean of samples 1 to 50 given a 5 Hz sampling rate. The next epoch would include the mean of samples 2 to 51, etc. Critically, this dynamic measure is affected by window size as we illustrate in Figure 2. We chose five sliding window sizes (5, 10, 15, 20, and 30 sec) to assess its effects on calculating the covariation between infant-mother dyads. Figure 2 provides a graphical depiction of our sliding window estimates of RSA and the traditional ‘epoch’ level estimates of RSA. Matlab and R code and data can be found here:

[https://osf.io/d5vq3/?view\\_only=40301356f66e493aad02c6940b311062](https://osf.io/d5vq3/?view_only=40301356f66e493aad02c6940b311062).

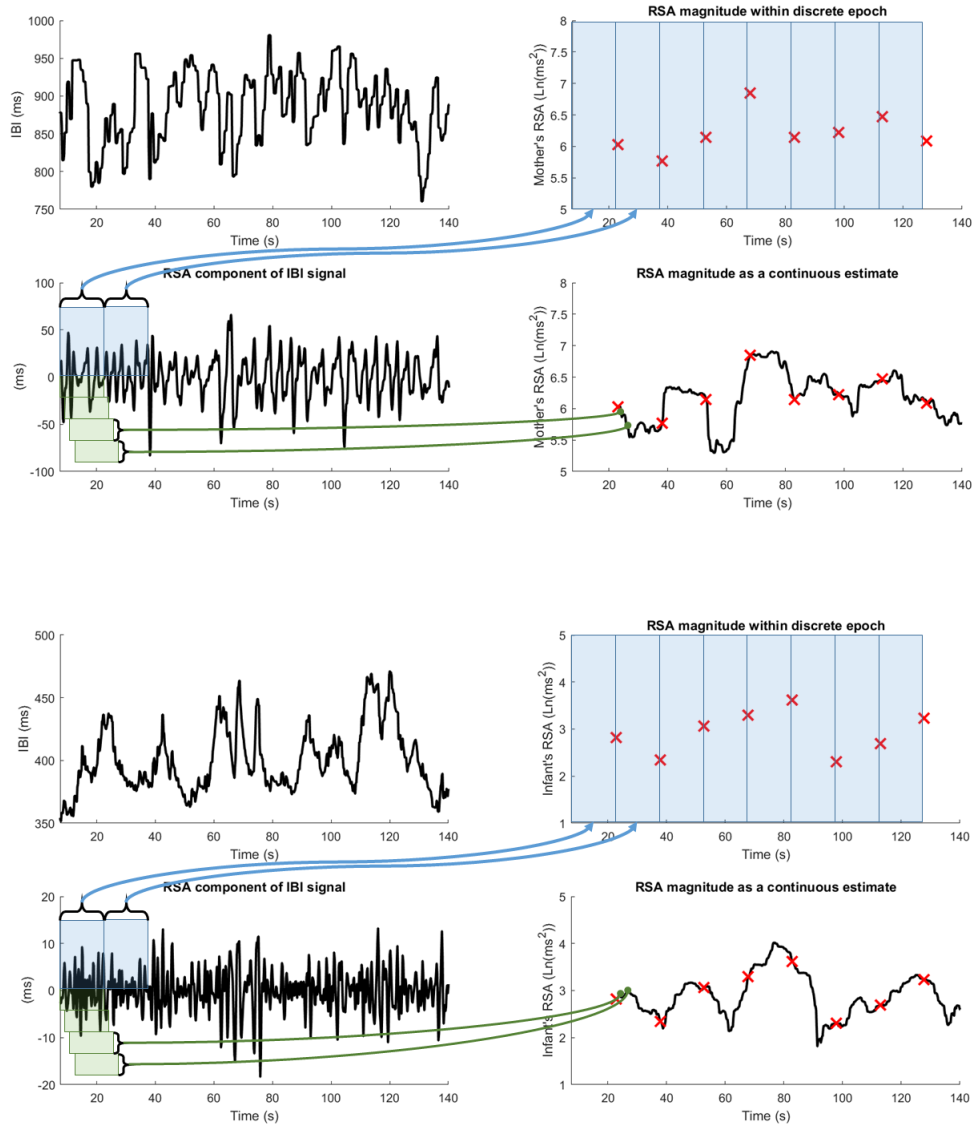


Figure 2: RSA magnitude extracted from mother and infant during the social play phase. Top-panels illustrate the result for an infant and bottom-panels for the mother. Green dots in the lower-right panels corresponding to infants' and mothers' data represent the sliding window estimate of RSA (updated at 5 Hz, reflecting 15-sec histories), while Red X symbols represent the 'epoch' level estimates of RSA magnitude at discrete intervals (e.g., 15-sec), as used in the Porges & Bohrer (1990) method of RSA measurement. Estimates are identical where the sliding window overlaps with the discrete epoch boundaries.

One of the main motivations for calculating a more continuous measure of RSA was to derive a measure of physiological synchrony between infants and mothers. Figure 3 includes

the cross-correlation functions using raw RSA signals and detrended RSA signals for measuring physiological synchrony. There are a number of techniques for measuring the covariation and synchrony of RSA signals such as multilevel modeling, correlation, regression, and ARIMA modeling (see Davis et al., 2018 for a systematic review). In the current paper, we chose to estimate the cross-correlation profile and the lag-0 coefficient as our estimate of synchrony because it not only provides clearer insight into the simultaneous correlation (i.e, at lag-0), but also the lagged correlations to the left and to the right of the lag-0 estimate. A comparison of the two cross-correlation functions reveals the importance of removing linear dependences by partialling out the 1<sup>st</sup>-order derivative from the original signal before calculating the cross-correlation at different time lags. As can be seen, the unfiltered time series reveals positive cross-correlations above .90 at zero-lag for each window size. By contrast, the results are dramatically different after removing the linear dependencies. The cross-correlations now range between -.20 and +.20 and the maximum cross-correlation occurs at different time lags for each window size.

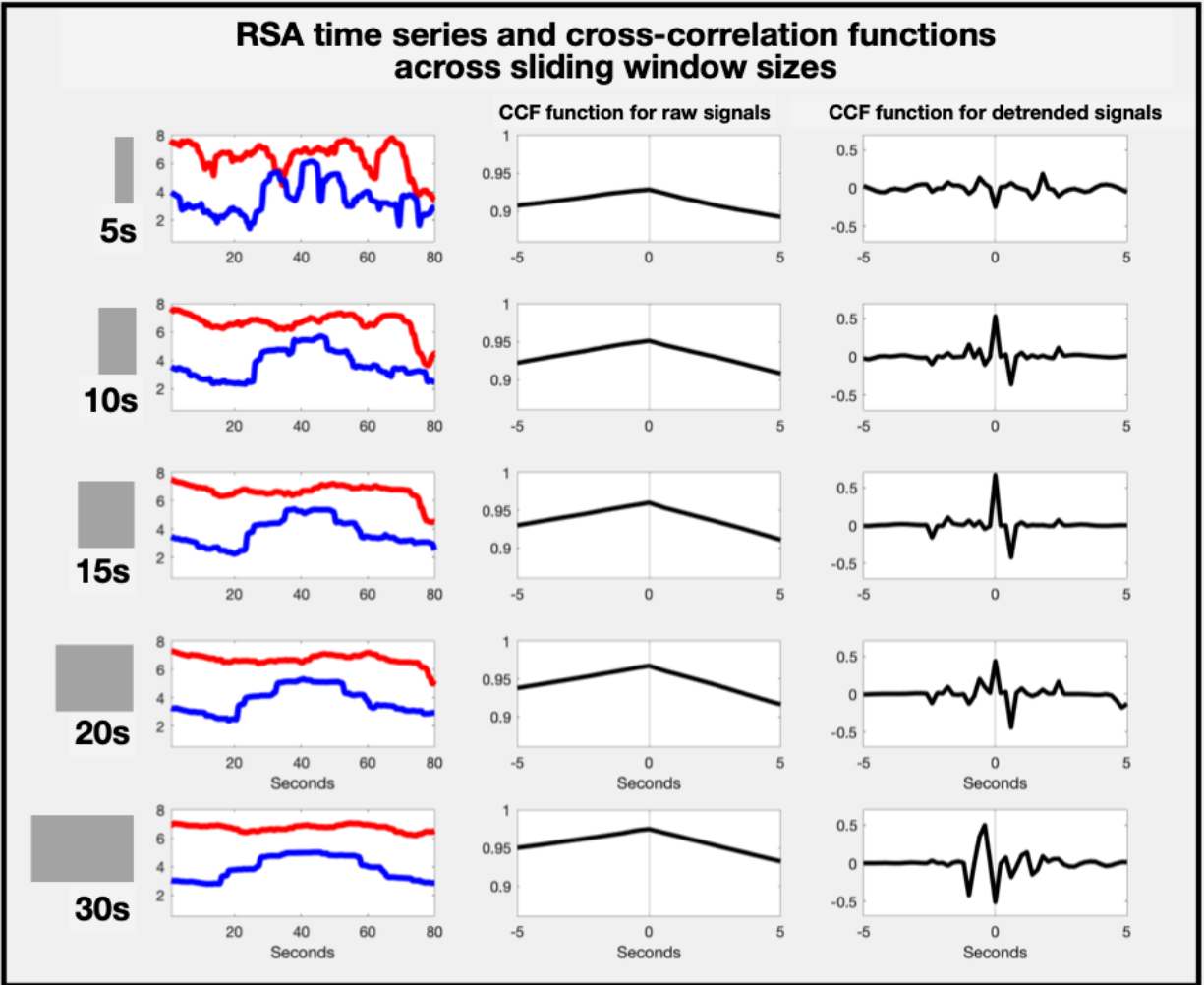


Figure 3. Sliding window size was parameterized from 5s, 10s, 15s, 20s, and 30s. Continuous RSA signals are displayed across window sizes in addition to example cross-correlation functions (CCF) for raw signals and detrended signals.

### 3. Results

#### 3.1 Properties of the RSA time series as a function of window size

There are many techniques for measuring the patterns and structure of time series data. One simple question that is afforded by an estimation of a continuous measure of RSA is whether or not RSA increases, decreases, or does not change throughout the play phase. To measure trends in RSA time series, we submitted each RSA time series (for infants and mothers and across sliding window sizes) to linear regression. We categorized each time series as



‘positive’ if  $p < .001$  and the regression beta was positive, (2) ‘negative’ if  $p < .001$  and the regression beta was negative, and (3) ‘no change’ if  $p > .001$ . Critically, these trends varied as a function of window size. As can be seen in the tile plot depicted in Figure 4, the three trend categories are represented by three colors (positive = red, negative = blue, no trend = green) for both infants (left panel) and mothers (right panel). Each row displaying one or more colors corresponds to an individual participant’s trend as a function of window size. For example, the top row in the ‘Infant’ facet window shows that for one infant, every RSA time series (across sliding window) was categorized as ‘positive’ resulting in a solid red line. In a second example, the 3<sup>rd</sup> row from the top of the ‘Mother’ facet window shows that her RSA time series at a sliding window of ‘5 sec’ was categorized as ‘no change’ (green) but the RSA time series estimated with larger sliding windows were categorized as ‘negative’ (blue).

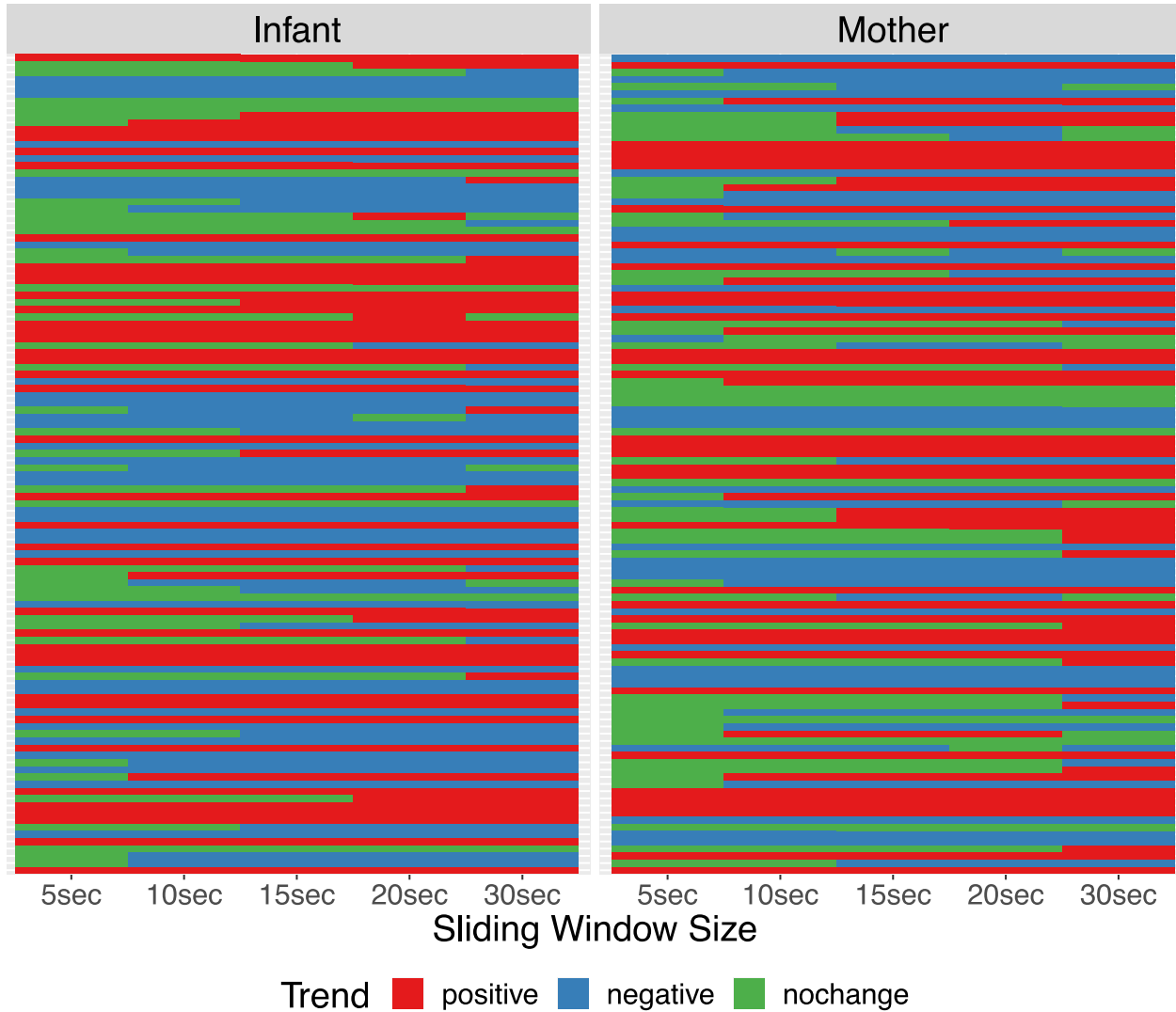


Figure 4: Tile plot showing linear trends of RSA signals estimated at different sliding windows increasing in size from left to right for infant (left) and mothers (right). Linear fit trends were determined as negative if  $b < 0$  and  $p < .001$ , positive if  $b > 0$  and  $p < .001$ , and no change is  $p > .001$ . Each row corresponds to one dyad.

One important observation from Figure 4 is that the stability of the RSA time series trends seems to depend on the size of the sliding window size. To calculate the stability and consistency of these categorizations, Markov chain models were computed separately for infants and mothers to determine whether trend categorizations changed or remained stable across sliding window sizes. The Markov chain models estimated the probability of transitioning from one trend category to another (or staying in the same trend category) across sliding window

sizes. We present the results in two matrices of transitional probabilities for infants and mothers, respectively, across the five sliding window sizes (see Figure 5). The rows represent the prior state and the columns represent the subsequent state. For both infants and mothers, the highest transitional probability involved the 15-sec and the 20-sec sliding windows. Conversely, for both infants and mothers, the lowest transitional probability (although still high at  $\sim 0.75$ ) involved the 5-sec sliding window and the 20-sec sliding window. This result may be partly due to the greater distance between the 5- and 30-sec windows than between any of the other window combinations. Still, the transitional probabilities increased systematically from one window size to the next (e.g., 5- to 10-sec, 10- to 15-sec) suggesting increasing stability in the RSA measure with increasing window size. Notably, transitional probabilities decreased going to the longest window size (30-sec). It is also noteworthy that the effect of window size was similar for infants and mothers.

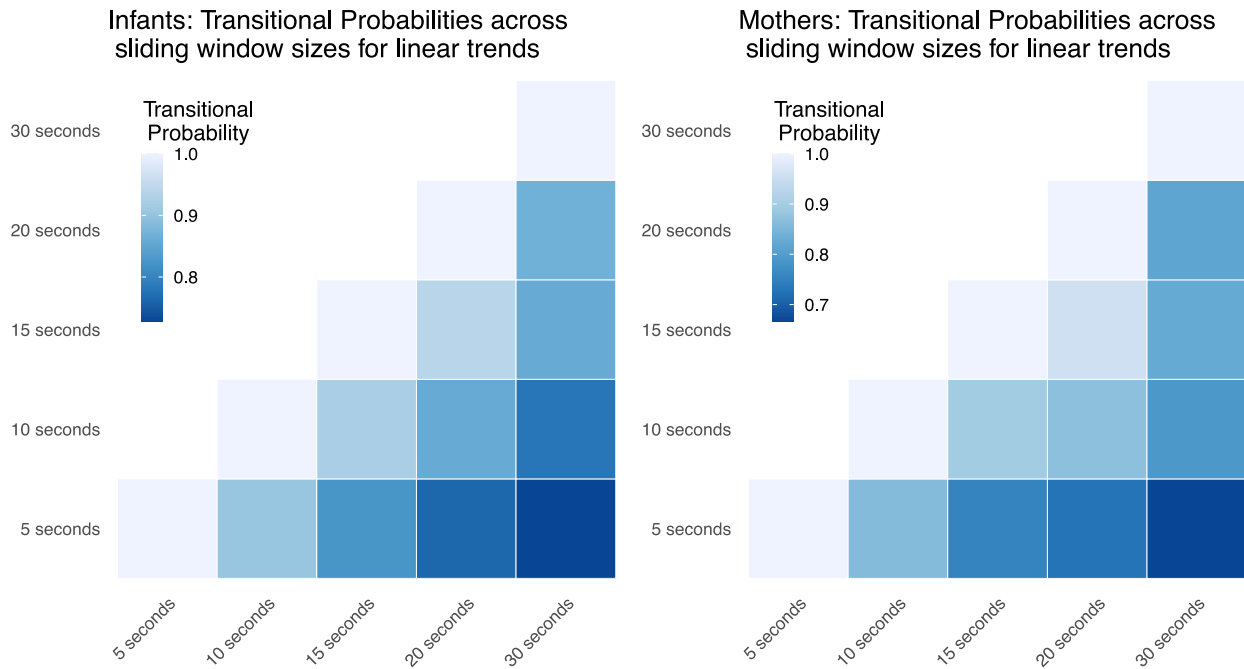


Figure 5. Matrices of group-level transitional probabilities for (left) infants and (right) mothers. Each cell in the matrix corresponds to one transitional probability across two sliding window sizes. For example, for the infant matrix, the bottom-right cell corresponds to the probability of the time series trend categorization to be the same at the 5-second sliding window and the 30-second sliding window. Lighter colors represent higher transitional probabilities.

One implication of the preceding results is that small window sizes are more likely susceptible to random noise and will therefore distort the RSA measure. By contrast, large window sizes will likely reduce the effects of noise because the noise averages out over increased sample sizes. To test these hypotheses, we calculated the standard deviations of the RSA time series across window sizes. As can be seen in Table 1, the standard deviations decrease as window size increases. We submitted the standard deviations to one-way ANOVAs with Sliding Window Size as a main effect for infants and mothers, respectively. For both infants ( $F[4, 564] = 43.13, p < .001$ ) and mothers ( $F[4, 564] = 61.18, p < .001$ ) there was a significant main effect of Sliding Window Size. Pairwise comparisons (with Bonferroni correction)

indicated that for both infants and mothers, the standard deviations from the 20 sec window size were significantly lower than the 5 sec and 10 sec window sizes ( $ps < .01$ ). Moreover, the standard deviations from the two largest window sizes (e.g., 20s and 30s) were not significantly different from each other.

Table 1: Averaged standard deviations across Sliding window sizes

Sliding Window Size	<i>Infant</i>	<i>Mother</i>
5 sec	1.05	0.99
10 sec	0.85	0.73
15 sec	0.75	0.65
20 sec	0.68	0.60
30 sec	0.58	0.55

### 3.2 Testing the Covariation Between Infants' and Mothers' RSA Time Series

When testing for physiological synchrony, it is especially important to ensure that the correlations between infants and mothers are not spurious. To determine whether or not these calculations go beyond patterns that could be explained by the natural frequency of the RSA time series, we compared the empirical cross-correlation coefficients to cross-correlation coefficients computed from shuffled RSA time series (i.e., randomly permute the observed infant and mother RSA time series). A non-parametric Kolmogorov-Smirnov (KS) test is used to compare the distributions of lag-0 coefficients (or coefficients from any time lag selected) from the empirical and shuffled dyads.

We submitted the empirical and shuffled cross-correlation coefficients from our data set to a separate KS test for each of the sliding window sizes. The KS test statistics are reported in Table 2. These results suggest that the empirical and shuffled distributions of cross-correlation coefficients were not sampled from the same population for any of the sliding window sizes, suggesting evidence for physiological synchrony across a large range of sliding window sizes.

Table 2: Results from Kolmogorov-Smirnov (KS) tests

Sliding Window Size	$D$	$p$
5 sec	0.47	<0.001
10 sec	0.46	<0.001
15 sec	0.49	<0.001
20 sec	0.47	<0.001
30 sec	0.48	<0.001

To determine the relative stability of cross-correlation coefficients across sliding window sizes, we correlated cross-correlation coefficients for each dyad across the four sliding window sizes. Table 3 presents the correlations between lag-0 cross-correlation coefficients across sliding window sizes. The key finding from this analysis is that there is reasonable stability (i.e., mild to strong correlations) between coefficients for sliding windows sizes 10 seconds and larger, but the stability is clearly highest ( $r = .89$ ) for window sizes between 15- and 20-sec. This finding thus converges with the findings in the last section suggesting poor stability for the smallest window sizes.

Table 3: Zero order correlations between lag-0 cross-correlation coefficients across sliding window size.

Sliding Window Size	5 sec	10 sec	15 sec	20 sec	30 sec
5 sec	-	0.35	0.19	0.21	0.19
10 sec		-	0.81	0.63	0.48
15 sec			-	0.89	0.71
20 sec				-	0.85
30 sec					-

Note. Cutoff for  $\alpha=.05$  when  $N=114$  is  $r=.18$ .

### 3.3 Testing the Significance of the Cross-Correlation Coefficients with Bootstrapping

Although it is important to assess whether empirical cross-correlation estimates are different from estimates of shuffled RSA time series between infants and mothers, it is also informative to assess which cross-correlation coefficients measured at different time lags differ from chance levels. To do so, we employed a bootstrapping analysis to estimate confidence

intervals of the cross-correlation function. For brevity and because we suggest that the 15-sec sliding window size is a good option, we performed a bootstrapping analysis only on the data processed using a 15-sec sliding window for RSA estimation.

Similar to testing for spurious cross-correlations, the bootstrapping analysis starts by first estimating the cross-correlation functions for random pairings of the RSA time series between infants and mothers. Cross-correlation functions were estimated for each random pairing, and spanned from 5-sec before lag-0 to 5-sec after lag-0, which resulted in a 50-sample (5 Hz) long vector for each function. Cross-correlation functions were estimated for 1,000 random infant-mother pairings, creating a 50 (samples per function) by 1000 (iterations) matrix. Confidence intervals (-/+95%) were then estimated for each function (see Figure 6).

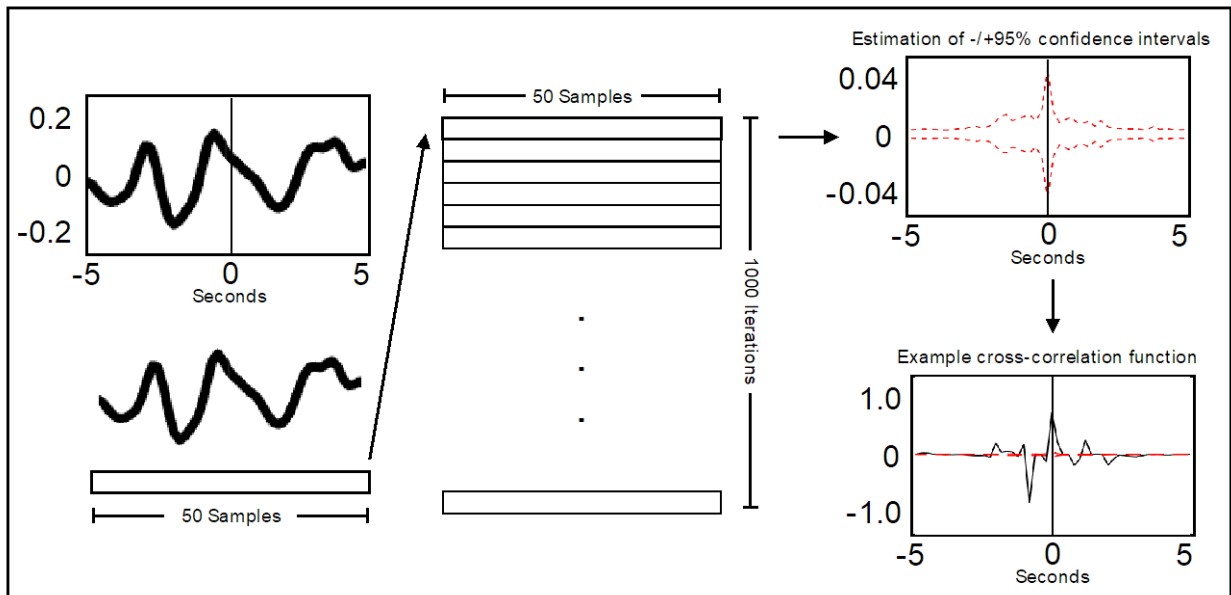


Figure 6. Schematic of the bootstrapping approach for calculating confidence intervals for the cross-correlation function. Note differences in y-axis ranges.

Also in Figure 6, we illustrate how this approach is useful for testing whether the 0-lag or any of the time lagged cross-correlation coefficients are significant. The bottom right panel

shows the empirical cross-correlation function for one dyad. By using the bootstrapping analysis to estimate confidence intervals, it is possible to identify where in the empirical cross-correlation function the dyadic covariation is beyond the threshold of chance covariation as determined by the bootstrapping analysis. As can be seen in the bottom right panel, there is a significant negative correlation approximately 1 sec before lag-0, suggesting that a decrease in infant's RSA leads to an increase in mother's RSA 1-sec later. Moreover, at approximately lag-0, there is a significant positive correlation suggesting evidence for positive physiological synchrony by this infant-mother dyad.

### **3.4 Similarities and Differences between RSA and IBI Synchrony**

As we briefly mentioned in the Introduction, previous research with infants and mothers reveals that they sometimes synchronize their heart-rate rhythms based on IBIs (Feldman et al., 2011). This is clearly a different measure than RSA because it is associated with multiple sources of heart rate variability, whereas RSA is associated primarily with the myelinated vagus nerve. Although RSA and IBIs are generally correlated, it is an empirical question as to whether physiological synchrony based on each of these two measures is correlated. This question was addressed by the last set of analyses. First, the average RSAs and average IBIs for both infants and mothers were correlated across the four sliding window sizes. As can be seen in Table 4, there are significant strong positive correlations between average RSA and average IBI for infants and mothers across sliding windows sizes. By contrast, the correlation of the two measures of physiological synchrony revealed no significant correlations between RSA and IBI synchrony across sliding window sizes (see Table 4) revealing no evidence of a relationship between RSA and IBI synchrony at the level of inter-individual covariation.



Table 4: Zero-order correlations for average RSA and average IBI and lag-0 cross-correlation coefficients for infants and mothers.

Sliding Window Size (sec)	<i>Average RSA v. IBI (Infant)</i>	<i>Average RSA v. IBI (Mother)</i>	<i>RSA Cross-Correlation v. IBI Cross-Correlation</i>
5	.48	.62	.15
10	.46	.61	.09
15	.44	.60	.14
20	.43	.59	.02
30	.41	.58	.01

Note. Cutoff for  $\alpha=.05$  when  $N=114$  is  $r=.18$ .

#### 4 Discussion

First and foremost, we have demonstrated that it is possible to calculate a continuous measure of RSA that can be used for comparing dynamic changes in physiological regulation of infants and mothers. In order to appreciate the value of this measure, it is important to emphasize that we measured dynamic changes across a 120-sec time interval of face-to-face play and correlated these changes in RSA between infants and their mothers. If we had used the standard method for calculating RSA (i.e., aggregating RSA over 30-sec or longer, e.g. Ham & Tronick, 2006; Moore et al., 2009; Waters et al., 2014), we would have at best obtained four samples of RSA for calculating the covariation between infants' and mothers' physiological responses (see Figure 2 for a graphical comparison). More importantly, we would have missed any changes that occurred in RSA during each epoch. This sort of dynamic information is critical to understanding physiological synchrony. Consider, for example, that infants' and mothers' RSA values increased and decreased in such a way that their mean RSA values did not change across the four epochs. This pattern of results suggests that physiological synchrony did not change during this period. By contrast, our measure of dynamic RSA could nevertheless reveal significant changes in synchrony within each epoch that was simply

obscured by aggregating the measure over the entire epoch. These dynamic changes might reveal that physiological regulation in infants and mothers was differentially entrained or coupled or uncorrelated within each epoch, which is considerably different than the conclusion suggested from analyzing time-averaged RSA values. This is why measuring RSA at a finer time-scale than is normally accomplished is so important: it reveals the moment-to-moment changes in physiological regulation that can occur during interpersonal interactions.

Our exploration of the parameter space for determining the optimal sliding window size suggested that 15- to 20-sec yielded a more consistent result than smaller window sizes or larger window sizes like 30 sec. This result was consistent with our expectation that smaller windows would be more susceptible to random noise, and thus would underestimate the covariations between infants' and mothers' RSA changes and larger windows (e.g., 30s) would be too course-grained to provide informative dynamic variability. There is a collection of evidence that impacts our assessment. First, the transitional probabilities between 15- and 20-sec windows for linear trends of the RSA time series were already very high (i.e., 0.96). Second, the correlation of the RSA synchrony measures between these two window sizes was  $r=.87$ , suggesting that additional differences between window sizes would be quite modest. Given these results, we recommend that continuous RSA should be measured with a sliding window size of 15-sec, although this comes with the caveat that this recommendation is based on the results of only one data set. It will be necessary to test window sizes with future data sets to establish the reliability of this recommendation.

An added consideration when measuring dynamic synchrony is that it reveals that RSA synchrony is not a binary measure (i.e., synchrony or no synchrony). Instead, infants and mothers can show either positive or negative synchrony or neither. For example, during face-

to-face play sessions, it is expected that dyads engage in positive synchrony (Bornstein & Suess, 2000; Lunkenheimer et al., 2015). Given the evidence for both positive and negative synchrony, it would be a mistake to assume that positive synchrony is always beneficial and negative synchrony is always detrimental. In some contexts, negative synchrony is both expected and adaptive for the infant. For example, during and following a stressor, mothers must respond sensitively and flexibly in different ways than during social play or caregiving routines in order to soothe a distressed infant (Moore et al., 2009; Leerkes, 2010; Leerkes, Weaver, & O'Brien, 2013). To support infant bio-behavioral regulation during a stressor, mothers have been found to initially increase their RSA, followed by RSA decreases (vagal withdrawal) during peak infant distress (Oppenheimer, Measelle, Laurent, & Ablow, 2013). Moreover, negative synchrony is observed when infants and mothers re-engage after the still-face. In particular, infants tend to increase in their RSA and mothers decrease their RSA, both in terms of their mean RSA levels relative to the still-face episode (Jones-Mason, Alkon, Coccia, & Bush, 2018) as well as in terms of the linear trend displayed during the reunion period (Ostlund, Measelle, Laurent, Conradt, & Ablow, 2017). The reason that negative synchrony is observed during the reunion episode is that mothers' withdrawal of RSA enables a mobilization response to facilitate her efforts to calm and re-engage her infant (Mills-Koonce et al., 2009). In sum, negative synchrony may be adaptive in some situations that require co-regulation (Creavy, Gatzke-Kopp, Zhang, Fishbein, & Kiser, 2020).

Although positive synchrony is generally beneficial, an important question to consider is whether the beneficial effects of positive synchrony follow a linear or a nonlinear function. We found evidence of a linear relationship between positive synchrony and infants' reduction of distress following a still-face stressor among infants with less developed physiological regulation

(Abney et al., under review). However, there is certainly some evidence to suggest that behavioral synchrony does not always follow a linear trajectory in which higher levels are necessarily better (Mayo & Gordon, 2020). It is generally assumed that synchrony demonstrates coordination and attunement, but too high levels of synchrony may reflect intrusive parenting or infants' dependence on caregivers at the expense of self-regulation (Isabella & Belsky, 1991; Taipale, 2016). Accordingly, some authors posit an 'optimum midrange model' where moderate levels of infant-caregiver synchrony are optimal for infant socio-emotional outcomes (Beebe & Steele, 2013; Galbusera, Finn, Tschacher, & Kyselo, 2019; Jaffe et al., 2001; Malatesta et al., 1989; Noe, Schluckwerder, & Reck, 2015). Moreover, it may not be the duration of synchrony that matters, but instead how flexibly the dyad can transition in and out of synchronous states (Mayo & Gordon, 2020). Although the preceding research is thought provoking, it needs to be considered as provisional since these investigations have focused on optimal levels of behavioral synchrony for infants' socio-emotional outcomes, rather than optimal levels of physiological synchrony.

Another consideration for future work is to examine whether infant-mother physiological synchrony changes across the first year. Here, we provide evidence for positive and negative physiological synchrony between 4- to 6-month-old infants and their mothers. This aligns with the time period of the greatest increase in myelinated vagal fibers, which is observed between 30-32 weeks gestational age and six months postpartum (Sachis, Armstrong, Becker, & Bryan, 1982). While baseline vagal tone stabilizes around six months (Fracasso, Porges, Lamb, & Rosenberg, 1994; Porter, Bryan, & Hsu, 1995), the parasympathetic system continues to develop throughout the second half of the first year (Alkon et al., 2006). Furthermore, as infants develop the capacity for self-regulation, they engage in different regulatory behaviors (Kopp, 1989;

Rothbart, Ziaie, & O'Boyle, 1992; Stifter & Braungart, 1995), which may further influence the type and degree of physiological synchrony with caregivers. For example, positive synchrony may be more beneficial for younger infants who still require co-regulation.

Although we believe that this new method is very promising, there is one limitation associated with calculating a continuous measure of RSA that will need to be resolved. Empirical studies of behavioral synchrony have identified that on average, mothers and infants are only in matched states approximately 20-30% of the time (Busuito et al., 2019; Tronick & Cohn, 1989; Tronick & Gianino, 1986). Moreover, these matched or similar states may be at a shorter timescale than the epoch length used to measure the states. Consider, for example, facial affect matching or mutual gaze, which are both typical behaviors coded when studying synchrony (e.g. Feldman et al., 2011; MacLean et al., 2014; Messinger, Mahoor, Chow, & Cohn, 2009). These behaviors may last for as little time as two to three seconds. This presents a problem for our proposed method which requires a sliding window of 15-sec. Accordingly, this method is not currently suited to the study of physiological synchrony over short time intervals. We are currently working to overcome this limitation so that in the future it will be possible to conduct investigations of behavioral and physiological synchrony on the same time scale.

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