Abbey Alkon
School of Nursing
University of California
San Francisco, CA 94143-0606
E-mail: abbey.alkon@nursing.ucsf.edu

Suzanne Lippert
School of Medicine
University of California
San Francisco, CA

Nicole Vujan
School of Nursing
University of California
San Francisco, CA 94143-0606

Marya Eugenya Rodriquez
Center for Children's Environmental Health, Natividad Medical Center
University of California, Berkeley, CA

W. Thomas Boyce
Institute of Human Development and School of Public Health
University of California, Berkeley, CA

Brenda Eskenazi
Center for Children's Environmental Health, School of Public Health
University of California, Berkeley, CA

The Ontogeny of Autonomic Measures in 6- and 12-Month-Old Infants

ABSTRACT: *The purpose of this study was to develop a standardized protocol to measure preejection period (PEP), a measure of sympathetic nervous system, and respiratory sinus arrhythmia (RSA), a measure of parasympathetic nervous system, during resting and challenging states for 6- and 12-month-old infants and to determine developmental changes and individual stability of these measures. A 7-min reactivity protocol was administered to Latino infants at 6 months (n = 194) and 12 months (n = 181). Results showed: (1) it is feasible to measure PEP and RSA in infants, (2) the protocol elicited significant autonomic changes, (3) individual resting autonomic measures were moderately stable from 6 to 12 months, but reactivity measures were not stable, and (4) heart rate and RSA resting and challenge group means changed significantly from 6 to 12 months. Findings suggest that although infants' autonomic responses show developmental changes, individuals' rank order is stable from 6 to 12 months of age. © 2006 Wiley Periodicals, Inc.* Dev Psychobiol 48: 197–208, 2006.

Keywords: *psychobiology; development; stability; infant; autonomic nervous system; Latino*

INTRODUCTION

The autonomic nervous system (ANS) can be assessed using valid and reliable measures of the parasympathetic (e.g., respiratory sinus arrhythmia (RSA)) (Alkon, Goldstein, Smider, Essex, Boyce, & Kupfer, 2003; Cacioppo, Uchino, & Berntson, 1994) and sympathetic nervous systems (e.g., preejection period (PEP)) (Oberlander, Grunau, Pitfield, Whitfield, & Saul, 1999; Snidman, Kagan, Riordan, & Shannon, 1995). Infant's autonomic resting, challenging, and reactivity measures are important measures of individual psychobiology since they are related to later physical and mental health problems (Doussard-Roosevelt, Porges, Scanlon, Alemi, & Scanlon, 1997; Porges, Doussard-Roosevelt, Portales, & Greenspan, 1996; Snidman et al., 1995). Resting measures of the ANS indicate an individual's physiologic state when they are not responding to a challenge. Challenging measures of the ANS indicate an individual's physiologic response to a stressor or challenging condition. Reactivity measures of the ANS are an individual's physiologic response to a discrete, environmental stimulus or challenge compared to a resting state (Matthews, 1986).

Individual stability and developmental changes in autonomic measures have not been studied thoroughly in children. Parasympathetic measures of individual differences have been shown as early as 2 months of age (Bornstein & Suess, 2000b; Snidman et al., 1995), but it is not known when these measures stabilize and resemble adult values. Studies of developmental, ontogenic changes

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of the ANS show that heart rate (HR) and parasympathetic tone decrease during the first year of life (Bar-Haim, Marshall, & Fox, 2000; Fox & Porges, 1985; Izard et al., 1991; Stifter & Jain, 1996). Furthermore, changes in the sympathetic nervous system have rarely been studied in young children (Oberlander et al., 1999; Snidman et al., 1995), so it is not known when these measures stabilize or what ontogenic changes occur during infancy.

Some studies suggest that infants' *resting* parasympathetic tone may stabilize by the end of the first year (Fox, 1989; Fracasso, Porges, Lamb, & Rosenberg, 1994; Izard et al., 1991; Porter, Bryan, & Hsu, 1995; Porges, Doussard-Roosevelt, Portales, & Suess, 1994b; Richards & Cameron, 1989), while others show less consistent findings about the stability of infants' parasympathetic responses to *challenging* conditions (Fox, 1989; Oberlander et al., 1999; Porges et al., 1994b; Stifter & Jain, 1996). These studies show moderate correlations between parasympathetic measures across two points in time but no patterns or trajectories over time emerge. Reactivity measures, changes from rest to challenge, may be less stable than resting measures alone, since they reflect the integration of multiple systems, including emotions, cognition, attention and physiology, with environmental demands (Bornstein & Suess, 2000a).

It is challenging to compare study results on the stability of infants' resting and/or reactivity measures of the parasympathetic nervous system (e.g., RSA, vagal tone), since the studies include different definitions and methodologies. The different methodologies include variations in elapsed time between data collection periods (months to years) (Bar-Haim et al., 2000; Bornstein & Suess, 2000a; Fracasso et al., 1994; Porter et al., 1995; Stifter & Jain, 1996), age ranges (infancy only or from infancy through early childhood) (Izard et al., 1991; Porges et al., 1994b; Richards & Casey, 1991), parasympathetic measures (e.g., RSA, vagal tone, or high frequency bands) (Oberlander et al., 1999; Porges, 1992; Porter et al., 1995; Stifter & Fox, 1990), statistical methods of summarizing the data (resting means, difference scores) (Bornstein & Suess, 2000a; Izard et al., 1991; Richards & Casey, 1991) and analyzing responses over time (ANOVA, repeated measures ANOVA, correlations) (Bornstein & Suess, 2000a; Fracasso et al., 1994).

A standardized protocol was developed to measure the parasympathetic and sympathetic nervous system using RSA and PEP during rest and under challenging conditions. The objectives of this study were: (1) to describe the development and validity of a standardized protocol administered to 6- and 12-month-old infants, (2) to determine if infants' autonomic measures (HR, RSA, and PEP) during rest, across challenging conditions, and changes from resting to challenge conditions differ by sex or age (developmental changes from 6 to 12 months), and (3) to examine if the autonomic measures (HR, RSA, and PEP) during rest, across challenging conditions, and changes from resting to challenge conditions are stable for individual infants from 6 to 12 months of age.

METHODS

The current study is part of a larger longitudinal birth cohort study of the potential health effects of pesticides (e.g., organophosphates) and other environmental exposures in a population of pregnant women and their children living in the agricultural community of the Salinas Valley in California (Eskenazi et al., 2003; Eskenazi et al., 2004). The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) enrolled pregnant women over a 1-year period (October 1999–October 2000). Four hundred eighty-two 6-month-old infants and 449 12-month-old infants participated in the study. For more detail on the sample and recruitment procedures, see Eskenazi et al. (2003). The majority of mothers were Spanish-speaking, recent immigrants from Mexico, and low income. Parents signed consent forms approved by the Human Subjects Committees at the participating institutions. Maternal interviews and medical record reviews were conducted to collect information on family and child demographics and health. Pesticide exposure, assessed by urinary levels of organophosphates, was collected at both 6 and 12 months of age. The organophosphate levels were similar at both time points.

A 7-min ANS protocol was administered by bilingual, bicultural staff to a subset of CHAMACOS infants at 6 and 12 months of age after they completed a 1-hr assessment including height, weight, and a neurobehavioral tests, including The Bayley Scales of Infant Development (Bayley, 1993) at 6 months and Preschool Language Scale (Zimmerman, Steiner, & Pond, 1992) at 12 months of age. Infants with even identification numbers or who enrolled in the study between May and October 2000 were eligible to complete the ANS protocol. Of the 482 infants enrolled in the CHAMACOS at 6 months, 241 infants were eligible to participate in the ANS protocol and 194 infants completed the 6-month ANS protocol. Of the 241 infants eligible to participate in the ANS protocol at 12 months of age, 181 infants completed the ANS protocol. There were technical and logistical problems (e.g., limited equipment available), which affected our ability to offer ANS to all eligible infants. For the current analyses, only infants with clean, scoreable data seen within 2 months of the target ages, 6 and 12 months, were included. Therefore, the 6-month data included 150 infants, ages 4–8 months old, and the 12-month data included 142 infants, ages 10–14 months old. Eighty-five of these infants had ANS data collected and scored at both 6 and 12 months of age. There were no demographic differences between those infants assessed with ANS and those not assessed by ANS.

The reactivity protocol was administered at a research office located next to the local hospital or in a recreational vehicle designed to accommodate data collection near the participants' home. The protocol was administered in the child's native language, Spanish or English. Interrater reliability was assessed four times during the year by two research staff through direct observations or videotape reviews of the reactivity protocol. Reliability was based on protocol administration, electrode placement, and data quality.

The reactivity protocol was modified from a previously standardized protocol administered to 3- to 8-year-old children (Alkon et al., 2003; Boyce et al., 2001). The modified protocol was shortened from 15 to 7 min to accommodate an infant's attention span. The data collection procedures were modified from using spot electrodes to band electrodes since spot electrodes are sensitive to movement and difficult to place on infants with short, chubby necks. The tetrapolar configuration of electrodes included placement of two bands on the infant's neck and two bands on the infants' trunk to collect impedance, electrocardiograph and respiratory measures (Allen & Matthews, 1997). The first band electrode was placed 3 cm above the suprasternal notch (Fig. 1). This band electrode was attached to a 2 cm nylon band with a Velcro closure so there was no adhesive on the infant's skin. The second band electrode was applied directly to the infant's skin around the upper chest crossing the clavicles and suprasternal notch. The third band electrode was placed across the torso crossing the xiphisternal junction and the fourth band electrode was placed 3 cm below the third band. These two bands, placed on the torso (#3, #4), were applied to a 5 cm nylon band with a Velcro closure. This configuration and application procedure minimized infant's skin reaction and discomfort with the bands (personal communication with Elizabeth Gilles, MD). After the electrode bands were in place, the psychometrician spent 5 min checking the bands and equipment to verify quality cardiac signals during which time no data was collected.

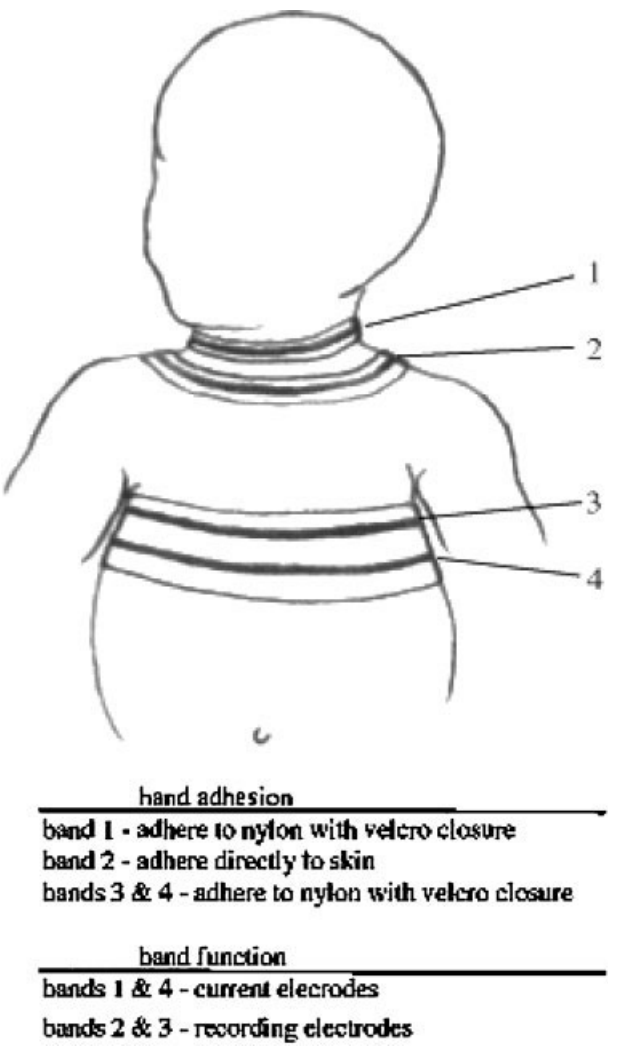

FIGURE 1 Electrode band configuration on infants (Alkon_-fig1.tif).

The electrode bands were attached to a Minnesota Impedance Cardiograph HIC-2000 and continuous measures of HR, ECG, Z_o (basal impedance) and dZ/dt (first derivative of the impedance signal) were collected during the protocol. A 4-mA AC current at 100 Hz was passed through the two outers, current electrode bands and Z_o and dZ/dt signals were acquired from the two inner voltage-recording electrode bands (Cacioppo et al., 1994) (Fig. 1). The data were filtered, extracted, and then scored using the ANSsuite software (Berntson, Cacioppo, & Quigley, 1993; Cacioppo et al., 1998).

RSA is the periodic oscillation in sinus rhythm occurring at the frequency of respiration and manifested as an increase in HR with inspiration and a decrease during expiration. As the parasympathetic influence on HR increases, referred to as parasympathetic withdrawal, the RSA index score decreases. RSA scores were calculated using the interbeat intervals on the ECG reading, respiratory rates derived from the impedance (e.g., dZ/dt) signal, and a bandwidth range of 0.24–1.04 Hz (Bar-Haim et al., 2000; Bazhenova, Plonskaia, & Porges, 2001; DiPietro, Porges, & Uhly, 1992; Porges, 1988).

PEP is an indirect, noninvasive cardiac measure of the sympathetic nervous system's influence on the cardiac cycle (Sherwood, 1995). PEP is the time interval, measured in milliseconds, of the duration of isovolumetric contraction in the left ventricle and it specifically represents the sympathetic influence on the contractility of the heart. As sympathetic activity increases, PEP scores decrease. PEP was measured in milliseconds as the interval between the ECG's Q wave and the B notch on the dZ/dt signal.

The standardized protocol administered to the infants included RSA and PEP measures at rest and during challenging conditions. The social, emotional, and physical challenges were intended to represent normative, common stressors experienced by infants. The protocol was administered in a standardized order. The 7-min protocol included three challenges (1 min each) preceded and followed by resting states (2 min each). During the first two resting minutes of the protocol, infants listened to digitally recorded lullabies in either Spanish or English. At minute 3, the infant watched a jack-in-the box wound up and jumping out of the box three times as the social/startle challenge. At minute 4, the infants' listened to a digital recording of a sick baby crying as the emotion challenge. At minute 5, the infants felt a vibrator on their leg, one at a time, as the physical challenge. During minutes 6 and 7, the infants again listened to digitally recorded lullabies in either Spanish or English to help them return to a resting state. The protocol was administered with the infant sitting in the mother's lap and facing the psychometrician.

Data Reduction

Four summary scores were calculated for HR, RSA, and PEP measures: mean resting, mean challenge, reactivity, and attenuation scores. HR, RSA, and PEP mean scores were

calculated for the resting minutes 1 and 2[1] and for the challenging tasks during minutes 3, 4, and 5. Reactivity or difference scores were calculated as the mean challenge minus mean resting score (Matthews, 1986). Responses to the challenges were regarded as one response, even though there were three distinct challenges. An attenuation or slope score was calculated as the standardized beta coefficient of the regression of minutes 3–7, including challenge tasks and the last lullaby (Boyce, Alkon, Tschann, Chesney, & Alpert, 1995). Attenuation can be considered a component of self-regulation, which measures an individual's physiologic ability to modulate their autonomic response to several sequential challenges over time.

Data Analysis

Descriptive statistics were calculated for all demographic data and autonomic measures. Chi-square analyses were conducted to compare the demographic characteristics of the infants who had complete data at both 6 and 12 months of age to the infants who completed the ANS protocol at either 6 or 12 months of age. To determine the construct validity of the protocol, repeated measures ANOVAs were conducted to compare HR, RSA, and PEP scores within subjects (minutes 1–7), between subjects (age, sex), and all interactions involving minute, age, and sex. Correlations were conducted to determine the construct and discriminant validity of the autonomic measures. All analyses were repeated for infants with data at both 6 and 12 months of age or either age.

The ontogeny of autonomic measures can be determined by individual stability and developmental group changes. According to Bornstein & Suess (2000a) stability is defined as the "...consistency in relative ranks of individuals in a group with respect to a function or process through time" and is operationalized using correlations. Individual stability was analyzed by correlating ANS measures at 6 months with ANS measures at 12 months. The stability of individual scores is determined by the strength of the correlation. High correlation coefficients reflect stability for the individual's rank over time (e.g., months and years) within the sample.

Developmental change or continuity is defined as the "...consistency in the absolute level of a function or process in a group through time" and operationalized using repeated measures ANOVA (Bornstein & Suess, 2000a). HR, RSA, and PEP summary scores (resting, mean task, difference scores, and attenuation scores) were analyzed by repeated measures ANOVA, using ANS mean scores as the within subjects factor, sex as the between subjects factor, and the interaction between ANS mean scores and sex.

Statistical significance was established a priori at $p < .05$ and analyses were conducted with SPSS 11.0.

RESULTS

Table 1 shows the infants' demographic characteristics for sex, birthweight, gestational age, mother's country of origin, and maternal tobacco and alcohol use during pregnancy for infants with scored data at 6 and/or 12 months of age. Infants who had only 6- or 12-month ANS data had similar demographic characteristics to infants who had both 6- and 12-month ANS data. The sample was reduced from 194 infants who were administered the reactivity protocol to 150 infants at 6 months of age due to data acquisition (e.g., noise due to movement; data not scoreable) and technical difficulties (e.g., equipment not available). Similarly, the sample of 181 infants who were administered the reactivity protocol at 12 months was reduced to 142 infants. Table 2 shows the means (SD) for all ANS summary scores for infants seen at 6 months, 12 months, and both 6 and 12 months with scored data.

Table 1. Demographic Characteristics of Sample with 6- and 12-Month ANS Data

	Data at 6 Months ($N = 150$)	Data at 12 Months ($N = 142$)	Data at Both 6 and 12 Months ($N = 85$)
Variable	*n* (%)	*n* (%)	*n* (%)
Sex			
Boy	81 (54)	66 (47)	43 (51)
Girl	69 (46)	76 (53)	42 (49)
Birthweight			
≥2500 g	145 (94)	133 (94)	78 (92)
<2500 g	5 (6)	9 (6)	7 (8)
Gestational age			
≥37 weeks	140 (94)	133 (94)	80 (94)
< 37 weeks	9 (6)	9 (6)	5 (6)
Mothers' country of origin			
Mexico	133 (89)	121 (85)	76 (89)
US	16 (11)	19 (14)	9 (11)
Other	1 (1)	2 (1)	0 (0)
Tobacco use during pregnancy			
No	141 (94)	134 (94)	79 (93)
Yes	7 (5)	7 (5)	5 (7)
Alcohol use during pregnancy			
No	123 (83)	115 (81)	71 (84)
Yes	26 (17)	27 (19)	14 (16)

Note: Numbers may vary slightly due to missing data.

Validity of Protocol

Construct, convergent and discriminant validity were analyzed to determine the validity of the protocol and measures. Repeated measures ANOVAs, controlling for age and sex, were conducted for HR, RSA, and PEP scores minute-by-minute of the protocol for the infants seen at

[1]Mean scores were calculated for the second resting task during minutes 6 and 7, but the resting scores for minutes 1 and 2 were significantly lower than minutes 6 and 7. Therefore, subsequent resting scores refer to minutes 1 and 2.

Table 2. Mean (SD) ANS Summary Measure for HR, RSA, and PEP

ANS Summary Score	6 Month Only ($N = 150$)[a] Mean (SD)	12 Month Only ($N = 142$)[a] Mean (SD)	Both 6 and 12 @ 6 Months ($N = 85$)[a] Mean (SD)	Both 6 and 12 @ 12 Months ($N = 85$)[a] Mean (SD)
Heart rate				
Resting (minutes 1, 2)	140.7 (11.4)	133.0 (12.8)	139.2 (11.2)	133.9 (13.0)[b]
Challenge (minutes 3–5)	142.1 (10.7)	132.5 (10.9)	141.7 (10.6)	132.9 (11.2)[b]
Reactivity (mean task-resting)	1.9 (5.1)	−0.3 (5.2)	2.6 (5.0)	−0.7 (4.5)[b]
Attenuation (minutes 3–7)	0.6 (.4)	0.6 (0.5)	0.6 (0.4)	0.5 (0.5)
RSA index				
Resting	3.3 (0.9)	3.8 (0.9)	3.3 (1.0)	3.7 (1.0)[b]
Challenge	3.2 (0.7)	3.7 (0.9)	3.2 (0.7)	3.7 (0.9)[b]
Reactivity	−0.1 (0.5)	−0.1 (0.5)	−0.1 (0.7)	−0.2 (.5)
Attenuation	−0.2 (0.6)	−0.3 (0.5)	−0.2 (0.6)	−0.3 (0.6)
PEP (ms)				
Resting	65.8 (8.9)	67.4 (10.4)	66.2 (8.8)	66.5 (10.0)
Challenge	65.6 (8.3)	68.0 (9.8)	66.2 (8.6)	67.7 (9.9)
Reactivity	−0.2 (3.4)	0.3 (3.8)	−0.1 (2.8)	0.8 (3.8)
Attenuation	−0.2 (0.5)	−0.2 (0.6)	−0.3 (0.5)	−0.1 (0.6)

[a]Numbers may vary by ANS summary score.

[b]Repeated measures ANOVA on 6- and 12-month ANS summary measures for infants seen at both 6 and 12 months, controlling for sex: HR resting $F(1,80) = 11.3$, $p < .01$, HR mean challenge $F(1,80) = 36.2$, $p < .01$, HR difference $F(1,77) = 25.8$, $p < .01$, RSA resting $F(1,80) = 9.9$, $p < .01$, RSA mean challenge $F(1,80) = 24.7$, $p < .01$.

6 months, 12 months, and both 6 and 12 months of age. Figure 2 shows the RSA and PEP means and 95% confidence intervals (CI) for infants with complete data for each minute of the protocol at 6 months and 12 months. For infants seen at 6 months of age ($n = 110–115$), there were statistically significant changes across protocol minutes for HR ($F(4,383) = 22.8$, $p < .01$), RSA ($F(5,475) = 6.6$, $p < .01$), and PEP ($F(5,402) = 2.7$, $p < .05$). For infants seen at 12 months of age ($n = 99–107$), there were statistically significant changes across protocol minutes for HR ($F(4,387) = 9.4$, $p < .01$) and RSA ($F(5,501) = 4.4$, $p < .01$), controlling for age and sex. There were no significant changes for PEP scores across the protocol. For infants seen at both 6 and 12 months of age ($n = 85$)[2], the pattern was similar except that there were statistically significant changes for *all* ANS measures at both 6- and 12-month visits: 6 months: HR ($F(4,195) = 20.5$, $p < .01$), RSA ($F(5,287) = 3.6$, $p < .01$), and PEP ($F(5,217) = 2.8$, $p < .05$); 12 months: HR ($F(4,199) = 16.1$, $p < .01$), RSA ($F(5,270) = 5.9$, $p < .01$), and PEP ($F(4,222) = 3.9$, $p < .01$).

At 12 months of age, the infants showed an overall dampened response to the challenges compared to 6 months of age. At 6 months of age, the greatest sympathetic activation (lowest PEP) and parasympathetic withdrawal (lowest RSA) occurred during the last minute of the protocol (second lullaby) intended to be the second resting measure.

[2]There were 74 infants with both RSA and PEP data at 6 and 12 months of age, compared to the 85 infants with either RSA or PEP data at 6 and 12 months of age. Results from repeated measures ANOVAs were similar for the 85 and 74 infants samples.

Convergent and Discriminant Validity

To validate the RSA and PEP measures, HR, RSA, and PEP were correlated (Tab. 3a and Tab. 3b). The correlation *between* ANS summary measures at either 6 or 12 months of age showed moderate to strong negative correlations for HR and RSA resting, HR and RSA mean challenge, HR and RSA reactivity, HR and RSA attenuation scores, HR and PEP resting, HR and PEP mean challenge, HR and PEP reactivity, and PEP attenuation scores. These findings supported the validity of the parasympathetic and sympathetic nervous system's influence on HR. RSA scores were negatively correlated with HRs such that as RSA decreased (parasympathetic withdrawal), HR increased. PEP scores were negatively correlated with HRs such that as PEP decreased (sympathetic activation), HR increased. There was no significant correlation between any of the RSA and PEP summary scores and, thus, there is minimal overlap between these measures. Therefore, RSA and PEP scores uniquely contribute to an integrated autonomic response at rest, during challenge, and as reactivity measures.

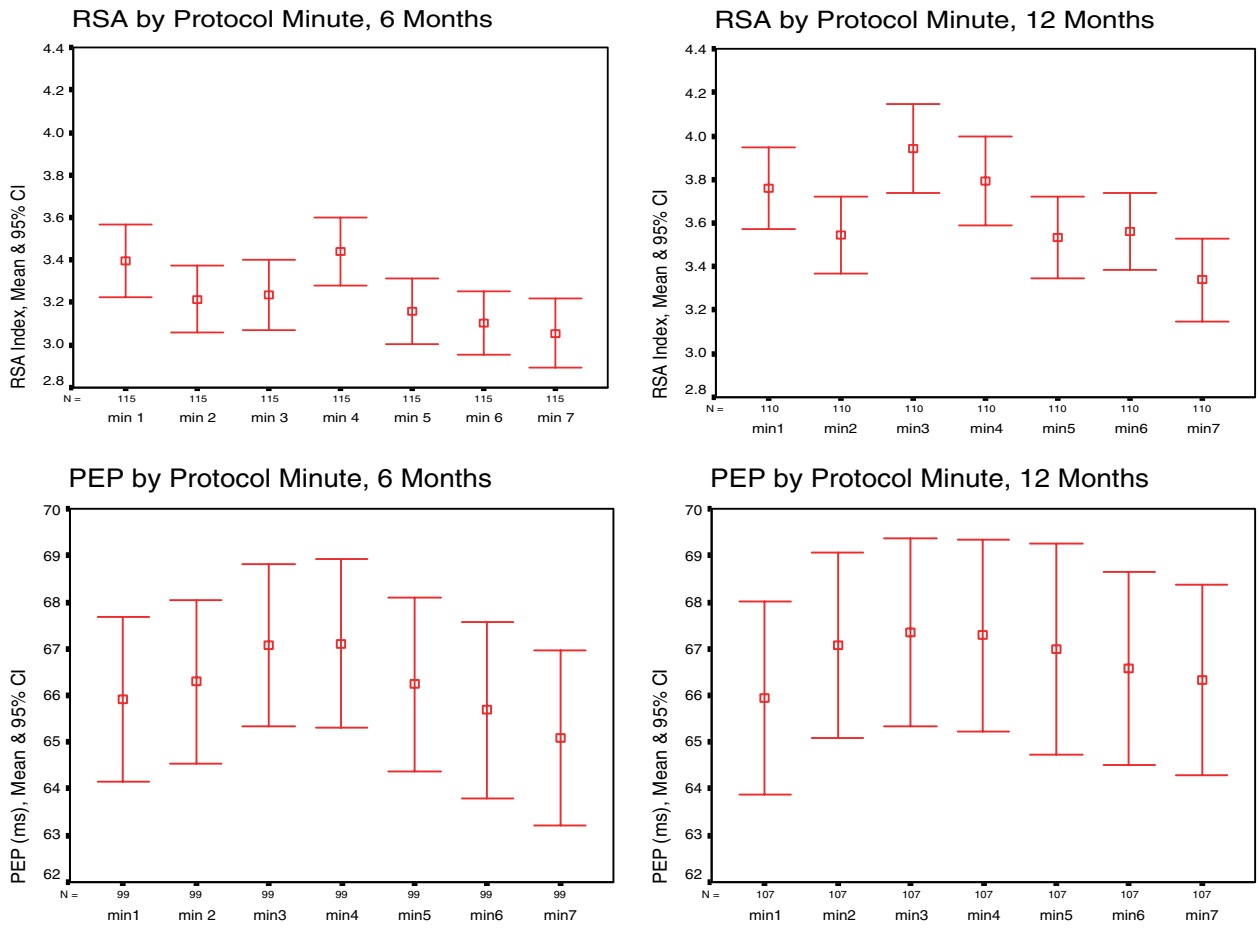

FIGURE 2 RSA and PEP by protocol minute (Alkon_fig 2. ppt).

Age and Developmental Changes

ANS measures minute-by-minute showed no statistically significant differences within each age group, 6, 12, and both 6 and 12 months of age using repeated measures ANOVA. ANS summary scores for each age group were analyzed for infants seen at both 6 and 12 months of age ($n = 85$) using repeated measures ANOVA (Tab. 2). Results showed significant changes in resting HR and RSA, HR and RSA challenge means, and HR reactivity scores from 6 to 12 months of age. As the infants matured, their mean HR decreased and mean RSA increased. PEP scores did not statistically differ between 6 and 12 months of age, but there was a nonsignificant trend showing mean PEP scores increasing from 6 to 12 months of age. Reactivity and attenuation scores did not significantly differ by age for RSA or PEP. The HR, RSA, and PEP reactivity scores were lower at 12 months of age compared to 6 months of age. The HR, RSA, and PEP attenuation scores were fairly stable from 6 to 12 months of age.

Table 3a. Correlations between ANS Summary Measures at 6 Months of Age ($n = 133–147$)

	RSA				PEP			
HR	Resting	Challenge	Reactivity	Attenuation	Resting	Challenge	Reactivity	Attenuation
Resting	−.47*				−.41*			
Challenge		−.49*				−.31*		
Reactivity			−.32*				−.38*	
Attenuation				−.27*				−.29*

*$p < .05$.

Table 3b. Correlations between ANS Summary Measures at 12 Months of Age ($n = 132–141$)

	RSA				PEP			
HR	Resting	Challenge	Reactivity	Attenuation	Resting	Challenge	Reactivity	Attenuation
Resting	−.64*				−.24*			
Challenge		−.66*				−.11		
Reactivity			−.47*				−.46*	
Attenuation				−.46*				−.24*

*$p < .05$.

Sex

Repeated measures ANOVA showed no statistically significant differences by sex for HR, RSA, or PEP minute-by-minute or by any of the summary scores.

Individual Stability

The correlations *within* ANS measures between 6 and 12 months of age showed that the resting measure was positively and moderately correlated from 6 to 12 months of age for HR, RSA, and PEP (Tab. 4). The mean challenge scores (minutes 3–5) were also positively and moderately correlated from 6 to 12 months of age for HR, RSA, and PEP. The correlations for HR, RSA, and PEP reactivity scores and attenuation scores were weak and not significant from 6 to 12 months of age. Thus, individuals maintained their rank within the group, for both parasympathetic and sympathetic resting and challenge measures from 6 to 12 months of age, but not for reactivity or attenuation measures.

DISCUSSION

This is the first study to simultaneously measure parasympathetic and sympathetic nervous system activity (via RSA and PEP, respectively) in infants. Band electrodes were used to acquire impedance and electrocardiogram measures in a cohort of primarily Latino infants while a standardized reactivity protocol, including rest and challenge conditions, was administered. Individual infants' autonomic systems responses during rest and under challenge conditions were stable from 6 to 12 months of age, but reactivity (difference between challenge and rest) and attenuation scores were not stable.

Changes in group means from 6 to 12 months of age for HR and RSA in both resting and challenging conditions suggest developmental or ontogenic changes in the parasympathetic nervous system during the first year of life; namely, as infants matured, the parasympathetic nervous system was activated and subsequently HR decreased. Sympathetic measures did not differ from 6 to 12 months of age.

This study is unique in its use of four separate scores (rest, challenge, reactivity, and attenuation) to summarize individual infants' dynamic responses to a standardized protocol. Many psychobiological studies of infants have limited their focus to HR and/or parasympathetic measures during only resting (Bar-Haim et al., 2000; Bornstein & Suess, 2000a; DiPietro et al., 1992; Doussard-Roosevelt et al., 1997; Fox, 1989; Fracasso et al., 1994; Huffman et al., 1998; Izard et al., 1991; Porter et al., 1995; Richards & Cameron, 1989; Stifter & Jain, 1996) or challenging conditions (Bazhenova et al., 2001; DiPietro et al., 1992; Fox, 1989; Oberlander et al., 1999; Richards & Casey, 1991). Few studies have included reactivity measures (Bornstein & Suess, 2000a; Calkins & Keane, 2004) and no previous studies have included attenuation measures.

Table 4. Correlations between 6- and 12-Month Old's ANS Summary Measures for HR, RSA, and PEP ($n = 74–82$)

	12 Months											
	Resting			Challenge			Reactivity			Attenuation		
6 Months	HR	RSA	PEP	HR	RSA	PEP	HR	RSA	PEP	HR	RSA	PEP
HR	.28*			.25*			.13			.21		
RSA		.40*			.31*			.13			.06	
PEP			.53*			.65*			−.11			−.05

*$p < .05$.

At the present time, findings on children's autonomic responses may differ depending upon the context in which the study occurred, the use of resting versus challenging protocols, and the choice of psychobiological measure (e.g., RSA, Vagal Tone, and PEP). To enhance comparability of autonomic findings across studies and populations, researchers should use consistent measurements and summary scores.

Protocol Validity

The validity of our findings is supported by other studies of primarily European–American infants, which used spot electrodes to measure the parasympathetic nervous system (RSA resting and reactivity measures) (Bazhenova et al., 2001; Bornstein & Suess, 2000a; Fracasso et al., 1994; Richards & Cameron, 1989). Two studies of 5-month-old infants had similar RSA resting indices of 3.26 (Bazhenova et al., 2001) and 3.02 (Fracasso et al., 1994), but one longitudinal study of 3- to 6-month infants had lower RSA resting indices of 1.96 (Richards & Cameron, 1989). Similar to our study (RSA rest = 3.3), a study of infants 13 months of age showed resting RSA indices around 3.7 (Bornstein & Suess, 2000a). One study of infants (Snidman et al., 1995) and another study of adults (Bar-Haim et al., 2000; Cacioppo et al., 1994; Snidman et al., 1995) showed that parasympathetic and sympathetic measures were distinct and nonoverlapping, which supports this study's findings that RSA and PEP are not correlated. There are no previous studies of PEP measures in 6- or 12-month-old infants.

In this study, infants generally showed more sympathetic activation and parasympathetic withdrawal during the last 2 minutes of the protocol (lullaby 2) than during the challenge conditions. Children with self-regulatory skills attenuate or decrease their state of arousal during a series of stressful conditions (Boyce et al., 1995; Lewis, 1992). Since the infants in this study did not return to a resting state during the second lullaby, their responses may have reflected a lack of self-regulation, difficulty sitting still for 7 minutes, or fatigue related to the length of the entire protocol (1-hr visit). Other studies showed that protocol length and infant's attention to challenges are important factors in eliciting autonomic responses (Bazhenova et al., 2001; Richards, 1987). Indeed, our reactivity protocol was longer than the 2–5 min protocols used for 4- to 9-month-old infants in previous studies (Bar-Haim et al., 2000).

The ontogenic changes in autonomic measures (HR, RSA resting and challenge) between 6 and 12 months of age shown in this study are supported by other studies; several studies have suggested that RSA increases and HR decreases during the first year of life (Bar-Haim et al., 2000; Fox, 1989; Porges et al., 1994b; Stifter & Jain, 1996). In a study of 5- to 10-month-old infants, resting HR rate decreased and RSA increased between these developmental periods (Fracasso et al., 1994). In a study of children from 4 months to 4 years of age, HR decreased from 4 to 9 months of age and then stabilized at 4 years of age. Parasympathetic activity increased from 4 to 14 months of age and then it moderately stabilized at 4 years of age (Bar-Haim et al., 2000). One study of younger infants (newborns to 5-month-olds) showed contrary changes; group means suggest increases in parasympathetic withdrawal and increased HR during this early period. This study too, however, showed an increase in parasympathetic activation between 6 and 12 months of age (Stifter & Fox, 1990). Overall, these studies indicate that there is an increase in parasympathetic activation, inhibition of the vagal influence on the heart, and a corresponding decrease in HR during the second half of the first year of life.

Since there are very few studies of the sympathetic influence on the ANS in the first year of life, it is not known how the maturation of the sympathetic nervous system versus the parasympathetic nervous system affects HR during the first year of life. Snidman et al. (1995) have shown that sympathetic activation influenced HR variability more than parasympathetic withdrawal for children under 2 years of age. Oberlander and Grunau (Oberlander et al., 1999) showed that the relative influence of the sympathetic (vs. parasympathetic) nervous system on HR was greater at 4 months of age than 8 months of age. On the other hand, Porges (1992) and Bar-Haim et al. (2000) have hypothesized that changes in HR in the first year of life may be due to maturational increases in the activity of the brainstem respiratory centers, shown by changes in parasympathetic activity. Thus, conflicting hypotheses remain about the sympathetic and parasympathetic influence on HR in the first year of life.

It is not known at what age autonomic resting and reactivity measures begin to show adult levels of individual and group stability. In one longitudinal study of mothers and children at 2 months of age and 5 years of age, children reached adult levels of resting parasympathetic activity by 5 years of age (Bornstein & Suess, 2000a). In studies of older children, increases in HR and parasympathetic activity continued until at least 7 years of age (Alkon et al., 2003; Marshall & Stevenson-Hinde, 1998).

ANS and Sex

Infant's autonomic resting and/or reactivity measures are similar for boys and girls in this study, as found in a previous studies of infants and toddlers (Bar-Haim et al., 2000; Bornstein & Suess, 2000a; Calkins & Keane, 2004; Huffman et al., 1998; Snidman et al., 1995).

ANS and Individual Stability

Individual stability of resting parasympathetic measures during infancy has been shown in our study ($r = .40$) and other studies (Bar-Haim et al., 2000; Bornstein & Suess, 2000a; Fox, 1989; Fracasso et al., 1994; Kagan, Reznick, & Snidman, 1988; Snidman et al., 1995). Resting RSA was moderately correlated from 2 to 5 months of age ($r = .55$) (Bornstein & Suess, 2000b), from 9 to 14 months of age ($r = .36$) (Bar-Haim et al., 2000), and from 5 to 10 months of age ($r = .60$) (Fracasso et al., 1994). Resting HR was also moderately correlated from 4 to 9 months of age ($r = .30$) (Snidman et al., 1995) and 5 and 10 months of age ($r = .43$) (Fracasso et al., 1994). Although there are no comparable studies of the stability of sympathetic measures, this study showed that PEP resting measures were moderately stable from 6 to 12 months of age ($r = .53$).

Findings from infant studies of HR and parasympathetic measures during challenging conditions were somewhat inconsistent. In this study, there was moderate stability for mean challenge responses from 6 to 12 months of age for HR, RSA, and PEP. In a study of infants from 5 to 14 months of age, HR and parasympathetic responses during challenges were stable (Fox, 1989). On the other hand, a study of infants assessed at 4, 9, 14, 24, and 48 months of age showed there was moderate stability for HR and parasympathetic responses during a visual stimulus from one assessment to the subsequent assessment, but not across the ages (Bar-Haim et al., 2000). Other studies of autonomic responses during challenges were cross-sectional (Bazhenova et al., 2001) or did not include correlations of challenge responses over time (Oberlander et al., 1999).

In this study, HR, RSA, and PEP reactivity measures (difference scores) were not stable for individual infants from 6 to 12 months of age. These findings were supported by two cohort studies of 2- to 5-month-old infants (Bornstein & Suess, 2000b) and children from 2 months to 5 years of age (Bornstein & Suess, 2000a,b), both of which showed that parasympathetic reactivity scores (e.g., vagal tone) were not stable.

Implications

The relationship between autonomic measures and behavior has been studied primarily with parasympathetic and not sympathetic measures. These findings are not consistent. Three-month-old infants with parasympathetic withdrawal during a resting state were rated by their mothers as being negative and difficult to soothe during a stressful paradigm (Huffman et al., 1998). In a study of 2- to 4-year-old children, boys with parasympathetic withdrawal at rest were more likely to have high risk behaviors (specifically, aggression and destructive behavior) compared to low-risk behaviors (Calkins & Dedmon, 2000). Four-year old children with observed reticent behavior (e.g., unoccupied time in group activity, not talking to peers, anxious) had more parasympathetic withdrawal during resting conditions than highly social children or children who engage in frequent solitary play (Henderson, Marshall, Fox, & Rubin, 2004). These studies show that young infants and children with low RSA scores at rest, reflecting parasympathetic withdrawal, display more negative behaviors and show more signs of difficulty with self-regulation than children with higher RSA scores.

On the other hand, some studies show that resting parasympathetic activation is associated with negative behaviors. Five-month-old infants who had parasympathetic activation during a resting state displayed more mother-rated fearful behavior and high activity than infants with parasympathetic withdrawal (Stifter & Fox, 1990). Nine-month-old infants who had parasympathetic activation during a resting state had more difficult temperaments than infants with less activation (Porges, Doussard-Roosevelt, Portales, & Suess, 1994a). Toddlers with high resting parasympathetic measures had negative temperament reactivity (Calkins, 1997).

Measures of parasympathetic and sympathetic reactivity, not resting measures, have been associated with behavior. In a study of low birthweight infants, RSA increases from 32 to 34 weeks of gestational age predicted social competence at 6–9 years of age (Doussard-Roosevelt, McClenny, & Porges, 2001). In a longitudinal study, 9-month-old infants who had parasympathetic withdrawal during a challenging task were more likely to have behavior problems at 3 years of age than infants with less parasympathetic withdrawal (Porges et al., 1996). In one study of sympathetic changes from a supine to erect position in 2-week-old infants, reactivity predicted fearful behavioral responses at both 14 and 21 months of age (Snidman et al., 1995).

Physiologic regulation, measured as autonomic reactivity or difference scores, may be an important component of behavioral- and self-regulation. Studies show that preschool-age children with high parasympathetic reactivity had better self-regulatory skills (Calkins, 1997), fewer behavior problems, and more positive emotions than other children (Calkins & Keane, 2004). On the other hand, school-age children with high parasympathetic reactivity had negative regulatory skills, including more sleep problems than other children (El-Sheikh, 2004). Future longitudinal studies of physiologic and behavioral self-regulation may help us to understand what affects infants' development during this critical period of life.

Although there are ontogenic changes in infants' autonomic responses across the first year of life, infants'

resting responses may stabilize by the end of the first year, as seen in moderate correlations for resting HR and RSA in the last half of the first year of life in this and other studies (Fracasso et al., 1994; Izard et al., 1991; Porges et al., 1994b; Porter et al., 1995; Richards & Cameron, 1989). This emerging stability in the infants' resting physiologic state may reflect genetic and/or biologic contributions toward internal homeostasis of the ANS (Bornstein & Suess, 2000a). Reactivity responses are not stable and may, thus, be determined by both biologically determined resting states in addition to repeated environmental exposures to stress and protective factors. Young children's autonomic responses to challenges may be shaped by their repeated exposure to stressors and their family's ability to cope with these stressors. Therefore, while children's resting autonomic states are fairly stable during infancy, their autonomic responses to stress are not yet fully developed.

Limitations

Although this study showed new, significant findings, there are several limitations. The same protocol was administered 6 months apart and there is a possibility that the infants recalled the tasks and, thus, exhibited dampened responses at 12 months of age. The 7-min protocol might have been too long for the infants to sustain their attention and too short to show recovery or a return to resting states. Lastly, these findings are not generalizable to all populations since the sample consists of low-income infants from primarily Latino farmworker families.

Future Studies

Additional longitudinal studies of infants' autonomic responses at rest and during challenging conditions are needed to delineate when autonomic measures stabilize for individuals and groups. Studies that examine the integrated effect of the sympathetic and parasympathetic nervous system during infancy and early childhood are needed to understand their influence on self-regulation, attenuation, attention, and behavior. Infancy is a critical period for autonomic development; neuron myelination is occurring and the nervous system is developing but self-regulatory behaviors have not yet developed. We gain a more thorough understanding of infants' autonomic development through studies of diverse populations that employ comparable psychobiologic measures and standard definitions of rest and challenge. These studies may lead the way to interventions for highly reactive infants at risk for socioemotional and/or behavioral problems later in life.

NOTES

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