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Distribution, Stability, and Continuity of Autonomic Nervous System Responsivity at 18- and 36-Months of Age

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Abstract

Objective: Cardiac autonomic nervous system (ANS) measures, respiratory sinus arrhythmia (RSA) and preejection period (PEP), are valid and reliable indicators of children's sensitivity to their environment; however, there are few studies of ANS measures in children less than three years of age. This study's aim was to summarize the distributions, stability, and continuity of RSA and PEP measures during resting, challenge, and reactivity for children at 18- and 36-months. **Methods:** This was a cohort study of racially- and ethnically-diverse, low-income children who completed a developmentally challenging protocol while we simultaneously assessed their RSA and PEP at 18-months ($N = 134$) and 36-months ($N = 102$). **Results:** The ANS resting, challenge, and reactivity measures at 18- and 36-months of age were normally distributed. The RSA resting ($r = 0.29$), RSA challenge ($r = 0.44$), PEP resting ($r = 0.55$) and PEP challenge ($r = 0.58$) measures were moderately stable but RSA ($r = 0.01$) and PEP reactivity ($r = 0.02$) were not stable from 18- to 36-months of age. There was no continuity in the ANS measures from 18- to 36-months of age with statistically significant changes in sample means for all of the ANS measures. **Discussion:** These developmental changes in ANS are shown at the sample level but there are individual differences in ANS responses from 18- to 36-months that may be affected by adversity or protective factors experienced early in life.

Keywords

development, stress, autonomic nervous system, cardiovascular reactivity, early childhood, respiratory sinus arrythmia, preejection period

Children's physiologic responses to everyday challenges differ for each individual and predict short- and long-term health problems, including learning, behavior, and physical health problems (Alkon et al., 2011; Beauchaine et al., 2013; Busso et al., 2017; Obradovic et al., 2010; Treadwell, Alkon, et al., 2010). Experiences early in life may contribute to these physiologic individual differences and 'get under the skin' to change children's brain architecture, immunologic responses, and genomic function affecting both current and future health (Hertzman, 1999). Adverse experiences during certain timepoints in childhood, known as sensitive developmental periods, may be particularly damaging and long-lasting and are explained by Hertzman as the theory of biological embedding.

The autonomic nervous system (ANS) is one of the most responsive physiologic systems to stress (Cacioppo et al., 2017). The parasympathetic nervous system (PNS) facilitates a "rest and restorative" state while sleeping or relaxing. The sympathetic nervous system (SNS) is the "fight or flight" system that is aroused to respond to emergencies or threatening situations. RSA and PEP can be measured under resting and challenge conditions and combined to describe reactivity, the difference between a challenge and a resting condition (Salomon et al., 2000). ANS resting and reactivity measures have been shown to be valid and reliable indicators of children's sensitivity to everyday stressors in their environment (Alkon et al., 2003; Matthews et al., 1990).

Respiratory Sinus Arrhythmia (RSA) is an index of the parasympathetic nervous system's influence on heart rate due to the frequency of respirations (Berntson et al., 1993). Decreases in RSA indicate PNS withdrawal and correspond to increases in heart rate. Low resting RSA is related to positive social engagement (Geisler et al., 2013; Porges, 2001) and

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greater RSA reactivity during emotion tasks is related to externalizing behavior problems (Beauchaine et al., 2013).

The preejection period (PEP), the time interval from the onset of ventricular depolarization to left ventricular ejection measured in milliseconds, reflects the influence of the SNS on cardiac activity (Cacioppo et al., 2017). Decreases in PEP, or shortened time intervals, indicate activation by the SNS and correspond with increases in heart rate. Decreased PEP reactivity has been associated with attention deficit hyperactivity disorder, oppositional defiant disorder, and conduct disorder in preschool-age children and delinquency, aggression, or obsessive behaviors in adolescents (Beauchaine et al., 2013; Crowell et al., 2006).

Stability and continuity provide different dimensions of the developmental changes in the ANS over time. Stability shows how individual's ANS changes over time, whereas continuity shows group changes in ANS mean scores over time. Although ANS measures provide important markers of stress physiology, few studies have examined their distribution, stability, and continuity among young children.

Distributions of ANS measures are normally distributed in sample populations across different ages and ethnic distributions (Alkon et al., 2003, 2011; Busso et al., 2017; El-Sheikh, 2005; Treadwell et al., 2011). Normal distributions are evidence of a pattern of individual differences showing a range from high to low responses. Although studies of ANS in children show normal distributions, many of these studies used different data collection methods (e.g., protocols), hardware (e.g., acquisition equipment), and analysis software (Beauchaine et al., 2013; Calkins et al., 2007; Elmore-Staton et al., 2012).

Two studies of RSA and PEP resting, challenge, and reactivity measures of preschool-age children reported similar means but they used different data collection procedures, protocols, and/or equipment and scoring software. In a cross-sectional study of 3- to 5-year-olds (N = 51) in child care centers, a 15-minute standardized protocol with resting and challenging conditions was administered while continuous measures of RSA and PEP responsivity were measured (Alkon et al., 2003). Their resting and challenge RSA and PEP means were similar to a cross-sectional study of children with attention deficit hyperactivity disorder and oppositional defiant disorder (N = 18, 7 girls) compared to a control group (N = 20, 9 girls; Crowell et al., 2006). The children, who had a mean age of 4.5 years, were administered a five minute resting baseline and 25-minute challenge of playing a "Perfection" game. Although the studies' protocol differed in length and challenges, they reported similar ANS findings. Two studies of infants also found similar ANS measures although they used different protocols, equipment, and scoring programs. A cohort study of low-income, primarily Latinx children included RSA resting and reactivity at 6- and 12-months of age using a 7-minute ANS protocol (Alkon et al., 2011). The ANS measures were acquired using the Minnesota Impedance Cardiograph HIC-2000 and scored using the ANS Suite software (Mindware Technologies, LTD). Another study of low-income, Mexican American infants at 6- and 12-months of age reported RSA during a 7-minute resting protocol (Jewell et al., 2018). The data were acquired using electrocardiogram (ECG) equipment from Forest Medical, LLC and scored using CardioBatch software.

Two studies that used the same protocol with 3- to 5-year-old children showed similar resting RSA and PEP values although they had different sample characteristics and they used different acquisition equipment. One study enrolled primarily Latinx, low-income children and they used Biopac equipment for data acquisition (Alkon et al., 2011). The other study included primarily White, middle to high income children in California and Wisconsin and they used the Minnesota Impedance machine for data acquisition (Alkon et al., 2003). In both studies, the children completed the same ANS protocol with four challenging tasks and a rest period and analyzed the ANS data using Mindware software (www.mindwaretech.org).

Stability of ANS Measures in Childhood

Stability is defined as the consistency in relative ranks of individuals with respect to a function or process through time (Bornstein & Suess, 2000) or their between-individual variability. Instability refers to the change in relative order in individuals over time (Bornstein et al., 2017). In early and middle childhood, the majority of studies show that RSA indices during resting and challenge (without accounting for baseline levels) is fairly stable while RSA reactivity is not stable. In a cohort study of 8- (N = 251) to 10- (N = 185) year-olds (162 White and 89 Black), PNS and SNS resting measures were positively and moderately correlated across three waves (8-, 9- and 10-years of age) showing stability (Hinnant et al., 2011). The children were given an acclimating period of six minutes to help adjust to their new environment, followed by a three-minute resting period. On the other hand, in a study of Latinx children from 6 weeks to 2 years of age (N = 312) of only RSA resting measures there was no stability (Jewell et al., 2018). In another longitudinal study of Latinx children, RSA and PEP resting measures were moderately correlated from infancy to five years of age (N = 297; Alkon et al., 2006). In the same study, there was moderate stability for the children's RSA and PEP during the challenge conditions, but not for RSA or PEP reactivity. In a study of older children, 8- to 10-year-olds (N = 201) who were 48% Black and 52% White, they also had moderate stability of RSA and PEP resting measures, but not RSA and PEP reactivity (Salomon et al., 2000). They participated in a protocol with three challenges: the reaction time task, a mirror tracing task, and the Social Competence Interview. Overall, these studies showed fairly consistent, moderate stability of RSA and PEP resting, but no stability of RSA and PEP reactivity.

Continuity refers to the consistency in the absolute level of ANS measures in a group through time (Bornstein & Suess, 2000) or the mean level change of the construct over time within a sample (Hinnant et al., 2018). Previous studies showed that resting RSA and PEP measures show mean level increases during the first six years of life (Alkon et al., 2011; Bar-Haim et al., 2000; Calkins & Keane, 2004). There are few studies of ANS reactivity over time. RSA reactivity increased in a cohort study of Mexican American children from 6 months through five years (Alkon et al., 2011), suggesting that the stress response became more robust as children developed (i.e., children exhibited PNS withdrawal in response to challenges versus rest). These findings were different than other studies where they found no significant change in RSA reactivity in 3 to 5-year-olds (Calkins & Keane, 2004; Perry et al., 2013). PEP reactivity increased in different cohort studies with 3- to 8-year olds (Alkon et al., 2003) and 8- to 17-year olds (Matthews et al., 2002). In a study of 5- to 7-year olds, PEP reactivity was moderately stable (Gatzke-Kopp & Ram, 2018). Overall, children's RSA and PEP reactivity levels appeared to increase with age, indicative of developmental changes or more responsivity to different challenge conditions as children got older. There seems to be some evidence that RSA and PEP reactivity responsivity may become more stable after 5 years of age.

Children's neurobiological development has been shown to demonstrate plasticity, sensitivity to their environment, and rapid growth particularly in the first three years of life. The studies included infants and preschool-age children, but not 18-month old toddlers. There were several studies that measured RSA and PEP during infancy using the Still Face Paradigm (SFP; Jones-Mason et al., 2018) and during the preschool-ages, but not toddlers. As we cannot assume a linear path of ANS development from 6 months to 3 years of age (Gatzke-Kopp & Ram, 2018), there is a gap in knowledge about toddlers that needs to be addressed. The aim of this study was to describe the distribution, stability, and continuity of RSA and PEP during resting, challenge, and reactivity states, in a sample of predominantly minority children from low-income families assessed longitudinally at 18 and 36 months of age.

Methods

This study is part of a larger birth cohort study, the Stress, Eating, and Early Development (SEED) study, which was a continuation of the Maternal Adiposity, Metabolism, And Stress (MAMAS) study (Vieten et al., 2018). The MAMAS study was a non-randomized control trial that examined the effects of a mindfulness-based stress reduction and healthy lifestyle intervention to reduce excessive gestational weight gain among overweight and obese pregnant women. The SEED study followed the children of MAMAS mothers from birth through five years of age, investigating the impact of prenatal and early childhood stress and eating habits on the children's behavioral, physiologic, and anthropometric development (Bush et al., 2017). This paper describes and analyzes the ANS data collected at the 18-month and 36-month visits. All study protocols and consent forms were approved by the Institutional Review Board of the University of California, San Francisco.

Participants in the MAMAS study were pregnant women recruited between August 2011 and June 2013 when they were between their 16th and 22nd week of pregnancy. Women were eligible to participate if they were of low to middle income and overweight or above. Recruitment took place at hospital-based clinics, community health centers, Supplemental Nutrition Program for Women, Infants and Children (WIC) offices, organizations providing services to pregnant women, and through online advertisements (e.g., Craigslist). In total, 220 women enrolled in the MAMAS study, of whom 215 remained involved in the study at delivery of a liveborn child. All of the 215 mothers were invited to enroll themselves and their newborns in the follow-up SEED study at delivery. In total, 162 mother-child dyads (75%) enrolled in SEED, of whom 134 children (62% of the enrolled delivery sample) and 102 children (47% of the delivery sample) participated fully at 18-month and 36-month study visits, respectively.

Data Collection

For both the 18- and 36-month visits, a trained research assistant met with the mother and child in the family's home and the dyad were together during the study visit. During these visits, the research assistant administered multiple questionnaires with the mother, took anthropometric measurements of the child, and conducted a standardized, age-specific Developmental Challenges Protocol (DCP) with the child while simultaneously monitoring their ANS activity (i.e., RSA and PEP).

The research assistant placed four spot electrodes on the child's neck and trunk to collect impedance and respiratory measures and three spot electrodes were placed on the right clavicle, lower left rib, and right abdomen for ECG measures (Bush et al., 2016). The ECG and impedance waveforms collected during the DCP yielded measures of heart rate, RSA, PEP, and respiratory rate. Data were acquired using Mindware Technologies, LTD hardware (www.mindwaretech.com); continuous ECG, Zo (basal impedance), and dZ/dt (first derivative of the impedance signal) waveforms were recorded. A 4-milliamp AC current at 100 Hz was passed through the two current electrodes; Zo and dZ/dt signals were acquired from the two voltage recording electrodes.

After the spot electrodes were in place for five minutes, the DCP was initiated. The DCP was designed to elicit ANS responses to developmentally-appropriate challenges across different domains: cognitive, physical, and socioemotional and a comparison resting state (Table 1). The DCP included age-appropriate resting and challenge activities (Bush et al., 2016). The resting conditions at 18-months was listening to a lullaby recorded on a computer and at 36-months it was listening to a story read aloud by the research assistant. The challenges at 18-months included watching and listening to a Jack in the Box as a startle response, tasting sour lemon juice on the tongue, and listening to a recording of a sick infant crying. The challenges at 36-months included naming a picture, tasting sour lemon juice on the tongue, and watching a scary video clip. These protocols were adapted from existing standardized protocols (Alkon et al., 2011; Bush et al., 2011) and were pilot-tested to assess that the modified protocols were engaging, developmentally-challenging, and tolerable for each age group. Ten 18-month olds and six 36-month-olds were not able to complete the DCP protocol.

Table 1. Developmental Challenge Protocols (DCP) at 18- and 36-Months of Age.

18-month-old DCP			
Task #	Task	Time	Task Type
T1	Lullaby #1	1 min	Rest
T2	Jack in the Box toy	1 min	Cognitive (startle)
T3	Lemon juice on tongue	30 sec	Physical (sensory)
T4	Sick infant cry recording	30 sec	Emotion (socioemotional)
T5	Lullaby #2	1 min	Rest
	Total	**4 min**	

36-month-old DCP			
Task #	Task	Time	Task Type
T1	Calm story read aloud #1	2 min	Rest
T2	Picture identification	90 sec	Cognitive (receptive vocabulary)
T3	Repeat after me	1 min	Control condition for cognitive task
T4	Picture naming	90 sec	Cognitive (expressive vocabulary)
T5	Water drink	30 sec	Control condition for physical task
T6	Lemon juice on tongue	30 sec	Physical (sensory)
T7	Calm video	2 min	Control condition for emotion task
T8	Scary video	2 min	Emotion (fear)
T9	Calm story read aloud #2	2 min	Rest
	Total	**13 min**	

Data Preparation and Scoring

Autonomic nervous system data were filtered, extracted, and then scored using Mindware Technologies, LTD software programs (HRV 3.1.0F and IMP 3.1.0 H). RSA indices were calculated using the interbeat intervals on the ECG waveform, respiratory rates derived from the impedance waveform (e.g., d*Z*/d*t* signal), and a bandwidth range setting of 0.15–1.04 (De Rogalski Landrot et al., 2007). PEP was measured in milliseconds as the time interval between the onset of ventricular depolarization (Q point on the ECG wave) and the onset of left ventricular ejection (B point on the d*Z*/d*t* wave).

Each 30-second interval was scored if there was a minimum of 20 seconds of clean data. The task scores were calculated as the mean of several 30-second intervals depending on the task length (Table 1). Outliers were children with RSA or PEP scores greater than three standard deviations from the sample mean for each 30-second interval or task. For the 18-month-olds, there were seven outliers at the interval level and five outliers at the task level. For the 36-month-olds, there were 16 outliers at the interval level and three at the task level. After reviewing the outlier's raw data with an ANS expert on our team, we kept 19 of them in the analyses because their other scores showed similar results and were consistent with their individual patterns of physiology.

For the 18-month data, RSA and PEP reactivity scores were calculated as the mean response across the three challenge tasks minus the mean of the first resting condition. As the 36-month-old protocol included task-specific matched control conditions, 36-month-old RSA and PEP reactivity scores were calculated as the mean of the three difference scores. Each difference score was calculated as the challenge minus the corresponding matched control condition response. The receptive vocabulary challenge in the DCP was not included in these analyses because there was no task-specific matched control condition, and its intent was to "warm up" children to the activity of engaging with the unknown examiner. Reactivity scores were only calculated for children with scorable data for at least two of the three different challenge/control difference scores. These reactivity scores were used in the analyses for this paper. Therefore, the reactivity scores for the 18-month-old and 36-month-old protocol were calculated differently because of the inclusion of task-specific control conditions in the 36-month-old DCP (Table 1).

To calculate a comparable reactivity at 18 and 36 months, we also calculated a 36-month reactivity score of the mean of the four challenges minus the first resting condition as we did for the 18-month old children. We used this second reactivity score for the 36-month old children in post-hoc analyses comparing reactivity scores across the two timepoints.

Data Analysis

SPSS 25 was used to analyze the data and create the figures. Descriptive statistics were calculated for all of the variables. The RSA and PEP means were plotted across the protocol to show the distribution at 18- and 36-months of age. Pearson product moment correlations were calculated to assess the stability of the RSA and PEP measures between 18- and 36-months and scatterplots were created to visualize these associations. Matched-paired t-tests were calculated to evaluate the continuity of RSA and PEP resting, challenge, and reactivity between 18- and 36-months of age respectively.

Results

There were 162 mothers enrolled in the study at the time of the birth of their newborn. The mothers' mean age at the time of delivery was 28 years of age (range 18–43; $SD = 5.8$; N = 162). Sixty-eight percent of the mothers were married or had a partner and 54% were multiparous. Thirty-one percent of the mothers had a high school education or less, 50% had some college or vocational training, and 19% had earned a college degree. The families' median annual household income was $19,000, ranging from $0 to $98,000 with the majority of the sample falling 100% below the federal poverty level at study enrollment. Eight-five percent of the mothers identified their ethnic or racial backgrounds as 39% Black, 31% Latina, 15% White, 2% Asian, and 13% other or multiracial. The mother's cesarean rate was 28%, and the infants' mean gestational age at birth was 39.6 weeks (SD = 1.4).

Children included in these analyses were 19.0 months old (SD = 1.3) on average, at the time of their 18-month-old visit (N = 134), and were 38.7 months (SD = 3.4) at the time of their 36-month-old visit (N = 102); 53% of participants at both visits were girls. Based on the parent's classification of their child's race, the participants included in the 18-month-old visit analysis were 32% White, 14% were Black, and 54% Other (e.g., Asian, Native American, mixed race); 42% were classified as Latinx. Among children included in 36-month-old visit analyses, 36% were White, 12% were Black, and 52% were Other, with 33% of the total further classified as Latinx. The mean household federal poverty level was less than 200% at the 18-month and 36-month-old visits.

Overall, RSA and PEP resting, challenge, and reactivity measures showed normal distributions at both age timepoints with little evidence of skewness or kurtosis at 18- and 36-months of age (Table 2). The mean RSA and PEP resting and challenge scores were lower at 18-months compared to 36-months of age. The mean RSA and PEP reactivity scores were positive at 18 months and negative at 36-months of age, indicating higher reactivity at the older age.

The mean levels of PNS and SNS activity across the DCP at 18- and 36-months of age indicate the change in response to the resting and challenge conditions at 18- and 36-months of age (Figure 1). At 18-months, RSA levels were lowest during the lullabies and lemon juice challenge and highest for the emotion challenge (infant cry). PEP was lowest for the first lullaby and highest for the startle challenge (e.g., jack-in-the-box). At 36-months, RSA was highest during the first resting period, reading a book, and the lowest for the taste challenge (e.g., lemon juice on the tongue). PEP was highest for the task–specific control of picture identification and lowest for the cognitive challenge (e.g., repeating numbers). At 18-months of age, the largest changes in reactivity were from the taste challenge exhibiting PNS withdrawal and the emotion video challenge exhibiting SNS activation. At 36-months of age, the

Table 2. RSA and PEP Resting, Challenge, and Reactivity at 18- and 36-Months of Age.

	RSA resting		RSA challenge		RSA reactivity		PEP resting		PEP challenge		PEP reactivity	
Months	18	36	18	36	18	36	18	36	18	36	18	36
N	134	102	133	98	133	97	127	100	128	96	124	96
Mean	4.99	6.53	5.28	6.05	0.27	−0.36	80.48	87.28	81.12	86.41	0.59	−0.05
SD	1.22	1.08	1.04	1.01	0.73	0.49	6.01	6.79	6.03	6.66	2.33	1.78
Range	2.12, 8.06	4.02, 8.87	2.86, 7.97	3.81, 8.41	−1.36, 3.19	−2.66, 1.37	68, 95	70.5, 101.5	67.83, 94.67	70.89, 101.42	−6.33, 7.17	−6.08, 7.75
Skewness	0.20 (0.21)	0.00 (0.24)	0.36 (0.21)	0.20 (0.24)	0.56 (0.21)	−0.75 (0.25)	−0.06 (0.22)	−0.32 (0.24)	−0.04 (0.21)	−0.17 (0.25)	0.11 (0.22)	0.82 (0.25)
Kurtosis	0.18 (0.42)$^{\#}$	−0.29 (0.47)$^{\#}$	0.03 (0.42)$^{\#}$	−0.04 (0.48)*	1.87 (0.42)	5.29 (0.49)	−0.40 (0.43)*	−0.28 (0.48)$^{\#}$	−0.68 (0.43)$^{\#}$	−0.45 (0.49)*	0.30 (0.43)*	4.54 (0.49)

Note. RSA = respiratory sinus arrhythmia; PEP = preejection period; SD = standard deviation.
Shape of distribution: * = peaked, $^{\#}$ = flat.

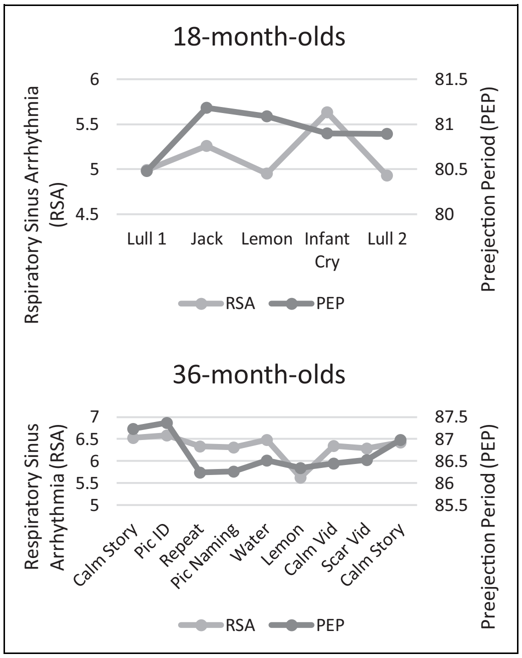

Figure 1. Mean levels of parasympathetic and sympathetic activity across the 18- and 36-month-olds developmental challenges protocol (DCP).

largest changes in reactivity were also from the taste challenge with PNS withdrawal and the cognitive challenge with SNS activation.

The correlations showed moderate stability of RSA and PEP resting measures, but not reactivity, from 18- to 36-months of age (Figure 2). RSA and PEP resting, challenge, and reactivity measures from 18- to 36-months of age were positively, moderately correlated: RSA resting ($r = 0.29$, $n = 96$), PEP resting ($r = 0.55$, $n = 90$), RSA challenge ($r = 0.44$, $n = 92$), and PEP challenge ($r = 0.58$, $n = 87$). RSA reactivity ($r = 0.01$, $n = 91$) and PEP reactivity ($r = 0.02$, $n = 84$) were not correlated at 18- and 36-months of age.

There was a lack of continuity of RSA and PEP resting, challenge, and reactivity at 18- and 36-months. There were statistically significant changes in sample means from 18- to 36-months of age for all of the ANS measures (Table 3). The resting and challenge measures increased from 18- to 36-months and reactivity measures decreased. The largest changes were PEP resting (M $= 6.5$, SD $= 6.0$; t-statistic (df) $= 11.4(89)$, $p < .01$) and PEP challenge (M $= 5.3$, SD $= 5.8$; t-statistic(df) $= 8.5(86)$, $p < .01$).

Discussion

This study is the first one that we are aware of to report on both RSA and PEP measures in children at 18-months of age. RSA and PEP measures at rest, during challenges, and as reactivity scores displayed normal distributions. There was moderate stability in the RSA and PEP resting and challenge responses within individuals over time but not in reactivity measures. There was a lack of continuity of the RSA and PEP measures at rest, challenge, and as reactivity scores across the ages with significant mean changes from 18- to 36-months of age.

Other studies also found RSA and PEP measures to be normally distributed in infants and toddlers (Alkon et al., 2011; Jewell et al., 2018), reflecting individual differences and a range of ANS responses across children at each age. There were approximately equal numbers of children with positive and negative RSA or PEP reactivity scores. Figure 1 illustrates that, on average, the children at 18-months may have begun the protocol stressed by the strange situation of having electrodes placed on their chest and starting an unfamiliar protocol. Developmentally, toddlers have a short attention span and thus, it is challenging to engage and sustain toddler's attention during the protocol. On the other hand, at 36-months of age the children appeared to relax at the start of the protocol when an adult read a story aloud to them and were engaged during the tasks. In addition, the mean responses showed that many of the preschool-age children were able to return to their resting levels after the challenges were over when the adult read another story aloud to them. Thus, this study contributes new knowledge as it compares ANS responsivity at 18- and 36-months of age.

In previous studies, RSA resting, PEP resting, and RSA challenge levels in infants and children have consistently shown stability over time (Alkon et al., 2011; Conradt et al., 2016; Gatzke-Kopp & Ram, 2018); whereas, PEP challenge, RSA reactivity, and PEP reactivity have not shown stability (Hinnant et al., 2011). The results of this study align with Hinnant and colleagues' findings of moderate stability in older children, 8- to 10-year-old except for our finding of moderate stability in PEP challenge measures. However, RSA reactivity was not stable between 18- and 36-months of age, consistent with other studies (Alkon et al., 2011; Hinnant et al., 2011). In this study, PEP reactivity was not stable between 18- and 36-months. This lack of stability in the PNS and SNS reactivity indicates the potential for plasticity in stress physiology during this period of early childhood. Plasticity indicates individual changes in a child's stress responsivity from one time point to another (within-person variability) or changes over time in the stress response from person to person (between-person variability; Hinnant et al., 2018). Our findings and others' support the theories of individual differences, such as biological sensitivity to context (BSC), in the stress response (Boyce & Ellis, 2005) as there are a range of ANS responses at each age group.

Because this was a longitudinal study, we needed to design an ANS protocol with similar challenges for each developmental domain but adjust the type and length of tasks (Table 1). At 36-months we were able to include task specific challenges so we calculated two reactivity scores. Similar to the 18-month reactivity score, we subtracted the first resting condition from the mean of the four challenge conditions. The 36-month reactivity score, not comparable to 18-months, was the mean of three reactivity scores computed as the challenge-specific control minus the challenge. Specifically, within measure, RSA and PEP reactivity are not correlated from 18- to 36-months of age no matter whether the 36-month-old reactivity was

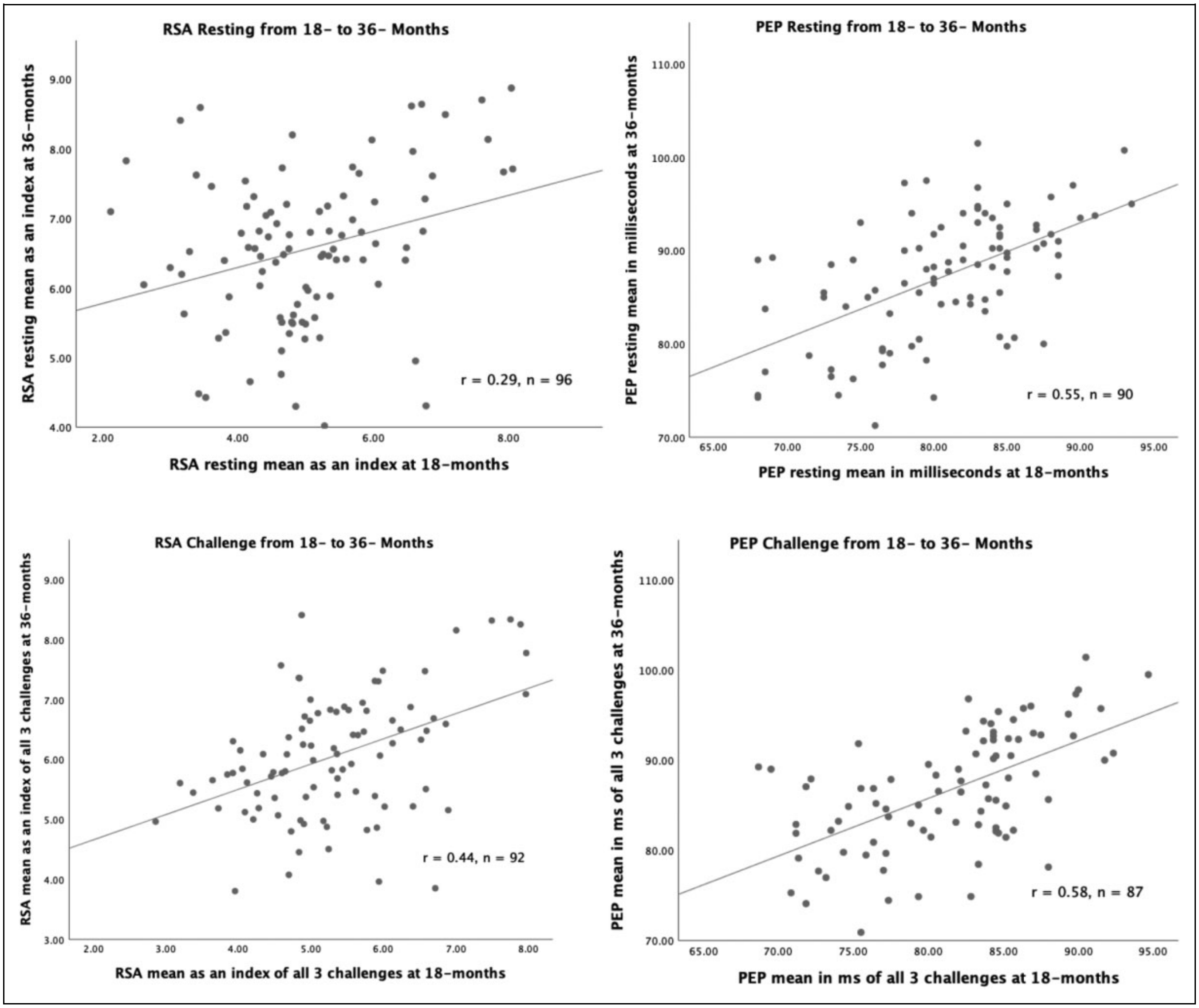

Figure 2. Correlations of RSA and PEP resting and challenge from 18-months to 36-months of age (n = 84–96).

Table 3. Mean Change of RSA and PEP Resting, Challenge, and Reactivity From 18- to 36-Months of Age.

	Mean Change	SD	t-statistic	df	*p*-value
RSA resting	1.5	1.4	10.8	95	0.000
RSA challenge	0.8	1.1	6.4	91	0.000
RSA reactivity	−0.6	0.9	−6.7	90	0.000
PEP resting	6.5	6.0	10.4	89	0.000
PEP challenge	5.3	5.8	8.5	86	0.000
PEP reactivity	−0.9	2.9	−2.9	83	0.005

Note. SD = standard deviation; RSA = respiratory sinus arrhythmia; PEP = preejection period.

calculated when controlling for or not controlling for task specific responses.

The statistically significant mean level changes of RSA and PEP resting, challenge, and reactivity from 18- to 36-months of age are indicative of developmental changes or a lack of continuity expected over time. Several other studies found more PNS withdrawal compared to rest for older children compared to younger children's longitudinal cohort samples (Alkon et al., 2011; Conradt et al., 2016; Gatzke-Kopp & Ram, 2018; Hinnant et al., 2011), PEP resting and PEP challenge had larger mean changes compared to the other measures, which may suggest that the SNS may become more responsive over time. From 18- to 36-months of age, the children showed greater physiological responsiveness to the challenges as evidenced by a negative RSA and PEP reactivity measures. This finding suggests that older children may be more biologically responsive or sensitive to the challenges presented during the protocol or that they were more engaged in the ANS protocol compared to the 18-month olds. Overall, the mean changes from 18- to 36-months of age are consistent with other studies of continuity from 6-months to 10-years of age (Alkon et al., 2006, 2011;

Bar-Haim et al., 2000; Bornstein & Suess, 2000; Calkins & Keane, 2004). Other studies with different sample demographic characteristics also support similar findings of increasing reactivity as the child ages (Alkon et al., 2006, 2014). Although earlier studies showed changes in RSA and PEP from infancy to 5 years of age, the level of change from 18- to 36- months was not known. This is the first study that shows mean RSA and PEP changes from 18- to 36-months. It is known that toddlers are especially challenging to study since they have a short attention span and are not comfortable with spot electrodes or any physiologic equipment.

Although this study contributed new findings to the field of ANS function in young children, there are several limitations to consider. These findings are limited to two time points of repeated measures. However, these data are described in light of two complementary constructs, stability and continuity, that are important to report in a longitudinal developmental study and only requires two waves of longitudinal data. While the SEED study does have RSA and PEP measurements from these same children at age 6 months, the 6-month ANS protocol utilized an attachment-based Still Face Paradigm (SFP), rather than a developmentally challenging protocol like the 18- and 36-month protocols, and funding delays led to only half the cohort being assessed for ANS. Thus, our analyses conducted here were not appropriate to model starting during infancy. We also note that the challenges in the DCP were not randomized and order of challenges may affect physiologic responsivity. Impedance cardiography is sensitive to movement and respiratory artifact (Bush et al., 2011; Zisner & Beauchaine, 2017). However, these ANS measures (RSA and PEP) offer an inexpensive, noninvasive estimation of central nervous system function as compared to EEG or fMRI studies.

We recommend that nursing scientists and developmental researchers use standardized, valid measures and methods for the assessment of ANS during resting and challenge conditions. For example, a protocol that assesses ANS responsivity including reactivity should be holistic and include a range of domains, such as the physical, emotional, cognitive, and social, and these protocols should be repeated in the same children across time. The 18-month-old protocol should be revised to illicit a better measure of rest as many children were not relaxed at the start of the DCP. Future analyses of challenge-specific reactivity may be helpful for investigating how children at a certain age responds to a specific developmental domain and possible environmental factors that may affect the response. Stability and continuity are important terms to define and report in a consistent manner across studies, especially longitudinal studies (Bornstein et al., 2017). Other researchers suggest that RSA and PEP measures be collected in conjunction with neuroimaging methods (such as fMRI, EEG, or PET) to assess neural correlates of ANS functioning (Zisner & Beauchaine, 2017). Adding measures of genetic predisposition, environmental risk, and protective factors that may be associated with ANS reactivity can tease out the interplay of endogenous and exogenous factors.

As strong advocates for children, pediatric nurses regularly assess stress using surveys, interviews, and physiologic measures. We know that adverse childhood experiences can negatively contribute to health problems later in life and that ANS measures are a valid measure of how these experiences get under the skin. To address issues of health inequities and the social determinants of health, nurses and researchers can assess young children's ANS responsivity and develop future interventions to support healthy physiologic responses to stressful experiences. Nurses are becoming leaders in this field since they are well-suited to identify and intervene to help families and young children adapt to an ever-changing and stressful world.

Conclusion

A child's neurobiological circuitry develops rapidly from birth to 5 years of age (Shonkoff et al., 2012). Understanding the measurable indicators that can affect the trajectory of these neurobiological and ANS pathways, including adversity and resilience factors, can reveal important information that may inform interventions during developmentally-sensitive time points. RSA and PEP are valuable measures to assess changes in ANS activity over time, with implications for understanding the biological embedding of early childhood experiences, self-regulation, and child adjustment, all of which can have important consequences for the health and wellness of an individual over their lifespan.

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