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Parasympathetic and Sympathetic Responses to the Strange Situation in Infants and Mothers From Avoidant and Securely Attached Dyads

ABSTRACT: *Vagal reactivity and salivary α-amylase (sAA) were assessed in infants (M age = 13.55 months) and their mothers during the Strange Situation Paradigm (SSP) to investigate differences in physiological responses in a sample of insecure-avoidant and securely-attached dyads (N = 132). Infants classified as insecure-avoidant had significantly higher vagal withdrawal during the SSP and higher sAA overall, suggesting that the avoidant attachment pattern is associated with a greater allostatic load. During separation episodes of the SSP, all mothers showed significant vagal withdrawal, suggesting greater attempts at regulation. During the last reunion, typically the most stressful episode for infants, mothers of secure infants showed greater vagal withdrawal than mothers of insecure-avoidant infants, suggesting greater attempts by these mothers at interactive repair. Results for mothers and infants supported the allostatic load theory. © 2008 Wiley Periodicals, Inc.* Dev Psychobiol 50: 361–376, 2008.

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INTRODUCTION

Contemporary biosocial models propose that the attachment relationship is a context in which the caregiver provides cues that influence and regulate the state of the infant at both biological and behavioral levels and that may foster the development of coping and social response behaviors (e.g., Calkins, 2004; Calkins & Hill, 2007; Hofer, 1994, 2006). Differential patterns of caregiver-infant attachment may set the stage for the development of characteristic patterns (e.g., hypo- vs. hyper-reactivity) of how infants' respond to stress, threat, and challenge. In fact, early adversity, social experience, and relationship quality are thought to shape individual differences in stress response characteristics at both the behavioral and biological levels (Anisman, Zaharia, Meaney, & Merali, 1998; Cicchetti & Walker, 2001; Gunnar & Cheatham, 2003; Gunnar & Donzella, 2002; Schneider, 1992). This study sought to identify patterns of stress responding in early life using a multiple-operational biological measurement approach (Granger & Fortunato, submitted for publication), an approach aimed to include multiple stress-related biological measures in contrast to the majority of multiple-level approaches that notably examine multiple measures, but usually only one at the biological level and perhaps one or more at the behavioral level. We examine physiological measures from the sympathetic and parasympathetic branches of the autonomic nervous system

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(ANS) during the Strange Situation Paradigm (SSP) in secure and insecure-avoidant infants and their mothers. The concomitants and correlates associated with insecure-avoidant attachment classification are understudied relative to other patterns of insecurity such as insecure–resistant and insecure-disorganized; however, infants who exhibit this pattern may be at high risk for stress-related adjustment problems. Moreover, we measure mothers' reactions and how mothers' and infants' physiological responses interrelate at the dyadic level.

The Case of the Insecure-Avoidant Attachment Relationship

We focus specifically in this study on contrasts between dyads that are classified as insecure-avoidant and secure. Whereas studies show that the insecure-resistant and disorganized attachment patterns are more likely to show physiological distress reactions to the SSP (e.g., elevated cortisol indicating hypothalamic-pituitary-adrenal axis, HPA, activation) and are more at risk for psychopathology when compared to secure infants (see reviews: Cassidy & Berlin, 1994; Van Ijzendoorn, Schuengel, & Bakermans-Kranenburg, 1999), overall less is known about the physiological reactivity and risk factors associated with insecure-avoidant attachment especially during the SSP itself (although see: Burgess, Marshall, Rubin, & Fox, 2003; Schieche & Spangler, 2005; Spangler & Grossman, 1993). Infants classified as insecure-avoidant, on average, display comparably less behavioral distress during SSP reunion episodes and more self-oriented regulation (e.g., self-comforting and exploration) strategies than do infants classified as securely attached, who may or may not show distress and are more likely to use more mother-oriented regulation strategies (e.g., looking at the mother) (Braungart & Stifter, 1991; Cassidy, 1994; Spangler & Grossman, 1993; Zelenko et al., 2005).

The appearance of less distress and more regulation may be misleading if only behavioral observations are considered and internal physiological measures are not examined. Studies reveal that infants classified as insecure-avoidant, in comparison to infants classified as secure, show similar increases in heart rate and higher cortisol during separations from their mothers despite displaying minimal distress behaviorally, suggesting a disassociation between behavior and physiology for the insecure-avoidant group (Spangler & Grossman, 1993; Zelenko et al., 2005). However, methods used to replicate this finding have collapsed across insecure-resistant and insecure-avoidant groups and other studies have shown that only infants with an insecure-resistant or insecure-disorganized pattern of attachment show heightened cortisol (Gunnar, Brodersen, Nachmias, Buss & Rigatuso, 1996; Hertsgarrd, Gunnar, Farrell, & Nachmias, 1995; Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996; Spangler & Schieche, 1998; Schieche & Spangler, 2005). One reason for these contradictory findings may be the physiological system that was measured. For instance, cortisol reactivity is thought to occur when stress is coupled with extreme or prolonged distress (Henry, 1973), perhaps more indicative of insecure-disorganized or insecure-resistant behavioral patterns. Other physiological systems (i.e., Parasympathetic Nervous System [PNS] via vagal regulation and Sympathetic Nervous System [SNS] activity via fight–flight reactivity) may be better measures of the internal reactivity of infants classified as insecure-avoidant. These physiological systems support stress responding and regulation processes important for the self-oriented regulatory behaviors characteristic of infants with avoidant attachments. Examining the physiological reactivity of infants classified as insecure-avoidant could illuminate an early-appearing stress-response style as a risk factor.

Within the realm of psychosomatic medicine, the insecure-avoidant attachment pattern in adults is viewed as a risk factor for vulnerability to, and maintenance of, physical disease due to the suppression of negative emotion and the avoidance of social support (Kotler, Buzwell, Romeo, & Bowland, 1994). This regulatory pattern may be costly at the metabolic level, resulting in high allostatic load. Allostatic load is the wear and tear the body sustains during fluctuations between stress reactions and maintenance of homeostasis (McEwen, 1998; Sterling & Eyer, 1988). While allostatic fluctuations are normal phenomena for coping with daily stress and challenge, individual differences in allostatic load overtime can lead to deleterious outcomes. For example, in adults, suppression of intense emotions and regulation of those emotions is associated with lower immune functioning and higher rates of stress-related illnesses (Christensen et al., 1996; Esterling, Antoni, Fletcher, Margulies, & Schneiderman, 1994; Maunder, Lancee, Greenberg, Hunter, & Fernandes, 2000; Petrie, Booth, Pennebaker, Davison, & Thomas, 1995; Picardi et al., 2003).

At least one study shows that insecure infants have an inadequate coping style. During play, infants with insecure-avoidant attachment, and not those with secure or insecure-resistant attachment, fail to show the normative drop in heart rate associated with focused play (e.g., object orientation), suggesting that while infants classified as insecure-avoidant may *appear* well regulated, in fact, this regulation is rigid, unfocused, and of poor quality (Spangler & Grossman, 1993). In the stress and coping literature there is a clear link between avoidant attachment, regulation of negative affect, and risk for negative health outcomes. It is not yet clear whether links among the attachment system, regulation processes, and physiology originate in infancy. In this study we address this possibility.

Mothers' Physiological Responses and Attunement With Their Infants

Several models of early development (Hofer, 2006; Schore, 2000) stress the importance of the caregiver's role in directly and indirectly regulating her child's behavioral and biological systems (Calkins & Fox, 2002). Yet, few studies have examined a mother's ability to regulate herself and how this regulation may enhance or hinder her interactions with her infant (for exceptions, see, Bornstein & Suess, 2000; Cumberland-Li, Eisenberg, Champion, Gershoff, & Fabes, 2003). Evidence from non-human primate models indicates that mothers may show physiological reactions to separations from their offspring (Levine, Wiener, & Coe, 1993). The physiological reactions of a mother to separation and her ability to effectively regulate those reactions may be associated with her capacity to respond appropriately to her child, and thus influence how her child learns to regulate his or her own distress.

In addition, co-regulation or attunement of mother and infant may be an important factor to consider. Attunement is a natural process by which behavioral or physiological systems, in particular those associated with stress responding, of the mother and child are naturally matched or in alignment (e.g., heart rate concordance between mother and child; Field, 1985). Several theorists believe that an attachment system characterized by security promotes behavioral and biological synchrony between the caregiver and the infant, creating a biobehavioral system that supports the development of the infant's own regulation systems (Field, 1996; Hofer, 2006; Riete & Capitanio, 1985). In fact, attunement has been found to vary by attachment classification. For example, only secure dyads experienced physiological attunement during the SSP (i.e., the mother's heart rate was concordant with that of her infant; Donovan & Leavitt, 1985; Zelenko et al., 2005). It may be that mothers of infants with secure attachments are more perceptive of their children's cues for soothing and may respond in sensitive and empathic ways that are in tune with their infants' needs. This behavioral attunement may reflect underlying physiological concordance. For example, after recovery from a stressful interaction, greater physiological concordance was related to greater behavioral synchrony within dyads and in another study, physiological concordance was found only for those dyads in which mothers were rated as more sensitive during free play episodes (Ham, Tronick, & Marci, 2007; Moore et al., in press). Moreover, because infants with an insecure–avoidant attachment pattern are actively minimizing their contact and interaction with their mothers (Cassidy, 1994), it is less likely that the dyad would be behaviorally, emotionally, or physiologically attuned. In fact, several studies have found that mothers of infants with an insecure-avoidant attachment pattern are less sensitive to their infants' needs and either more rejecting or intrusive (Isabella, 1993; Isabella & Belsky, 1991; Isabella, Belsky, & von Eye, 1989). The current study investigated whether infants' attachment classification predicted mothers' physiological reactivity and regulation and whether attunement varied by secure or avoidant attachment pattern.

Vagal Reactivity: Index of Parasympathetic Stimulation of the Heart

Porges' polyvagal theory proposes that the vagal system plays an important role in social interaction, helps to control facial expressions and display of emotions, and is a measure of stress responsiveness (Porges, 1992, 2007). Thus, parents' and infants' vagal regulation should be an important component of a challenging interactive sequence such as the SSP. Under conditions of challenge, the Parasympathetic Nervous System (PNS) *withdraws* vagal control of the heart and allows for the organism either to engage and attend to the challenge and/or prepare for reciprocal sympathetic (SNS) influence on the heart resulting in a fight-flight response (Berntson, Cacioppo, & Quigley, 1993; de Geus, Veerman, Hoogstraten, & Nieuw Amerongen, 2003; Huffman et al., 1998; Porges, 1992). More specifically, vagal tone is a measure of heart rate variability that is based on the vagus inhibitory control of the heart. It is measured as the amplitude of respiratory sinus arrhythmia (RSA), a measure of heart rate acceleration and deceleration during the respiratory cycle. Typically, under conditions of stress or challenge, RSA decreases from baseline levels indicating the withdrawal of vagus stimulation (i.e., vagal withdrawal) and change from homeostasis in order to support regulatory processes and coping responses (Bazhenova, Plonskaia, & Porges, 2001; Calkins, 1997; Porges, 1991; Porges, Doussard-Roosevelt, & Maiti, 1994). Vagal regulation (Berntson et al., 1993; Hirsch & Bishop, 1981; Porges, 1992) may provide new evidence for early differential patterns of psychophysiological support for coping with the stress of separation, for both the infant and mother. In particular, if vagal regulation of infants or mothers varies by attachment pattern then it is possible that some behavioral response patterns could be interpreted as requiring greater internal effort or as more metabolically costly to support.

Salivary α-Amylase: Surrogate Marker of Sympathetic Nervous System Arousal and Reactivity

SNS activity or the fight–flight response is an important individual difference variable to consider when investigating responses to challenging social interactions such as the SSP. Traditional psychophysiological assessments of the SNS involve electrodes, computerized recording apparatus, and sophisticated data reduction algorithms. Recently there has been renewed interest in monitoring the SNS subcomponent of the stress response non-invasively in saliva (e.g., Granger et al., 2006; Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007). The most common serological measures of autonomic (sympathetic) activation are catecholamines. Unfortunately, however, direct measurements of catecholamines in saliva seem not to reflect SNS activity (Schwab, Heubel, & Bartels, 1992). Epinephrine (EPI) and norepinephrine (NE) are released by sympathetic nerve endings in tissues and glands through the body as well as in response to HPA activation via the adrenal medulla. The salivary gland, glandular duct cells, and vascular bed of the salivary glands are rich with beta-adrenoreceptors (Nederfors & Dalholf, 1992). SNS activation affects the release of catecholamines from nerve endings and these compounds' action on adrenergic receptors influences the activity of the salivary glands. A small but rapidly growing literature suggests that an enzyme released from the salivary gland, salivary α-amylase (sAA), might serve as a non-invasive and an easily obtainable surrogate marker of SNS activity (see Granger et al., 2006, 2007). Several studies with adults and adolescents have shown that sAA increases in response to stressors in social and academic domains (e.g., Trier Social Stress Test, free speech in front of an audience) (Gordis, Granger, Susman, & Trickett, 2006; Kivlighan & Granger, 2006; Nater et al., 2006; Stroud, Handwerger, Granger, Kivlighan, & Solomon, 2006; van Stegeren, Rohleder, Everaerd, & Wolf, 2006). Much less research has examined sAA in infants or young children and no research has been done in relation to patterns of attachment.

With regard to research on sAA with infants and children, Davis, Kannan, Marucut, Granger, and Sandman (2007) report that sAA levels can be detected as early as 2 months of age, levels remain lower than those for adults until 24 months, and that sAA reactivity to stress (i.e., inoculation during a well baby visit) was not evident until 6 months. Furthermore, evidence shows that responses to stress as indexed by sAA are different from responses to stress as indexed by salivary cortisol in terms of the speed of response, the underlying systems of activation, and in the qualitative meaning of the response (for a review see Dickerson & Kemeny, 2004; Gordis et al., 2006). For example, more children showed increases in sAA than in salivary cortisol in response to the same battery of tasks, suggesting that sAA might be a more sensitive measure of reactivity to everyday stressors and challenges than is the HPA system and salivary cortisol (Mize, Lisonbee, & Granger, 2005).

Given that dissociations among various measures of physiological reactivity, even those activated by a common system such as the SNS, are frequently found (Quas, Hong, Alkon, & Boyce, 2000), and the fact that ANS responses are subject to the individual specificity phenomenon (Engel, 1972) in which children may consistently react in one specific physiological modality, the examination of a salivary measure of SNS activity is likely to provide unique information in addition to knowledge based on other physiological measures related to the SNS (e.g., pre-ejection period). The addition of sAA as another measure of physiological response will strengthen the description of physiological profiles of patterns of attachment and may provide further support for the idea that stress loads may vary in infants and mothers depending on their attachment classifications.

Summary and Goals of the Current Study

The current study had three main goals. First, we sought to clarify the role of physiology in insecure-avoidant infants in comparison to secure infants in order to identify an early biobehavioral pattern of stress responding that could have implications for later social and emotional development, adaptive functioning, and mental and physical health. Specifically we expected that infants classified as insecure-avoidant would exhibit greater vagal withdrawal and sAA in response to the SSP, indicating a greater allostatic load, than would infants with a secure attachment.

The second and third goals of the current study were more exploratory and sought to investigate the caregiver's own physiological reactivity to the SSP and physiological attunement with her infant. Specifically we expected that mothers would show physiological reactions to separations from their infants during the strange situation procedure and that those physiological reactions may vary by attachment classification. For example, it may be that mothers of secure infants are more likely to show physiological reactions to separations from their infant than are mothers of insecure-avoidant infants who show comparably less distress. Finally, based on past studies (Donovan & Leavitt, 1985; Zelenko et al., 2005), we expected to observe greater physiological attunement among mothers and infants in securely attached dyads. Investigation of mothers' own physiological reactions and attunement with their infants might help to explain maternal influences on attachment patterns and how the attachment system supports and reinforces certain biobehavioral patterns, such as developing regulatory systems.

METHODS

Participants

Participants were drawn from the Durham Child Health and Development Study, a longitudinal sample consisting of 206 healthy, full-term infants recruited at 3 months of age. Recruitment procedures specified an approximately equal number of European American and African American families sampled from both lower- and higher-income groups. The infant's race was determined by the race of the mother (or primary caregiver), while income status was determined by whether families were above or below 200 percent of the federally established poverty threshold. Families were drawn from a largely urban community via fliers and postings at birth and parenting classes, as well as through phone contact via birth records. There was a 13% rate of attrition by the 12 months assessment, which is the focus of the current study, due to a family having withdrawn from the study, moved out of state, or the inability to schedule an assessment that fit within the family's schedule.

The current study included 132 infants (mean age = 13.55, SD 1.2) classified as either insecure-avoidant or secure and their mothers. Sixty five of the infants were male and 67 were female. Seventy four of them were African American and 58 were European American. Sixty-seven families were low income (below 200% of the poverty level). Mothers' mean age was 28.4 years. Approximately 12% of mothers had no high school degree, 20% had either a high school diploma or a G.E.D., and 67% had some college or more. Distribution of race and income across secure and avoidant dyads was relatively equal, and chi-square analyses indicated there were no significant differences between attachment classifications on demographic variables (race χ^2 $(1, N = 132) = 1.62$, $p = .203$; income χ^2 $(1, N = 132) = .007$, $p = .932$).

Procedures and Measures

General Overview. Infants and their mothers participated in several joint and individual tasks followed by a standardized interview and completion of demographic questionnaires by mothers. All tasks were videotaped through a one-way mirror for later coding and analysis.

SSP Procedure and Attachment Classification. Mothers and their infants participated in the Ainsworth Strange Situation Paradigm (SSP). This procedure followed the protocol developed by Ainsworth, Blehar, Waters, and Wall (1978) for observing and classifying infants into discrete categories of attachment quality.

All videotapes were coded by two researchers who were trained by the Sroufe laboratory at the University of Minnesota. Thirty percent of the tapes were coded for reliability with a Cohen's kappa of .85. The distribution of attachment classifications in the present sample consisted of 95 children classified as secure, 37 children classified as insecure-avoidant, and 15 classified as insecure-resistant.[1] For the purposes of the current study, we were particularly interested in avoidant and secure dyads' regulation strategies.

[1]As part of a larger longitudinal study this sample was also coded for disorganized attachment classification which may reclassify infants from each group (secure, avoidant, resistant) into the disorganized group. In our case, less than 10% of insecure-avoidant infants reclassified as disorganized had relevant physiological data and were not included in the current study focused on insecure-avoidant attachment.

Mother and Infant RSA. At the start of the assessment, the experimenter placed two disposable pediatric electrodes on the infant's chest while the infant was seated in a baby seat or mother's lap. The mother also had electrodes attached to her chest within a belt that was situated over her heart. The electrodes from mother and infant monitors were connected to a preamplifier, the output of which was transmitted to a heart rate monitor (Mini Logger; Mini-Mitter/Respironics, Bend, Oregon) for R-wave detection. The infant wore a smock with a large pocket in which the heart rate monitor was placed, while the mother was asked to place the monitor in a pocket or on the table next to her. Heart inter-beat-interval data was continuously collected during the SSP, and manual electronic signals to the heart rate monitor were employed to mark the start and end of each episode of the SSP.

Data files containing infant heart interbeat intervals (IBIs) were edited for bodily movements, tugging on electrodes, and physical force and then analyzed using Mxedit software (Delta Biometrics, Inc., Bethesda, MD) by two Mxedit-certified reliable researchers. Data files that required editing of more than 10% of the data were not included in the analyses and were considered missing data. To calculate RSA, Porges's (1985) algorithm was employed, which uses a moving 21-point polynomial to detrend periodicities in HP slower than RSA and a bandpass filter of (.24–1.04) for young children (Huffman et al., 1998; Porges, 1996; Stifter & Fox, 1990). This estimate of RSA is derived by calculating the natural log of this variance and is reported in units of ln $(ms)^2$. RSA was calculated every 30 sec for each episode of the SSP. However, for episodes 6 and 7 of the SSP, 15 s epochs were used because these episodes were often shorter than 2 min. These epoch durations are typical for studies of short duration tasks (Hamilton, Mckechnie, & Macfarlane, 2004; Huffman et al., 1998). For the purposes of the current analyses, the second (first full 3-min) episode of the SSP served as the baseline measure of vagal tone. The mean RSA of the 30 or 15 s epochs within each episode was used in subsequent analyses. Those data points (numbers in parentheses) derived from less than two total epochs (6), or with a standard deviation above 1.5 (2), were considered to be outliers and excluded from the current analyses.

Mother and Infant sAA. sAA was examined before and after the dyads participated in the SSP. This study is part of a larger longitudinal study (Durham Health and Development Study), and the data collection procedures for saliva samples were originally designed for cortisol collection. However, these samples can be easily re-assayed for salivary α-amylase, which only requires small amount of saliva (10 μl) (Granger et al., 2006). The first saliva sample (baseline) was collected approximately 5 min after arrival to the lab (M [time of day] = 12:52 P.M.; SD = 2.91), with 75% of the sample providing the first sample in the afternoon (11:00 am–5:30 pm). Because of possible effect of diurnal fluctuations (Nater, Rohleder, Sclotz, & Kirschbaum, 2007) time of day for first collection is controlled in all analyses including sAA as a variable. The second sample was collected 15 min following the final reunion episode (post-15) to assess for change from baseline (i.e., reactivity), and a third sample was collected 45 min after final reunion episode (post-45) to assess recovery back to baseline levels. Samples were collected from infants and mothers by having them gently mouth a 3 inch cotton dental roll (Talge, Donzella, Kryzer, Gierens, & Gunnar, 2005). Once the tip was soaked with saliva, it was placed in a needleless syringe, and sampled was expressed into collection vials. Samples were immediately placed in a freezer and then frozen within hours at −20 C. They were stored frozen until batched and shipped on dry-ice overnight to Salimetrics Laboratories (State College, PA) where they were stored frozen at −80 C until re-assay. On the day of testing, samples were brought to room temperature, centrifuged at 3,000 RPM for 15 min, and the clear top-phase of the sample was pipetted into appropriate test tubes.

All samples were assayed for sAA following Granger et al. (2006) using a commercially available kinetic reaction assay (Salimetrics Laboratories). The assay employs a chromagenic substrate, 2-chloro-p-nitrophenol, linked to maltotriose. The enzymatic action of sAA on this substrate yields 2-chloro-p-nitrophenol, which can be spectrophotometrically measured at 405 nm using a standard laboratory plate reader. The amount of sAA activity present in the sample is directly proportional to the increase (over a 2 min period) in absorbance at 405 nm. Results are computed in U/ml of sAA using the formula: [Absorbance difference per minute × total assay volume (328 ml) × dilution factor (200)]/[millimolar absorptivity of 2-chloro-p-nitrophenol (12.9) × sample volume (.008 ml) × light path (.97)]. Intrassay variation (CV) computed for the mean of 30 replicate tests was less than 7.5%. Inter-assay variation computed for the mean of average duplicates for 16 separate runs was less than 6%.

sAA measures were moderately skewed and were therefore transformed using a square root transformation (Tabachnick & Fidell, 2001). In all analyses, outliers (number in parentheses) were removed from each of the following variables: infant baseline (2), infant post-stress (1), and infant follow-up (2) and mother baseline (2), mother post-stress (1), and mother follow-up (1).

Missing Physiological Data. Participants were excluded only if they were completely missing all physiological data. This included the small insecure-resistant group ($N = 15$), eleven of which were missing most RSA measures and ten of which were missing most sAA measures. As a result this group was not included in analyses. For the dyads classified as insecure-avoidant and secure (N = 132) some data were completely missing for both cardiac and saliva measures. Specifically, 21 infants and 24 mothers were completely missing cardiac data because the monitor failed to detect an accurate heart rate due to equipment malfunction or participant physical activity that dislocated the electrodes. Furthermore, 24 infants and 13 mothers were completely missing heart rate data because their files required more than 10% editing. Thirty-six infants and 34 mothers were completely missing sAA either due to not having sufficient saliva to assay ($N = 3$ infants, $N = 5$ mothers) or to control variables, such as being sick or on medication, eating immediately prior to or during the laboratory assessment or infants who had not been awake an hour prior to the lab assessment. In all, RSA data was available for 87 infants and 95 mothers and sAA data were available for 96 infants and 98 mothers.

Chi Square tests were performed and indicated that there were no systematic differences on measures of race, income, or infant sex between groups missing all physiological data and those with complete data. In addition, Chi Square tests were performed to determine if missing data varied according to attachment classification. In the analysis comparing insecure-avoidant and secure groups only there were no significant differences in amount of missing RSA or sAA data for either infants or mothers. However, not surprisingly, when the insecure-resistant group was added to the analysis there were significant differences in missingness for infant RSA (χ^2 (2, $N = 147) = 9.7$, $p = .00$), infant sAA (χ^2 (2, $N = 147) = 12.9$, $p = .00$), and mother sAA (χ^2 (2, $N = 147) = 7.1$, $p = .02$). The greater distress of the insecure-resistant infants resulted in less opportunities to obtain clean physiological data in part due to greater movement, refusal to chew on the cotton dental cord, and removing electrodes from their bodies. However, as stated above, insecure-resistant infants and their mothers were not part of the current study analyses or hypotheses, in part due to the missingness of physiological data.

RESULTS

Means and standard deviations for all physiological measures are presented in Table 1 for infants and mothers.

Model Specification

To examine changes in RSA and sAA across the SSP for infants and mothers, a general linear model with repeated measures analysis was employed. Data were analyzed using the PROC MIXED procedure in SAS version 9.1. This data analytic method handles repeated measures efficiently by modeling the covariance structure (Little & Rubin, 1987), assumes that the data included within the model are missing at random rather than missing completely at random (e.g., listwise deletion), and is recommended by methodologists as an appropriate way to accommodate missing data (Schafer & Graham, 2002). Restricted maximum likelihood (REML) was used in reporting model parameters and degrees of freedom were estimated using the between-within method.

For all models, the dependent variables were RSA and sAA for infants or for mothers (separate models) across the SSP with the sampling occasions of the physiological measure as the within-subjects factor. Specifically, for RSA, there were seven sampling occasions across the SSP (each episode of the SSP), and for sAA there were three collection occasions: baseline, post-15 and post-45 SSP (See Tab. 1). Because reactivity (i.e., change from baseline) of physiological systems were the variables of interest, a reference cell coding was employed such that the intercept in the model represented the baseline

Table 1. Mean RSA and sAA at Each Sampling Occasion

	Infants			Mothers		
	N	M	SD	*N*	M	SD
RSA (30s) episode 2—mother, infant	85	3.70	1.03	89	5.91	1.26
RSA (30s) episode 3—stranger, mother, infant	86	3.71	1.06	90	6.18	1.17
RSA (30s) episode 4—stranger, infant	84	3.64	1.10	82	5.40	1.12
RSA (30s) episode 5—mother, infant	85	3.53	.90	83	5.86	1.01
RSA (15s) episode 6—infant alone	50	3.39	1.16	47	5.60	1.24
RSA (15s) episode 7—stranger, infant	64	3.50	.98	72	5.29	1.13
RSA (30s) episode 8—mother, infant	76	3.66	1.07	84	5.80	1.10
sAA U/ml baseline	67	51.91	41.66	119	86.72	71.39
sAA U/ml post 15 min	90	61.18	50.57	114	79.40	59.98
sAA U/ml post 45 min	86	61.08	41.52	110	93.75	87.26

Note: Numbers in parentheses represent the time in seconds for each epoch for which RSA was calculated. Epochs were averaged to calculate mean RSA for each sampling occasion. Means for sAA are pre-transformations.

estimate of the physiological variable and all other episodes represented change from baseline (i.e., a difference score) of physiological reactivity. For RSA, baseline for the SSP was the first (i.e., acclimation episode) 3 min episode of the SSP. For sAA, baseline was the first sample of saliva collected within the first 5 min of the laboratory visit. Thus, each model included either infant or mother physiology as the dependent variable (RSA or sAA), the sampling occasions of that physiological variable coded to represent change from baseline, attachment classification, and the interaction of attachment classification by sampling occasion as independent variables.

To test for physiological attunement between mothers and infants, two additional models were examined with infants' physiology as the dependent variable (one model for infant RSA and one model for infant sAA), and in addition to the previous predictors two time-varying covariates were added, mother's physiology and mothers' physiology by attachment status (interaction term). The addition of these covariates tested for the possibility of physiological attunement between mothers and infants. In total, six models were tested, one for each physiological measure for infants and mothers separately and two additional models including mothers' physiology as a predictor of infants' physiology.

Given the stratification of the sample, income and race were included in all models as demographic controls. In addition, infant age in months and infant sex were also included in all infant models. Binary variables were coded to ease interpretation: race was coded as 0 for African Americans and 1 for European Americans, gender was coded as 0 for boys and 1 for girls, income was coded as 0 for below 2 times the poverty line and 1 for above 2 times the poverty line, and attachment was coded as 0 for insecure-avoidant and 1 for secure. Thus, estimates obtained for these variables are interpreted for the group coded as 1.

Infant Physiology and Attachment Status

In order to test our first hypothesis that we would observe RSA and sAA reactivity (i.e., change from baseline) to the SSP in infants and that change in RSA and change in sAA would vary by attachment classification, two models were performed.

Infant RSA ($N = 87$). For infants, there was an effect for race on RSA. Specifically, European American infants had significantly lower levels of mean RSA than did African American infants (see Tab. 2). In addition, there was a main effect of sampling occasion, revealing that, on average, infants had significant decreases from baseline to episodes 5, 6, and 7 of the SSP, after the first separation and throughout the episode when the stranger was alone with the infant. There was no main effect for attachment status; however, as anticipated, there was an attachment classification by sampling occasion interaction (see Tab. 2). As can be seen in Figure 1, mean values of RSA were lower in infants classified as avoidant, suggesting greater RSA reactivity in response to the SSP. More specifically, within the avoidant group, RSA first significantly decreased from baseline levels during episode 5 (after first separation), reached its lowest peak during episode 6 (when the infant was alone), and by episode 8, the final reunion, had not fully returned to baseline levels.

Infant sAA ($N = 96$). Results indicated there were main effects for both poverty level and race for infants' sAA. Specifically, infants from higher income families (200% above poverty line) had higher sAA levels than did infants from lower income families and African American infants had higher sAA levels than did European American infants (See Tab. 3). There was no effect for time of day of collection on sAA. In addition to the demographic findings, there was a main effect for attachment

Table 2. Change in RSA in Infants as a Function of the SSP and Secure Versus Avoidant Attachment Status

	Coefficient	SE	*T*-value	df	*p*-value
Fixed effects					
Intercept-baseline M-I	4.00	.28	14.35	81	.0001*
Poverty level	−.13	.21	−.59	81	.5585
Race	−.51	.21	−2.47	81	.0157*
Gender	.05	.20	.24	81	.8085
Infant age	−.07	.09	−.79	81	.4306
Episode 3 M-S-I	−.14	.13	−1.08	431	.2802
Episode 4 S-I	−.24	.13	−1.82	431	.0697
Episode 5 M-I	−.39	.13	−2.91	431	.0038*
Episode 6 I	−.66	.14	−4.65	431	.0001*
Episode 7 S-I	−.35	.14	−2.51	431	.0126*
Episode 8 M-I	−.20	.14	−1.53	431	.1278
Attachment group	−.02	.26	−.09	81	.9280
Episode 3 × attach group	.20	.15	1.32	431	.1883
Episode 4 × attach group	.28	.15	1.84	431	.0670
Episode 5 × attach group	.34	.15	2.19	431	.0289*
Episode 6 × attach group	.64	.17	3.71	431	.0002*
Episode 7 × attach group	.33	.16	2.02	431	.0436*
Episode 8 × attach group	.27	.16	1.72	431	.0860
	Coefficient	SE	*Z*-value	*p*-value	
Random effects					
Intercept	.85	.13	6.31	.0001*	

Note: Episodes refer to episodes of the SSP, capitalized letters refer to the configuration of people involved in that episode: M, mother; S, stranger; and I, infant. "×" represents an interaction term, here episode by attachment group.
*$p = <.05$.

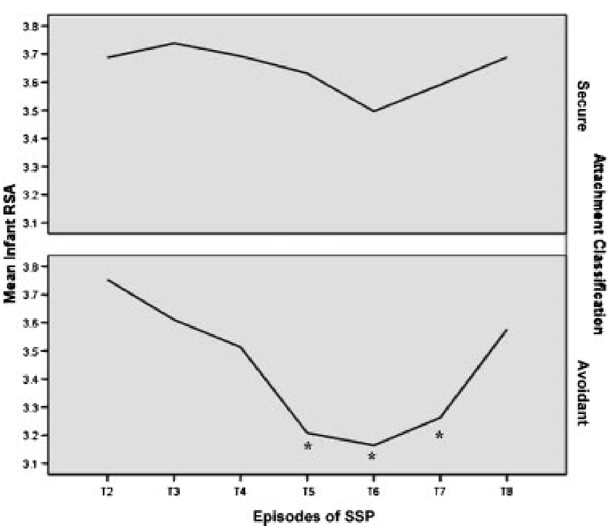

FIGURE 1 Change in mean levels of RSA in infants by attachment classification across the episodes of the SSP. Asterisks represent the interaction effect of sampling occasion by attachment status when RSA reached a significant level of decrease from baseline levels, indicating vagal withdrawal for insecure-avoidant infants.

classification indicating that, on average, children in the insecure-avoidant group (M(*untransformed*) = 74.6 U/ml, SD = 38.8) had higher sAA levels than those in the securely attached group (M(*untransformed*) = 49.46 U/ml, SD = 39) (See Fig. 2).

Mother Physiology and Attachment Status

In order to explore the possibility that mothers would show physiological reactivity to the SSP and that this reactivity may vary by their infants' attachment classification, two models were performed.

Mother RSA (N = 95). There were no effects for demographic variables on mothers' RSA. There was a main effect for sampling occasion (See Tab. 4). Specifically, mothers showed significant changes from baseline at episodes 3, 4, 6, and 7. At episode 3, when the mother, infant and stranger are interacting, mothers showed an increase in RSA from baseline. However, at episodes 4, 6, and 7, mothers showed significant decreases in mean levels of RSA, suggesting that mothers showed greater vagal withdrawal during those episodes when they observed their infant through a one-way mirror when the infant was alone or with the stranger (See Fig. 3).

Table 3. Change in sAA in Infants as a Function of the SSP and Secure Versus Avoidant Attachment Status

	Coefficient	SE	*T*-value	df	*p*-value
Fixed effects					
Intercept-baseline	8.7	2.0	4.19	89	.0001*
Poverty level	1.52	.50	3.03	89	.0032*
Race	−.99	.49	−2.02	89	.0462*
Gender	.11	.48	.22	89	.8225
Infant age	−.20	.20	−1.00	89	.3198
Time of day	.13	.08	1.49	89	.1391
15 min post-SSP	.69	.58	1.18	141	.2397
45 min post-SSP	.60	.59	1.03	141	.3071
Attachment group	−1.9	.71	−2.71	89	.0080*
15 min post × attach group	−.15	.72	−.21	141	.8356
45 min post × attach group	.42	.73	.57	141	.5670
	Coefficient	SE	*Z*-value	*p*-value	
Random effects					
Intercept	3.67	.84	4.36	.0001*	

Note: "×" represents an interaction term, here episode sampling occasion by attachment group.
*$p = <.05$.

There was no main effect for attachment status. One interaction effect of sampling occasion by attachment status was found indicating that mothers of secure infants had lower RSA, indicating vagal withdrawal, during the final reunion episode than did mothers of insecure-avoidant infants (See Tab. 2 and Fig. 3).

Mother sAA ($N = 98$). A parallel model was performed with mother's sAA as the dependent variable. There were no significant effects for mother's sAA reactivity during the SSP.

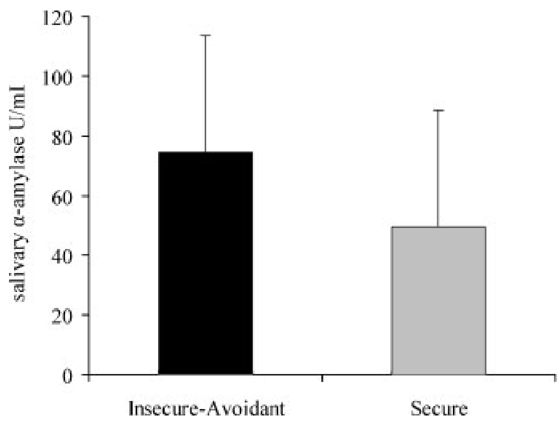

FIGURE 2 The main effect for attachment group on sAA in infants. Bars represent the mean levels of sAA for insecure-avoidant infants and secure infants.

Mother and Infant Physiological Attunement During the SSP

In order to test our final hypothesis that mothers and infants may show physiological attunement during the SSP and that this concordance may vary by attachment, two models were performed.

RSA Attunement. As can be seen in Table 1, mothers had significantly higher RSA than did infants across all sampling occasions $t\ (66) = -12.23$, $p = .001$. The infant RSA model, as described above, was rerun with two additional independent variables, mothers' RSA and mothers' RSA by attachment status (interaction term), which were entered as time-varying covariates to determine whether mothers' RSA covaried with infants' RSA overall or by attachment status. There were no significant results.

sAA Attunement. As can be seen in Table 1, mothers had significantly higher sAA than did infants across all sampling occasions $t\ (93) = -4.43$, $p = .00$. The infant sAA model, as described above, was rerun with two additional independent variables, mothers' sAA and mothers' sAA by attachment status, which were entered as time-varying covariates to determine whether mothers' sAA covaried with infant's sAA overall or by attachment status (interaction). There were no significant results.

DISCUSSION

The goal of this study was to further understand biobehavioral functioning within the attachment system

Table 4. Change in RSA in Mothers as a Function of the SSP and Secure Versus Avoidant Attachment Status

	Coefficient	SE	*T*-value	df	*p*-value
Fixed effects					
Intercept-baseline M-I	4.71	.78	6.01	90	.0001*
Poverty level	−.08	.22	−.36	90	.7165
Race	.32	.22	1.44	90	.1547
Gender	.25	.22	1.17	90	.2462
Episode 3 M-S-I	.36	.15	2.32	440	.0207*
Episode 4 S-I	−.43	.15	−2.77	440	.0058*
Episode 5 M-I	.14	.15	.89	440	.3735
Episode 6 I	−.44	.17	−2.49	440	.0131*
Episode 7 S-I	−.53	.15	−3.40	440	.0007*
Episode 8 M-I	.17	.15	1.06	440	.2882
Attachment group	.04	.27	.16	93	.8715
Episode 3 × attach group	−.21	.18	−1.12	440	.2628
Episode 4 × attach group	−.23	.18	−1.25	440	.2125
Episode 5 × attach group	−.26	.18	−1.40	440	.1628
Episode 6 × attach group	.05	.21	.23	440	.8202
Episode 7 × attach group	−.25	.19	−1.28	440	.2000
Episode 8 × attach group	−.46	.18	−2.44	440	.0149*

	Coefficient	SE	*Z*-value	*p*-value
Random effects				
Intercept	1.02	.16	6.31	.0001*

Note: Episodes refer to episodes of the SSP, capitalized letters refer to the configuration of people involved in that episode: M, mother; S, stranger; and I, infant. "×" represents an interaction term, here episode by attachment group.

$^{*}p = <.05$.

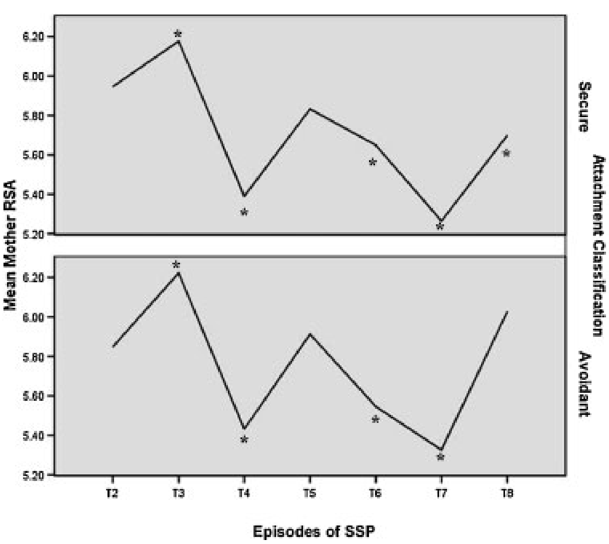

FIGURE 3 Change in mean RSA in mothers of infants with secure attachments and mothers of infants with insecure-avoidant attachments across the episodes of the SSP. Asterisks represent points that reached significant decreases from baseline levels (for episodes 4, 6, and 7 these effects were the same for both groups of mothers) indicating vagal withdrawal.

by examining both parasympathetic and sympathetic measures in avoidant and securely attached dyads. Towards this end vagal reactivity and sAA reactivity in response to the SSP were assessed in mothers and their infants. The results supported the hypotheses that infants' physiological responses to the SSP varied as a function of attachment classification, that mothers also showed physiological responses to the SSP, and that mothers' physiological responses varied as a function of infant attachment classification in one instance. However, there was no evidence for mother-infant physiological attunement. These findings provide support for the idea that allostatic load varies according to different patterns of infant attachment for both infants and mothers.

Infant Physiology as a Function of the SSP and Attachment Status

In terms of vagal reactivity, there was a main effect indicating that all infants showed significant vagal withdrawal after the first separation and throughout the episode when the infant was alone with the stranger. As expected, attachment classification moderated this relationship. Specifically, within the avoidant group, there were significant decreases in RSA after the first separation and throughout the episode when the stranger was alone with the infant, indicating that insecure-avoidant infants showed significantly greater vagal withdrawal during the SSP than did secure infants. These results supported the hypothesis that infants classified as insecure-avoidant show greater internal effort when compared to infants classified as secure. There was a non-significant trend indicating greater vagal withdrawal within the avoidant group during the final reunion with the mother. The lack of strength of this finding during the reunion episode was somewhat surprising given it is during this period when avoidant infants are thought to be most actively engaged in minimizing their distress and self-oriented regulation. It may be that this finding would reach significance given the power of a larger sample. It is also possible that the return of the primary caregiver provides some regulatory repair (Hofer, 2006) and psychological comfort even in an insecure attachment pattern. In other words, being with the mother after separation is less work than being separated.

In terms of sAA, our results showed that overall sAA rather than sAA reactivity differentiated secure from avoidant infants. This finding indicated that across all sampling occasions insecure-avoidant infants showed significantly greater SNS activity than did secure infants, including the baseline or resting measure. The fact that reactivity to the SSP (i.e., change from baseline) was not found could be due to the fact that our sampling occasions were originally designed for the collection of salivary cortisol data that follows a slower time course than sAA (Granger et al., 2007). Specifically, sAA has been found to peak approximately 10 min post stressor (Gordis et al., 2006; Stroud et al., 2006). Our second measurement occasion was 15 min post stressor, and thus we may have missed the reactivity pattern of sAA. However, despite this limitation, the results show that insecure-avoidant infants have significantly higher levels of sAA across all measurement occasions (baseline, 15 post, 45 post), indicating that, in comparison to secure infants, these infants are consistently showing greater sympathetic arousal. Thus, the pattern of insecure-avoidance is associated with greater levels of stress in general. This study suggests that socio-emotional factors such as attachment status are related to sAA and including such measures in our studies could contribute greatly to our understanding of patterns of behavior.

Taken together these results suggest that infants classified as insecure-avoidant showed greater allostatic load than did secure infants. It may be that it is more physiologically challenging to minimize distress than to openly communicate it, and thus insecure-avoidant infants show a greater physiological response. In the short term, relying on internal resources to support proximity to a caregiver is a functional response. Indeed, vagal withdrawal and SNS activation are indications of a defense response in which an individual recruits internal resources in order to *effortfully* and *actively* cope with an external challenge (Cannon, 1914; Henry, 1973). However, over time a high allostatic load may have a cost. One possible consequence is that hyperactivation of a system leads to a *burn out* phenomenon, such that in later development, physiological systems are underaroused perhaps leading to undercontrolled behavior. For example, children who have been classified as having an insecure-avoidant attachment in infancy have been found to show later uninhibited, less fearful, and in some cases aggressive behavior in comparison to children with secure and especially insecure-resistant patterns of attachment (Burgess et al., 2003; Calkins & Fox, 1992; Kochanska, 1998; Renken, Egeland, Marvinney, Mangelsdorf, & Sroufe, 1989; Shaw, Owens, Vondra, Keenan, & Winslow, 1996). In fact, one study showed that infants classified as insecure-avoidant at 14 months of age were more likely to have lower HR and high RSA at 4 years of age and more aggression, indicating physiological underarousal and undercontrolled behavior (Burgess et al., 2003).

Another possible negative outcome is physical *wear and tear* that increases propensity for disease. This relation between physiological hyperactivation and disease may be strengthened by the possibility that these infants are developing an early stress response that is based on the suppression of emotions and the avoidance of social support. It is well documented that emotional expression, disclosure, and social support are crucial factors for physical and psychological well-being (for reviews see Cohen & Willis, 1985; Frisina, Borod, & Lepore, 1995). Thus, these results suggest that whereas physiological arousal in infancy may support the capacity to minimize distress, this pattern may become maladaptive over time and may be a risk factor for later aggression or negative health outcomes. Future research using multiple physiological measures should examine patterns of insecure attachment over time and how they relate to coping with stress and overall physical and psychological health.

Mother Physiology as a Function of the SSP and Attachment Status

The expectation that mothers would show a physiological response to the SSP and that this response might differ by their infants' attachment classification was partially supported. In the current analyses, both groups of mothers had higher RSA from the acclimation episode to second episode when the stranger joined the mother and child in the room, perhaps indicating that mothers calmed down a bit once the procedure started. Additionally, both groups of mothers showed decreases in RSA, indicating vagal withdrawal, during the separation episodes of the SSP.

During these episodes when the infant was either alone or with the stranger, the mother left the infant and entered an adjoining room where she was able to watch her infant through a one-way mirror. This finding supports other work showing that the majority of mothers are found to worry about separations from their infants, regardless of attachment classification (Bretherton, Birngen, Ridgeway, Maslin, & Sherman, 1989). While we found no differences in attachment group on mother's physiological reactions during separations as was hypothesized, there was a difference in mother's physiology during the final reunion of the SSP. Specifically, mothers of secure infants exhibited greater vagal withdrawal than did mothers of insecure-avoidant infants. This finding suggests that mothers of infants classified as secure may have experienced a perceived challenge during the final reunion, one in which they needed to rely on internal resources to cope. It is possible that these mothers, in comparison to mothers of infants classified as insecure-avoidant, were engaged in more active soothing and interacting with their children, behaviors associated with greater vagal withdrawal. This interpretation fits with Porges' polyvagal theory (2007) that vagal withdrawal is neuroanatomically linked to nerves that control both facial expression and vocalization associated with social engagement behaviors.

Mother-Infant Physiological Attunement During SSP

The hypothesis that mothers and infants may be physiologically attuned during the SSP, particularly in secure dyads, was not supported. It may be the case that mothers and infants may have to regulate in different ways during the episodes, and separations may serve to disrupt attunement as well (Field, 1994; Hinde & McGinnis, 1977; Hofer, 2006). Future research should examine whether dyads show more attunement sometime after the reunion in another setting, such as free play. The time to recovery for attunement may vary by attachment status as well. Past research has supported the idea that secure dyads are more attuned; however, these studies did not compare secure dyads to insecure-avoidant dyads. One study collapsed across the two insecure groups and the other study was unable to include insecure-avoidant infants in the analysis (Donovan & Leavitt, 1985; Zelenko et al., 2005). Thus it may be the case that differences in attunement are observed in secure dyads only when they are compared to insecure-resistant dyads.

Race and Income Effects on Physiology

In addition to findings related to project hypotheses, there were some associations involving race and income. We replicated previous findings that African American infants at ages 5–10 months have higher sAA levels than European American infants (Granger, Blair, et al., 2007). Specifically, we observed that African American infants (age 13 months) had higher RSA *and* sAA when compared to European American infants. These results suggest that African American infants show different overall patterns of physiological functioning that may suggest greater levels of stress in these infants but also may suggest a more responsive, attentive, and reactive disposition, perhaps representing a better preparedness for challenge (Beauchaine, 2001). However, over time there could be wear and tear effects due to consistently higher physiological stimulation. Research with adults has shown consistently that there are racial differences in resting and stress-induced cardiac activity that are risk factors for essential cardiac hypertension, a condition African Americans more often acquire (Anderson, 1989). With this developmental perspective in mind, it is of more than passing interest that Granger et al. (Granger, Blair, et al., 2007) did not find that ethnic status was associated with differences in sAA for the mothers of the infants mentioned in the study above. Thus, the present findings should not be ignored and have been corroborated. At the same time, however, they should also be interpreted with extreme caution. Group comparison of race does not account for several confounding factors (McLoyd & Ceballo, 1998), and whereas an income measure was included in the present analyses, other relevant factors such as racial discrimination, parenting stress, etc. were not. In addition, the current study found that higher income (200% above the poverty line) was related to higher sAA. This result was surprising given that lower SES is usually associated with greater stress. There is some evidence that higher income is correlated with higher stress, particularly among African American groups. This is thought to be due to the loss of social support and networking found in lower-income primarily African American neighborhoods (Tatum, 1997). Thus, future research exploring stress in African American infants should include multiple measures of physiology and within group analyses of these factors is sorely needed.

Limitations and Benefits of the Current Study

There were several limitations of the current study. First, we were unable to assess the physiological responses of insecure-resistant infants to the SSP. We had great difficulty obtaining reliable physiological measurements from this subgroup of children. This was in part due to the greater physical display of distress that created more artifacts in cardiac data. In addition, these children were more likely to refuse to wear electrodes or chew on the cotton dental cord (Granger, Kivlighan, et al., 2007). Thus, one major limitation to this study is the possibility that this subgroup of infants were not included due to non-random factors leading to potential bias in this sample. Overall, missing physiological data for all attachment classifications was an issue in this paper and a limitation to the study. Improvement in collection techniques and technological advances will help to reduce missing data in future research. It will be important in the future to refine strategies to obtain reliable physiological measurements in order to retain more data from infants who display more challenging behaviors and reduce meaningful-missing data due to data collection difficulties. For example, perhaps introducing physiological data collection techniques on a separate occasion would help to reduce the novelty of the situation, especially if this were attempted during a game or play like activity session. This reduction in novelty may increase the likelihood that one could obtain a measure after a stressful circumstance. In the past, one solution to small or missing data in attachment groups has been to collapse across patterns of insecurity and include insecure-avoidant and insecure-resistant infants in one group. This method would have obscured our main question that was to assess whether insecure-avoidant infants show greater allostatic load due to their specific stress response style as opposed to their broadly defined status of ‘‘insecure.’’ One benefit of the current study is that results suggest that collapsing across groups may result in loss of important information. Future research should aim to include all attachment groups and to have multiple physiological measures.

CONCLUSION

In conclusion this study provides evidence for different parasympathetic and sympathetic functioning for infants with insecure-avoidant attachments compared to secure attachments, suggesting that the insecure pattern of avoidance results in greater allostatic load. This finding suggests that early experiences may play an important role in shaping the stress responsivity of an individual to psychosocial situations. It should also be noted that separate analyses from this study have found no main effect of cortisol reactivity to the SSP as a function of attachment status (Mills-Koonce & Cox, Personal Communication), suggesting that parasympathetic and sympathetic measures of autonomic activity may indeed be more sensitive to the emotional reactivity of avoidant versus securely attached infants. Future longitudinal work is needed to provide links between this early work in infancy and later work that shows insecure-avoidant individuals are more susceptible to negative health outcomes stemming from greater and (and perhaps) prolonged allostatic load.

In addition, results from this study reveal that mothers from secure attachment dyads are more likely to rely on internal resources when soothing their children, who themselves are less likely to show a pattern of physiological stress and perhaps more reliance on their caregivers. The long term effects of these early experiences are still unknown. However, discerning the origins of these biobehavioral patterns of functioning can help in understanding developmental trajectories of behavioral and biological reactivity and regulation.

NOTES

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