

The Pennsylvania State University
The Graduate School
College of Health and Human Development

**PHYSIOLOGICAL BASES OF EMOTION REGULATION IN YOUNG CHILDREN
LIVING IN LOW-INCOME, RURAL COMMUNITIES**

A Dissertation in
Human Development and Family Studies
by
Kristin M. Voegtline

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Submitted in Partial Fulfillment
of the Requirements
for the Degree of
Doctor of Philosophy

December 2010

The dissertation of Kristin M. Voegtline was reviewed and approved* by the following:

Cynthia A. Stifter
Professor of Human Development and Psychology
Dissertation Advisor
Chair of Committee

Clancy B. Blair
Associate Professor of Human Development

Lisa M. Gatzke-Kopp
Assistant Professor of Human Development

Michelle L. Frisco
Assistant Professor of Sociology and Demography

Douglas M. Teti
Professor of Human Development and Psychology
Professor-in-Charge of Graduate Programs in Human Development and Family Studies

*Signatures are on file in the Graduate School.

ABSTRACT

The expression and regulation of emotion at the behavioral level are functionally dependent on physiological state, and an increasing number of studies have examined physiology-behavior associations to identify precursors for childhood psychopathology. This dissertation advances the literature on autonomic nervous system (ANS) development in infancy and toddlerhood and ANS-behavior associations by investigating patterns of parasympathetic and sympathetic nervous system activity with a latent profile modeling approach, at a single measurement occasion and over time, and the prediction of emotion regulation and early childhood behavior problems by ANS profile. In addition, predictors of ANS profiles were examined, including features of the early social environment and prenatal cigarette use. Data was drawn from the Family Life Project, an ongoing longitudinal study of children and families living in low-income, non-urban communities. Study 1 explored latent profiles of autonomic nervous system functioning indexed by respiratory sinus arrhythmia (RSA) and salivary alpha-amylase (sAA) in children ages 7-, 15-, and 24-months, and the relationship of ANS profile at each measurement occasion with concurrent emotion regulation behavior (7-month $n=325$; 15-month $n=341$; 24-month $n=330$). A three profile solution of child ANS activity, indicated by baseline RSA, RSA suppression, and baseline sAA, was supported at 7- and 15-months of age. At 7-months, children in the “coactivation” profile showed a greater proportion of self-soothing behavior during a fear- and frustration-eliciting task in comparison to children in the “PNS inhibition” and “PNS activation” profiles. At 15-months, children in the “SNS activation” profile showed a greater proportion of attention regulation behavior during a fear-eliciting task in comparison to children in the “PNS activation” or “Average” profiles. There was no evidence for multiple ANS profiles at 24-months of age.

Study 2 investigated longitudinal latent profiles of RSA and sAA across 7-, 15-, and 24-months, the prediction of longitudinal profiles by social advantage, income, geographic isolation, and maternal parenting behavior, and differences in 3-year problem behavior by longitudinal profile ($n=309$). Four longitudinal ANS profiles represented the optimal solution to describe patterns of RSA and sAA development from 7- to 24-months of age. Membership in the longitudinal profiles was predicted by

social advantage, and contrary to expectations, not significantly predicted by maternal parenting behavior. Further, membership in Profile 2, a longitudinal pattern of average increasing RSA and average increasing sAA from 7- to 15-months, but deviated from the overall sample pattern from 15- to 24-months showing minimal increase in sAA predicted 3-year total behavior problems and emotional symptoms in comparison to the other longitudinal profiles.

Study 3 examined the prediction of latent physiological profiles of RSA, sAA, and L-HPA activity at 7-months by prenatal exposure to cigarette smoking, and sex differences in the probability of smoking in each of the physiological profiles (n=144, 72 children of prenatal smokers). A two profile solution for child RSA, sAA, and L-HPA activity was supported at 7-months of age. Children exposed to cigarette smoking prenatally had significantly increased odds of being classified in the “ANS coinhibition, L-HPA non-reactor profile” in comparison to the “ANS coactivation, L-HPA reactor” profile. Further, the probability of being in the prenatal exposure smoking group and classified as a member of the “ANS coinhibition, L-HPA non-reactor” profile was higher for males than females; however the odds ratio test was not significant.

In conclusion, the findings across the three studies from this dissertation highlight the advantage of a latent profile approach for exploring patterns of multi-system physiological response dynamics of the PNS and SNS, and ANS profile-behavior associations.

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ACKNOWLEDGEMENTS

This dissertation was made possible by data from the Family Life Project, supported by the National Institute of Child Health and Human Development Grant P01 HD39667, with cofunding from the National Institute on Drug Abuse. The Family Life Project key investigators include Lynne Vernon-Feagans, Martha Cox, Clancy Blair, Peg Burchinal, Linda Burton, Keith Crnic, Ann Crouter, Patricia Garrett-Peters, Mark Greenberg, Maureen Ittig, Stephanie Lanza, Roger Mills-Koonce, Debra Skinner, Cynthia Stifter, Emily Werner, and Michael Willoughby. I would also like to thank the many families participating in this longitudinal project and the numerous research assistants who traveled near and far collecting data in the home environment. I am tremendously grateful for the invaluable research and teaching skills I have gained during my graduate education at Penn State, and was honored to be the recipient of the 2009-2010 Joachim Wohlwill Endowment in Human Development and Family Studies dissertation award. To my dissertation chair, Cindy, thank you for being an incredibly supportive mentor. You have enabled me to grow as a scholar, offering guidance on my selected coursework, research activities, and writing, encouraging me to push my ideas further, and promoting my ability to think critically and scientifically about human development. You are an exemplary scholar and role model. To my other dissertation committee members, Clancy Blair, Michelle Frisco, and Lisa Gatzke-Kopp, you are a stellar team of scholars and I am fortunate that you have each shaped my research interests and imparted your wisdom on me through coursework and this dissertation project. I would also like to thank John Graham for his consultation on missing data in the current dissertation project. Finally, I want to thank my friends and family for being an unwavering source of support and joy in my life. To Mom, words cannot express how much your love and support has helped me get to where I am today. And to Mike, I do not know how I would do it all without you. Thank you.

INTRODUCTION

The ability to self-regulate emotion is one of the primary developmental tasks in early childhood, serving to control emotional arousal, support social relationships, and enable readiness for learning (Kopp, 1982, 1989; Thompson, 1994; Kopp & Neufeld, 2003). Young children who develop maladaptive patterns of emotion regulation, or emotion dysregulation, are at-risk for early mental health problems. The expression and regulation of emotion at the behavioral level are functionally dependent on physiological state (Porges, Doussard-Roosevelt, & Maiti, 1994), and an increasing number of studies have examined physiology-behavior associations to identify precursors for childhood psychopathology. *Inhibitory* activity of the vagal system, and *excitatory* activity guided by the sympathetic–adrenomedullary (SAM) system and related neuroendocrine processes, have been empirically substantiated as predictors of individual differences in emotion self-regulation. However, the sum of past studies has focused on the functioning of a single physiological system. Given the interactive roles of inhibitory and excitatory activity in responding to environmental demands and reestablishing physiological homeostasis, measuring multiple physiological systems is vital, as it will provide a more complete picture of nuanced physiological activity underlying individual differences in emotion regulation and early mental health risk. Further, these physiological systems exhibit sensitivity to context, and the response dynamics of inhibitory and excitatory physiological activity are calibrated early in life in part by the social environment.

The following sections serve as a background for the dissertation project, which will take the form of three empirical papers. First, a) the guiding framework of developmental systems theory will be introduced. Second, b) the development of emotion self-regulation in infancy and early childhood will be reviewed, as well as, how emotion dysregulation, or maladaptive patterns

of emotion regulation may predict early behavior problems. Next, the functioning of the c) vagus, d) SAM, and L-HPA as independent physiological systems predicting individual differences in emotion regulation will be presented. Following this, e) theoretical perspectives highlighting interacting physiological systems will be detailed. Finally, to lead into the empirical papers, the f) broad aim of each paper will be stated, g) the Family Life Project will be described in brief, and the h) advantages of the proposed person-oriented methodological approach, latent profile analysis and latent growth mixture modeling, will be discussed.

Developmental systems theory

“The abyss between biological and social levels of organization is a human construction, however, one that must be bridged to achieve a complete understanding of human behavior,” (Cacioppo, Berntson, Sheridan, & McClintock, 2000, p.830).

Introduced by Gilbert Gottlieb (1991), the developmental systems theoretical model of individual development is depicted as mutual interactions among multiple, hierarchically organized levels (e.g. genetic, neurophysiological, behavioral, social environment, cultural environment; see Figure 1). One feature of this model is the emphasis on coaction among levels, which Gottlieb defines as some bi-directional relationship between two or more components of the developmental system. Coactions may serve to maintain a steady-state of development, facilitate temporally when a feature of development appears, and induce shifts in the trajectory of development (Gottlieb, 1991; 1996).

Developmental systems theory directly challenges reductionist, unidirectional views of causality for individual development, and espouses that studying only one level of analysis, or

solely bottom-up or top-down processes among levels, is insufficient to fully capture individual development. One example can be found with regard to genetic determinism of development proposed by Waddington (Gottlieb, 1991). Waddington (1942) believed that one's genes predetermined development, to the extent that the environment could only create small, transient deviations away from a fixed pathway of normal or species-typical development specified by genetic make-up. As Gottlieb (1991) states, "genetic activity does not by itself produce finished traits; differentiation occurs as a consequence of events above as well as below the cellular level, necessarily involving [social] factors in addition to genetic influences," (p. 4). Similarly, developmental systems theory challenges classic psychoanalytical theory, which argues that individual development is decisively determined by the social environment. Watson (1930), a proponent of this theory, states, "give me a dozen healthy infants, well-formed and my own specific world to bring them up and I'll guarantee to take any one at random and train him to become any type of specialist I might select—doctor, lawyer, artist, merchant-chief, and yes, even beggar man, and thief, regardless of his talents, tensions, tendencies, abilities, vocations, and race of his ancestors" (p. 104). Currently within the field of developmental psychology, and as is captured within the developmental systems model, individuals are recognized as active agents in their environments.

A second feature of developmental systems theory is that it emphasizes individual development as being characterized by *probabilistic* epigenesis (Gottlieb, 1991; Gottlieb, Wahlsten, & Lickliter, 1998). In other words, depending on the unique coaction of one's genetic activity, physiological activity, behavior, and environment over time, several developmental pathways are possible. To this end, Gottlieb asserts that probabilistic epigenesis is akin to the concepts of equifinality and multifinality proposed by Cicchetti and Rogosh (1996). Equifinality

refers to the potential for several unique developmental pathways, or framed with developmental systems theory and probabilistic epigenesis, series of coactions, to lead to the same outcome. For example, it is possible that bidirectional influence between one or more of the following factors: genetic risk, emotion dysregulation, family conflict, and neighborhood violence could impact a child's development of aggression. On the other hand, multifinality suggests that any one factor predicting development may function differently depending on characteristics of other levels within the developmental system, such that multiple outcomes are possible. For example, if a young infant shows high emotional reactivity, depending on the coaction with factors in the environment such as parental sensitivity, stress, mental health, and social support, the child may have experiences which help or hinder her ability to develop self-regulations skills to modulate emotion in early childhood.

Guided by developmental systems theory, the current dissertation project aims to advance the understanding of the physiological bases of emotion regulation by examining individual differences in activity across multiple physiological systems, and plausible mechanisms for the etiology of emotion dysregulation that may place young children at risk for developing early behavior problems. Further, the influence of the early social environment on physiology-behavior will be explored.

Emotion regulation and early mental health risk

The ability to appropriately monitor, evaluate, and modify emotional reactions is an emergent skill that develops in the first years of life, and individual differences in emotion regulation represent a complex interaction of intrinsic and extrinsic factors over time

(Thompson, 1994; Fox & Calkins, 2003). At birth, there are notable individual differences in infant emotional reactivity, a hallmark feature of infant temperament. Emotional reactivity is characterized by the infant's latency to respond, threshold of responsiveness, and intensity of response to sensory stimulation (Rothbart & Derryberry, 1981). In the first months of life, caregivers figure prominently as external regulators of infant emotion reactivity (Kopp, 1982). Although, depending on infant emotional reactivity, there may be differential opportunities for external intervention. Later in infancy, the relative contribution of infants and caregivers to emotion regulation shifts, as repeated interactions with parents in emotion-inducing contexts and parental modeling support infants' learning of behavioral strategies to modulate their own emotional arousal (Kopp & Neufeld, 2003; Fox & Calkins, 2003). For example, an infant may shift attention, self-comfort, or exhibit avoidance to reduce distress and the expression of negative affect (Stifter & Braungart, 1995). The repertoire of behavioral strategies used for emotion regulation changes and expands with development, as language becomes available, and rapid gains in cognitive ability support an understanding of self-awareness (Kopp, 1982, 1989). In early childhood, more sophisticated emotion regulation strategies include compliance to adult demands, the ability to delay gratification, and inhibitory control, or the ability to suppress a dominant impulse (Kopp, 1982).

Thus, emotion regulation, and in particular the ability to self-regulate negative emotional reactivity, has significant effects upon the young child's capacity to engage in adaptive, organized behavior that supports positive interaction with the social environment. However, it is important to note that negative emotions, like fear or anger, have survival value, motivate goal-oriented behavior, and are healthy and necessary to some degree (Cole, Michel, & Teti, 1994). Still, under certain conditions, emotion regulation patterns develop that jeopardize or impair

functioning, and such patterns may be precursors or symptoms of psychopathology (Cole, et al., 1994; Cicchetti, Ackerman, & Izard, 1995; Keenan, 2000). Maladaptive patterns of emotion regulation, or emotion dysregulation, and in particular the inability to control negative emotionality, has been linked to both externalizing (e.g. disinhibited, aggressive, or antisocial behaviors) and internalizing (e.g. depression, anxiety, or social withdrawal) behavior problems (Cicchetti et al, 1995; Eisenberg et al., 2001; Mullin & Hinshaw, 2007). Although, it is hypothesized that the association between emotion regulation and behavior problems varies as a function of the type of negative emotion being regulated, such that dysregulated expression of anger predicts externalizing problems whereas dysregulated expression of fear predicts internalizing problems (Rothbart & Bates, 1998). For example, infants that were highly reactive to anger- or frustration-inducing events during infancy showed more defiance to social demands in toddlerhood (Stifter, Spinrad, & Braungart-Rieker, 1999) and aggression in childhood (Rothbart, Ahadi, & Hershey, 1994). Further, both overregulated and underregulated patterns of emotional expression or intensity are implicated in most forms of childhood psychopathology (Mullin & Hinshaw, 2007). In an investigation of 79 preschoolers, Cole and colleagues (1996) found that, compared to children who appropriately modulated their emotions during an induction task, inexpressive children, who showed little facial activity, and expressive children, who displayed strong negative emotions, had higher rates of behavior problems, especially externalizing behaviors. Further, at a school-age follow-up visit after the completion of the first grade, children who were inexpressive appeared to present more depression and anxiety symptoms.

Physiological bases of emotion regulation

Considerable attention has been directed at understanding the development of adaptive emotion self-regulation and the etiology of emotion dysregulation, exploring both intrinsic and extrinsic factors involved in monitoring and modulating emotions (Fox & Calkins, 2003). Given that the expression and regulation of emotion are functionally dependent on the state of the autonomic nervous system, a growing body of research addresses the goal of exploring the neurophysiological underpinnings of emotion regulation in physiological systems that regulate arousal. To this end, *inhibitory* activity of the vagus system, and *excitatory* activity guided by the sympathetic–adrenomedullary (SAM) system and related neuroendocrine processes have been established as physiological bases of emotion regulation.

The inhibitory vagus system

The ventral vagal complex (VVC), or smart vagus, is a parasympathetic physiological signaling system for inhibiting arousal, promoting calm behavioral states, and engaging in regulatory and social affiliative behaviors (Porges, 1995, 2001, 2007). Individual differences in the dynamic influence of the smart vagus are quantified by a continuous measure of respiratory sinus arrhythmia (RSA) amplitude (Porges, 2007). Individual differences in the functioning of the smart vagus may be quantified by respiratory sinus arrhythmia (RSA), a measure of heart rate variability (HRV). RSA is a naturally occurring rhythm in heart rate (HR) pattern that occurs at approximately the frequency of spontaneous respiration and varies as a function of environmental demands. Thus, RSA represents the PNS influence on the heart via the vagus nerve (Porges, 1995). The VVC dynamically regulates cardiac output, withdrawing PNS input to

rapidly increase cardiac output and promote short-term mobilization of metabolic resources resulting in RSA suppression, or conversely, increasing PNS input to decrease arousal and support state regulation and calm resulting in RSA augmentation (Porges, 2007).

Past research examining the function of the inhibitory vagus system would suggest that individuals with low baseline RSA, as well as those who show limited or no RSA suppression in response to a demanding context, may have difficulty expressing appropriate emotions, and modulating emotional state (Porges, 2007). Empirical research examining relations between baseline RSA and emotion reactivity in the first year of life has shown that higher baseline RSA is associated with parent and observer reports of greater emotional greater displays of negative reactivity to anger-eliciting events and positive reactivity to joy-eliciting events (Fox, 1989; Stifter, Fox, & Porges, 1989; Stifter & Fox, 1990). Further, several studies have also examined individual differences in RSA suppression in response to a cognitively challenging or attention-demanding tasks. Empirical linkages between greater vagal inhibition during a challenge task, indexed by RSA suppression, and better state regulation in infancy (DeGangi, DiPietro, Greenspan, & Porges, 1991; Huffman et al., 1998; Stifter & Corey, 2001), as well as, fewer behavior problems in early childhood (Porges, Doussard-Roosevelt, Portales, & Greenspan, 1996; Calkins, 1997; Calkins & Keane, 2004; Calkins, Blandon, Williford, & Keane, 2007) have been supported. For example, in a sample of 60 3-month old infants, Huffman and colleagues (1998) showed that children with greater RSA suppression in response to a series of visual, auditory, and tactile stimuli were rated by parents as displaying more attentional control and self-soothing behaviors.

Further, other work has examined the protective effects of RSA in adverse contexts. High RSA and greater RSA suppression appears to serve as a buffer for externalizing,

internalizing, and health problems in children exposed to marital conflict and hostility (Katz & Gottman, 1995, 1997; El-Sheikh, Harger, & Whitson, 2001). Conversely, in samples of anxious and antisocial children, altered vagal functioning has been shown, where either no decrease in RSA is observed in the presence of an environmental challenge or a counterproductive augmentation in RSA occurs (Mezzacappa et al., 1997). Overall, individuals with higher baseline RSA, and greater RSA suppression, present physiological flexibility to match environmental demands, showing marked arousal that is short in latency followed by rapid recovery. This transitory pattern of parasympathetic response is adaptive, and protects the individual from prolonged physiological arousal (Porges, 1995).

The excitatory sympathetic-adrenomedullary (SAM) system and limbic hypothalamic-pituitary-adrenal (L-HPA) axis

Dating back to the seminal work of Cannon (1914) and Selye (1949, 1956), which called attention to stress physiology, and pointed to aspects of adrenal functioning as the primary physiological index of stress and covariate of emotion, scientists have investigated *excitatory* mechanisms through which adaptation of the organism to acute or chronic challenges in the environment takes place (Levine, 2005). In humans, along with other mammalian species, there are two major biological systems that orchestrate the stress response (Gunnar & Davis, 2003; Chrousos & Gold, 1992). They are the sympathetic–adrenomedullary (SAM) system, and the limbic hypothalamic–pituitary–adrenal (L-HPA) axis. The SAM and L-HPA systems interact at all levels of organization, exerting a coordinated influence on cardiovascular activity, metabolic regulation, immune functioning, and emotional state (Gunnar & Davis, 2003). The fast-acting response of the SAM system involves ‘fight/flight’ functions including the secretion of

epinephrine (Epi) and norepinephrine (NE) which serve to increase heart rate, cardiac output, and to mobilize energy stores like glucose (Gunnar & Quevedo, 2007; Chrousos & Gold, 1992). These physiological functions of SAM system arousal supports heightened vigilance and attentional acuity in the short term (Gunnar & Quevedo, 2007). In contrast to the rapid release of Epi and NE by the SAM system and the immediacy of the associated physiological changes, activation of the L-HPA axis follows a much slower time course (approximately 20-25 minutes for peak arousal) (Gunnar & Davis, 2003). A cascade of events occurs to produce glucocorticoids; the end-product of the L-HPA axis (Gunnar & Quevedo, 2007; Gunnar & Davis, 2003; Chrousos & Gold, 1992). Beginning with the release of corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) in the hypothalamus, CRH and AVP act on the anterior pituitary to trigger the release of adrenocorticotrophic hormone (ACTH). In turn, ATCH stimulates the release of cortisol into the bloodstream by the adrenal cortex.

Recent advancements in the non-invasive collection and assessment of salivary analytes have supported a small, but growing, literature on salivary α -amylase (sAA), an enzyme released by glands in the oral cavity, as a viable marker of SAM functioning (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007). Specifically, the action of NE released by the sympathetic nerve terminals on adrenergic receptors influences the protein-to-fluid ratio secreted by salivary glands (Granger et al., 2007). Likely due to the fact that sAA aids in digestion of macromolecules, this enzyme is not present at birth; it can be reliably measured in the second half of the first year of life, paralleling the introduction of solid foods. However, once apparent in saliva secretions, individual differences in sAA exist, and show a robust response to physical and psychosocial stressors (approximately 10 minutes for peak arousal) (Chatterton et al., 1996; Kivlighan et al., 2005; Nater et al., 2005,

2006; Stroud et al., 2006). For example, young children show increases in sAA in response to maternal separation (Hill et al., 2006) and frustration-eliciting tasks (Spinrad et al., 2009). In contrast, salivary cortisol, which is passively diffused to saliva from cortisol circulating in the bloodstream, has been widely validated as an endocrine marker of L-HPA axis functioning (for review, see Gunnar & Quevedo, 2007). Individual differences in salivary cortisol and cortisol reactivity are present at birth. Even in the first weeks of life, infants are capable of mounting a cortisol response to pain stimuli (e.g. heel-stick, circumcision) (Gunnar & Quevedo, 2007). However, the diurnal rhythm of cortisol production, with peak levels occurring shortly after waking and gradually declining throughout the course of the day is not established until sleep patterns become less sporadic (Gunnar, Broderson, Krueger, & Rigatuso, 1996; Gunnar & Quevedo, 2007).

As noted, sAA is a relatively new and understudied marker of SAM functioning. Very few studies have investigated emotional correlates of sAA during early development (Kivlighan et al., 2005; Shea et al., 2006; Hill et al., 2006). Those that have investigated sAA focused on mother-infant dyads, generating support for a positive association in maternal and infant levels of sAA in response to emotionally challenging tasks presented to the infant (Kivlighan et al., 2005; Shea et al., 2006), and higher levels of sAA characterizing infants categorized as insecurely attached via the Strange Situation procedure (Hill et al., 2006). Although there is no investigation to date that has specifically examined the relation of sAA and the development of emotion regulation, there is reason to pursue this linkage. Keller and El-Sheikh (2009) have found that levels of sAA predicted externalizing symptoms in a sample of school age children, measured during the 3rd and 5th grade, in a curvilinear fashion. Children with low or high levels of sAA had higher externalizing symptoms compared to children with moderate levels.

A number of investigations have linked L-HPA activity, indexed by salivary cortisol, and emotional distress in the first months of life, supporting concordant physiological and behavioral arousal, such that increased cortisol reactivity is associated with paralleled emotional reactivity and increased behavioral distress (Gunnar et al., 1996). Although, there is also some evidence to suggest an increasing dissociation between L-HPA functioning and emotions manifested at the behavioral level, especially after the first year of life (Gunnar et al., 1996; Gunnar & Donzella, 2002; Ramsay & Lewis, 2003). For some infants, distress-eliciting events, such as an inoculation procedure, that continue to evoke negative emotional reactivity later in infancy and early childhood (i.e., 12, 15, or 18 months) no longer produce pronounced elevations in cortisol reactivity (Gunnar & Davis, 2003). This developmental pattern of decreasing cortisol reactivity to stressors may be better understood in light of the ascending influence of the vagal system (Huffman et al., 1998). Maturation of the vagal system and an ability to suppress RSA may modulate L-HPA activity, and therefore support emotional expression and regulation that are independent of cortisol reactivity (Porges, 2007). It is also noteworthy that although this pattern of declining cortisol reactivity describes most infants and young children, marked individual differences do exist. On one hand, Gunnar and colleagues (1996) have shown that at 15 months of age, some children continue to exhibit marked cortisol reactivity to the inoculation procedure, which was similar, or more pronounced than at 6 months. Exaggerated or prolonged cortisol reactivity without matched recovery may be a marker of acute stress exposure, and is associated with emotion dysregulation and internalizing problems (Granger, Weisz & Kauneckis, 1994; Stansbury & Gunnar, 1994; Repetti, Taylor, & Seeman, 2002; Boyce & Ellis, 2005). Conversely, other research has shown that some older infants and young children show a paradoxical suppression, or blunting, of L-HPA activation under conditions of stress. Cortisol

blunting, which may be a marker of chronic stress exposure (Heim, Ehlert, & Hellhammer, 2000; Gunnar & Vazquez, 2001), is related to emotion dysregulation and increased levels of externalizing behaviors (Tout, de Haan, Campbell, & Gunnar, 1998; van Goozen, Matthys, Cohen-Kettenis, Buittelar, & van Engeland, 2000; Beauchaine et al., 2007). Therefore, a curvilinear relationship between cortisol levels and emotion regulation is suggested, where moderate levels of cortisol are most adaptive.

Theories of interacting physiological systems

Although excitatory and inhibitory physiological activity underlying emotions manifested at the behavioral level and the ability to self-regulate such emotions have been theoretically and empirically substantiated, limited research has attempted to integrate concurrent activity of these systems. Furthermore, single physiological measurements may be misleading, and do not capture nuanced individual differences in physiological arousal and regulation. For example, “an increase in heart rate in a behavioral context may be the result of sympathetic activation, parasympathetic withdrawal, reciprocal sympathetic activation together with parasympathetic withdrawal, or even sympathetically dominant co-activation of both autonomic branches” (Berntson, Cacioppo, & Sarter, 2003, 1108). While there is a uniform cardiac response in each of these autonomic profiles, the varied symmetry and intensity of response may account for unique associations of physiological functioning with the ability to regulate emotion at the behavioral level, and have differential implications for early mental health risk. As stated in theories of interacting physiological systems, namely Berntson’s doctrine of autonomic space (1991), and Porges’ Polyvagal Theory (1995), there is much to be gained by an investigation that

considers functioning across both branches of the autonomic nervous system, as these systems do not operate in isolation, but exert coordinated and flexible responding in accordance with environmental demands.

The doctrine of autonomic space

Berntson and colleagues (1991; 2004) proposed a two-dimensional model of autonomic functioning, considering the joint action of parasympathetic (PNS) and sympathetic nervous system (SNS) activity. Within this model, physiological activity across the two branches may be reciprocal or nonreciprocal. Prior views of autonomic functioning adopted a simplistic view that PNS and SNS activity acted only in opposition, so that activation in one branch lawfully was associated with diminished activity in the other. The doctrine of autonomic space moves beyond this conceptualization, taking into account that more complex patterns of autonomic activity exist where, for example, both branches show activity of varying magnitudes. In this model, reciprocal activation represents more traditional views of autonomic branches in opposition, and is characterized by unidirectional changes in physiological activity (Berntson & Cacioppo, 2004). *Reciprocal parasympathetic activation* occurs with PNS activation and SNS inhibition, decreasing physiological arousal and promoting calm behavioral states. On the other hand, *reciprocal sympathetic activation* occurs with SNS activation and PNS inhibition and serves to increase physiological arousal. Nonreciprocal activation represents parallel activity in both branches. *Coactivation* is a pattern of PNS and SNS activation, whereas *coinhibition* is a pattern of PNS and SNS inhibition. In both of these cases, the net effect on autonomic activity is ambiguous, depending on magnitude and latency of response across both branches (Berntson & Cacioppo, 2004).

Several studies have explored the two-dimensional model of autonomic space. In clinical samples, children with externalizing problems, such as aggression, impulsivity, and hyperactivity, often exhibit coinhibition indexed by SNS measures of skin conductance level (SCL) and/or cardiac pre-ejection period (PEP), and the PNS measure of RSA (Boyce et al., 2001; Crowell et al., 2006; Beauchaine, Gatzke-Kopp, & Mead, 2007). In a recent monograph, El-Sheikh (2009) presented a collection of three studies examining interactions among SCL and RSA in the prediction of children's externalizing problems. Results showed that marital conflict was associated with children's externalizing problems under conditions of PNS/SNS coactivation or coinhibition. The authors suggest that these physiological patterns of activity may represent a maladaptive form of stress response, as functioning of the PNS and SNS do not exert a coordinated and unidirectional influence on ANS arousal. Conversely, reciprocal sympathetic and reciprocal parasympathetic patterns of activity operated as a protective factor, attenuating the association between marital conflict and child externalizing problems.

Polyvagal Theory

Polyvagal Theory (Porges, 1995) introduced a new perspective to frame the relation of autonomic function and behavior, specifying three hierarchically organized autonomic subsystems: 1) the dorsal vagal complex (DVC) or vegetative vagus, that originates in the dorsal motor nucleus of the vagus and is rooted in primary survival function of immobilizing and conserving metabolic resources in the face of threat or danger, 2) the SNS which adaptively mobilizes physiological resources to support fight or flight behaviors, and 3) the ventral vagal complex (VVC) or smart vagus, that originates in the nucleus ambiguus and is a distinctly mammalian signaling system for inhibiting arousal and engaging in regulatory and social

affiliative behaviors. The smart vagus, positioned at the top of the hierarchy and representing the most recent subsystem from an evolutionary perspective, supports the ability to dynamically regulate cardiac output in relation to changing environmental demands (Porges, 2001). Porges (1995) argues while past studies have either conceptualized this integration as singularly driven by arousal of the SNS, or as antagonizing SNS and PNS branches in opposition, Polyvagal theory is unique in the emphasis on a hierarchically organized autonomic nervous system. To this end, the inhibitory role of the smart vagus predominates SNS and DVC functioning, thus cardiac output can be increased by withdrawal of PNS input without engagement of the SNS. However, if PNS input is not withdrawn, or functioning in an insufficient manner to meet environmental demands, there is potential for recruitment of other neural (SAM) and endocrine (L-HPA axis) systems. However, reliance on these excitatory mechanisms is physically demanding, and may be associated with emotion dysregulation and early behavior problems.

Several recent studies by El-Sheikh and colleagues (2005, 2009) have focused on interactions of physiological systems in line with the Polyvagal perspective. El-Sheikh and colleagues (2005) studied the relationship of RSA and salivary α -amylase reactivity in children who were exposed to a stressful laboratory procedure in which they had to perform a cognitively challenging mirror–star tracing task while listening to an adult argument. Findings showed that higher levels of salivary α -amylase were related with deficits in RSA suppression, and in fact, RSA augmentation; a pattern of physiological co-activation (El-Sheikh, Mize, & Granger, 2005). In addition, Keller and El-Sheikh (2009) also found that RSA moderated the association between salivary α -amylase and externalizing symptoms in a sample of school age children. Children exhibiting lower salivary α -amylase combined with RSA augmentation (a counter-predicted increase in RSA) had higher levels of externalizing symptoms. Lower levels of externalizing

symptoms were predicted in the case when only the PNS was activated, represented by lower salivary α -amylase combined with RSA suppression.

Taken together, these theories highlight that functioning of the VVC, SAM, and L-HPA axis does not operate in isolation, but rather, these physiological systems form an integrated autonomic and neuroendocrine network, exerting coordinated responding in accordance with environmental demands (Bauer, Quas, & Boyce, 2002; El-Sheikh et al., 2009). No study to date has examined indicators of functioning across all three of these interacting physiological systems, and this is a noted gap in the literature. To better specify how physiological functioning supports the ability to regulate emotion at the behavioral level, a longitudinal investigation of VVC, SAM, and L-HPA axis response dynamics is essential.

Aims of the dissertation project

Across the three empirical studies proposed, the current dissertation project aims to utilize person-oriented methodologies to explore unique multi-system patterns of physiological arousal and regulation in infants and young children that may predict emotion regulation and operate as vulnerability or protective factor for early mental health risk. Guided by developmental systems theory, the focus the three papers will be to link physiology and behavior, accounting for social environment influences on this association. Despite universality in the structure and phylogeny of the VVC, SAM system, and L-HPA axis, there is considerable variation between individuals in the pattern of response to challenges in the environment (Ellis, Jackson, & Boyce, 2006; Porges, 2007). While some individuals may mount vigorous activation of these systems in response to a stressor, others may show minimal responsivity. Individual

differences in these patterns of physiological arousal and regulation emerge early in life, and are both inherited and *acquired through experience* (Keller & El-Sheikh, 2009). Importantly, these physiological systems are functionally immature at birth, and develop during infancy and early childhood. During this critical period of development, the maturation and organization of physiological systems is malleable, exhibiting heightened sensitivity to environmental influence (Beauchaine et al., 2007; Repetti et al., 2007). Describing patterns of physiological functioning across multiple systems utilizing person-oriented methodologies, and exploring physiological sensitivity to context, or how various factors in the early environment calibrate physiological responsivity, will be unifying themes across the three studies. Broadly, the aims of each study are:

Study 1: To explore interacting physiological systems by describing cross-sectional profiles of autonomic and neuroendocrine functioning at 7-, 15-, and 24-months of age, and to relate physiological profiles to concurrent emotion regulation. Individual differences in the synergistic functioning of the specified physiological systems (vagus, SAM, L-HPA) is not directly observable and impossible to estimate a priori; thus, meaningful differences in patterns of functioning must be inferred from the data. Latent profile analysis was used to distinguish subgroups of children with specific latent profiles of physiological functioning, separately, at 7-, 15-, and 24- months of age. In addition, at each assessment point, physiological profiles were associated with behavioral manifestations of emotion regulation.

Although it was originally proposed to create latent physiological profiles with RSA, sAA, and cortisol indicators, cortisol responsivity did not statistically differentiate membership in the profile. Further, the interpretation of the physiological profiles became difficult with a

larger host of indicators that deviated from the guiding theoretical models of the doctrine of autonomic space (Berntson et al., 1991) and Polyvagal theory (Porges, 2007). Therefore, the analysis for Paper 1 was restricted to the indicators of baseline RSA, RSA suppression, and pre-task sAA at each measurement occasion. In addition, although not previously proposed, level of negative behavioral reactivity was examined by physiological profile and controlled for in the analysis of emotion regulation behavior, as this informs the functional significance of these regulatory behaviors in reducing negative distress.

Study 2: To explore longitudinal trajectories of child physiological functioning across the first two years and physiological sensitivity to early experience. Insight into physiological bases for the ability to self-regulate emotion was enhanced significantly by the inclusion of early environmental effects of the social environment and parenting behavior on the maturation and organization of malleable physiological systems. Early environments characterized by poverty appear to shape the expression of autonomic and neuroendocrine activity, often heightening reactivity. However, past research would suggest that the primary mechanism of risk linking poverty to poor child outcomes is through parenting, and specifically maternal behavior. To test this, it was proposed that mutually exclusive developmental patterns, or trajectories, of physiological functioning would be explored across 7-, 15-, and 24-months of age using latent growth mixture modeling. Social level variables and parenting behavior were entered as predictors of latent trajectory, and the prediction of child emotion regulation behavior at 2-years and behavior problems at 3-years by physiological functioning trajectory would be examined.

Findings from Study 1 were used to inform Study 2, and as such, for model parsimony and interpretation, only the baseline RSA and sAA indicators were assessed over time. RSA

suppression was not included as an additional indicator of longitudinal physiological profile as no equivalent sAA measure of change in response to a challenge perturbation was assessed in the Family Life Project. Further, although growth mixture modeling was the proposed analytic approach, due to only having three measurement occasions, a longitudinal profile analysis was conducted. A minimum of four measurement occasions are recommended for growth models to have sufficient model power and flexibility to free selected model parameters (Muthén, 2004). Therefore, classes are referred to as *longitudinal profiles* rather than *trajectories* throughout the paper to indicate that classes are compared on the basis of mean levels across the host of ANS indicators over time and not by growth factors. In addition, 2-year regulation behavior was not significantly predicted by longitudinal ANS profile membership, and thus was not retained as an outcome in the paper so as to present a more cohesive and tighter analysis.

Study 3: To examine the association between prenatal exposure to cigarette smoking and infant patterns of physiological arousal and regulation across the vagus, SAM, and L-HPA systems at 7-months of age. Prenatal exposure to cigarette smoking is well-recognized as a neurotoxin to the developing fetus, with the potential to compromise autonomic and neuroendocrine function in young infants. To examine the fetal origins of individual differences in infant physiological arousal and regulation, prenatal exposure to cigarette smoking was examined as a predictor of infant physiological profiles at 7- months of age utilizing latent profile analysis. Further, sex differences in the effect of prenatal exposure to cigarette smoking were explored.

The Family Life Project

Data will be drawn from the Family Life Project (FLP) a longitudinal study of 1292 children child development designed to study children and their families from three Eastern North Carolina and three Central Pennsylvania counties, respectively in the Black South and Appalachia— regions of high child rural poverty. The overarching goal of the project is to examine the complex ways poverty, rurality, ethnicity, family contexts, parent-child relationships, and individual differences in children interact over time to shape child development during infancy and early childhood. Over two-thirds of the sample is low-income, living below 200% of the poverty line, and approximately 40% of the sample is African-American.

For recruitment, the FLP adopted a developmental epidemiological design. Study eligibility criteria included English-speaking families, residing in one of the target counties, with no intent to relocate from the area in the next 3 years. A cohort sampling method was used to over sample for low-income families and, specifically in the Black South, African-American families (with the expectation that families in Appalachia would be almost exclusively Caucasian). In total, across 10 obstetric clinics, FLP recruiters identified 5,471 (57% Black South, 43% Appalachia) women who gave birth to a child during the 1-year recruitment period, 3939 (72%) of whom fit the study eligibility criteria. Of those eligible, 2,678 (68%) women were willing to be considered and provided informed consent, a random selection lottery system was employed to fill planned sampling cells (race, income, and site) and 1,553 (58%) families were formally invited to participate. Finally, of those invited to participate, 1,292 (82%) families enrolled and completed their first home visit, when infants were 2-months of age.

Families were again visited in the home when infants were 7-, 15-, 24-, and 35-months of age.

Information for the current proposal will be drawn from data that were collected from demographic records, physiological measures, observational assessments of emotion regulation and parenting behavior, and parent-report questionnaires obtained in the home when the child was 7-, 15-, 24-, and 35-months of age. The current analyses are restricted to a sub-sample of children from the larger study in which electrocardiogram (ECG) heart period data was collected (N=321, 35% African American).

Person-oriented analytic approach: Latent profile analysis

Individual differences in the synergistic functioning of the specified physiological systems (vagus, SAM, L-HPA) is not directly observable and impossible to estimate a priori; thus, meaningful differences in patterns of functioning must be inferred from the data. Mixture modeling, specifically, latent profile analysis, will be used to identify distinct subgroups of children with similar patterns of physiological arousal and regulation. Latent profile analysis assumes that person-oriented subgroups can be defined such that members in a subgroup are more similar to each other than to members of another subgroup (Bergman & Magnusson, 1997). This method is superior to population level aggregate associations, which are faulted in their potential to obscure group-based differences, and variable-centered grouping techniques, which are limited by using arbitrary cutoffs to demarcate population subgroups (Bergman & Magnusson, 1997). Latent profile analysis improves upon these approaches by utilizing continuous indicators to generate the latent profile solutions within a formal statistical model, therefore, not requiring any classification decisions be made by the investigator where individuals may be forced into groups in error (Muthén, 2001). It also utilizes a maximum

likelihood estimation procedure which assumes all missing data are missing at random, avoiding typical listwise deletion (Little & Rubin, 1987). Further, latent profile analysis simultaneously estimates the latent profile solution while testing the prediction of the probability of membership in a given profile by other variables specified. Finally, longitudinal profiles may also be estimated by including repeated measures indicators, examining developmental patterns of physiological activity over time.

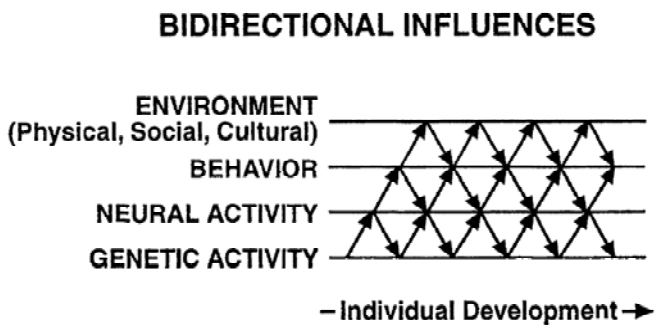


Figure 1. Theoretical model of probabilistic epigenesis, depicting bidirectional influences over four levels of analysis -genetic, neural, behavior, and environment. (from Gottlieb, 2006)

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STUDY 1:

Latent Profiles of Autonomic Functioning and Emotion Regulation in Young Children Living in Low-Income Communities

The ability to self-regulate emotion is one of the primary developmental tasks in early childhood, serving to control emotional arousal, support social relationships, and enable readiness for learning (Kopp, 1982, 1989; Thompson, 1994; Kopp & Neufeld, 2003). Young children who display emotion dysregulation, or the inability to effectively self-regulate emotions, are at-risk for developing early mental health problems (Cole, Michel, & Teti, 1994; Cicchetti, et al., 1995; Keenan, 2000). Considerable effort has been directed at understanding correlates of adaptive emotion regulation as well as identifying potential markers for emotion dysregulation early in life. Given that the state of the autonomic nervous system (ANS) supports the expression and modulation of emotion at the behavioral level, a growing body of research has investigated physiological substrates of emotion regulation. Inhibitory activity of the vagus system and excitatory activity of the sympathetic–adrenomedullary (SAM) system have been associated with the capacity to regulate emotional arousal at the behavioral level. However, the majority of studies have examined the activity of single physiological systems. This is a recognized gap in the literature as physiological systems do not operate in isolation, but rather exert coordinated and flexible responding in accordance with environmental demands (Bauer, Quas, & Boyce, 2002; El-Sheikh et al., 2009). The current study aims to describe patterns of autonomic functioning across multiple physiological systems in infancy and toddlerhood, and explore the association between physiological functioning and concurrent emotion regulation behavior.

The inhibitory vagus system

The ventral vagal complex (VVC), or smart vagus, is a parasympathetic physiological

signaling system for inhibiting arousal, promoting calm behavioral states, and engaging in regulatory and social affiliative behaviors (Porges, 1995, 2001, 2007). Individual differences in the functioning of the smart vagus may be quantified by respiratory sinus arrhythmia (RSA), a measure of heart rate variability (HRV). RSA is a naturally occurring rhythm in heart rate (HR) pattern that occurs at approximately the frequency of spontaneous respiration that varies as a function of environmental demands. Thus, RSA represents the PNS influence on the heart via the vagus nerve (Porges, 1995). The VVC dynamically regulates cardiac output, withdrawing PNS input to rapidly increase cardiac output and promote short-term mobilization of metabolic resources resulting in RSA suppression, or conversely, increasing PNS input to decrease arousal and support state regulation and calm resulting in RSA augmentation (Porges, 2007).

Past research examining the function of the inhibitory vagus system would suggest that individuals with low baseline RSA, as well as those who show limited or no RSA suppression in response to a demanding context, may have difficulty expressing appropriate emotions, modulating emotional state, and attending to social cues and gestures (Porges, 2007). Empirical research examining relations between baseline RSA and emotion reactivity has shown that higher baseline RSA is associated with parent and observer reports of greater displays of negative reactivity to anger-eliciting events and positive reactivity to joy-eliciting events (Fox, 1989; Stifter, Fox, & Porges, 1989; Stifter & Fox, 1990). Further, a linkage between greater RSA suppression during a cognitively challenging or attending-demanding task and better state regulation in infancy (DeGangi, DiPietro, Greenspan, & Porges, 1991; Huffman et al., 1998; Stifter & Corey, 2001), as well as, fewer behavior problems in early childhood (Porges, Doussard-Roosevelt, Portales, & Greenspan, 1996; Calkins, 1997; Calkins & Keane, 2004; Calkins, Blandon, Williford, & Keane, 2007) has been supported. Other work has also examined

the protective effects of RSA in adverse contexts. High RSA and suppression appears to serve as a buffer for externalizing, internalizing, and health problems in children exposed to marital conflict and hostility (Katz & Gottman, 1995, 1997; El-Sheikh, Harger, & Whitson, 2001). Conversely, in samples of anxious and antisocial children, altered vagal functioning has been shown, where either no decrease in RSA is observed in the presence of an environmental challenge or a counterproductive augmentation in RSA occurs (Mezzacappa et al., 1997). Overall, individuals with higher baseline RSA, and greater RSA suppression, present physiological flexibility to match environmental demands, showing marked arousal that is short in latency followed by rapid recovery. This transitory pattern of parasympathetic response is adaptive, and protects the individual from prolonged physiological arousal (Porges, 1995).

The excitatory sympathetic-adrenomedullary (SAM) system

The fast-acting sympathetic response of the SAM system involves the release of the catecholamines epinephrine (Epi) and norepinephrine (NE) which generates a ‘fight/flight’ physiological response, increasing heart rate, cardiac output, and mobilizing glucose energy stores, supporting heightened vigilance and attentional acuity in the short term (Gunnar & Quevedo, 2007; Chrousos & Gold, 1992). SAM functioning has traditionally been indexed by plasma catecholamines, systolic blood pressure, skin conductance level, or pre-ejection period (PEP). However, with recent advancements in the non-invasive collection and assessment of salivary analytes, individual differences in SAM activity can be measured by salivary α -amylase (sAA), an enzyme released by glands in the oral cavity (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007). Salivary AA levels show a robust response to physical and psychosocial stressors (approximately 10 minutes for

peak arousal) known to also increase traditional indicators of sympathetic activity (Chatterton et al., 1996; Kivlighan et al., 2005; Nater et al., 2005, 2006; Stroud et al., 2006).

Few studies have investigated emotional correlates of sAA in infants or young children (Kivlighan et al., 2005; Shea et al., 2006; Hill et al., 2008; Fortunato, Dribin, Granger, & Buss, 2008). In response to the administration of emotional challenge tasks designed to elicit negative reactivity, 7-month old infants' baseline sAA levels showed an inverse relation with positive reactivity, such that infants who displayed positive reactivity during the negative-emotion inducing tasks had the lowest baseline sAA levels (Kivlighan et al., 2007). Conversely, across a series of 12 tasks designed to elicit negative and positive emotions in toddlers, Fortunato and colleagues (2008) found that children with high baseline sAA levels displayed more approach behavior and positive affect. Although, a similar inverse relationship to that found by Kivlighan and colleagues (2007) was supported in an investigation examining the association between sAA reactivity and positive affect in young children. In a sample of 4-year old children, Spinrad and colleagues (2009) found that higher sAA reactivity was associated on a trend level with lower positive affect during a 'not-sharing' frustration-task where candy that is supposed to be divided equally among the experimenter and the child is unequally distributed in the experimenter's favor. Interestingly, higher sAA reactivity was also associated with lower expressions of anger during the tasks and fewer externalizing problems, particularly for girls (Spinrad, et al., 2009). Further, in a recent study Keller and El-Sheikh (2009) examined levels of sAA, in response to listening to an inter-adult argument and performing a challenging mirror-star tracking task, and externalizing in school-age children, and found evidence for a curvilinear association between sAA levels and externalizing problems. Moderate sAA levels predicted lower externalizing symptoms whereas low or high sAA levels predicted higher externalizing symptoms. Given the

mixed findings on sAA and the limited number of studies that have examined the sAA and the regulation of negative emotion in infants and young children, strong conclusions cannot yet be drawn. However, since emotion dysregulation may be a precursor or symptom of externalizing behavior problems, the findings of Spinrad et al. (2009) and Keller and El-Sheikh (2009) suggest that sAA may be a relevant predictor of emotion regulation.

Interacting physiological systems

Although inhibitory activity of the VVC and excitatory activity of the SAM system has been empirically substantiated as predictors of emotions manifested at the behavioral level and the ability to self-regulate emotion, limited research has attempted to integrate activity of these systems. An investigation of this nature is essential. As Bauer et al. (2002) suggest, substantial advances in the prediction of emotion regulation and early behavior problems can be made by examining patterns of physiological responses across multiple systems in place of independent functioning of a single physiological system. Single physiological measurements may be misleading, and do not capture nuanced individual differences in patterns of physiological arousal and regulation. Berntson and colleagues propose a theoretical model of autonomic space (1991, 2004) in which physiological interactions across the two branches of the ANS may be reciprocal or non-reciprocal and show activity of varying magnitudes (see Table 1.1). For example, when an individual's heart rate increases in response to an environmental challenge, it may represent SNS activation, PNS withdrawal, both, or SNS dominant co-activation (Berntson, Cacioppo, & Sarter, 2003). Without measurement of activity in both branches, it would be impossible to distinguish these patterns of physiological functioning. Similarly, Porges (2007) has also outlined a theoretical model for interacting physiological systems. Polyvagal

theory suggests a hierarchical organization of the physiological systems in which the ascending influence of the VVC predominates and may modulate SAM activity.

A few recent empirical investigations have considered interacting physiological systems, and disruptions in vagal or sympathetic regulation appear to be common among young children with behavior problems. In a clinical sample, Beauchaine and colleagues found that children with externalizing problems, such as aggression, impulsivity, and hyperactivity, often exhibit coinhibition indexed by measures of skin conductance level (SCL) and or cardiac pre-ejection period (PEP), and RSA, suggesting that low or non-response across the SNS and PNS may characterize these children and predict emotion dysregulation (Crowell et al., 2006; Beauchaine, Gatzke-Kopp, & Mead, 2007). Similarly, El-Sheikh et al. (2009) explored SCL and RSA interactions in predicting children's externalizing problems in the context of marital conflict, finding that externalizing problems were predicted by patterns of physiological coactivation (high SCL, high RSA) or coinhibition (low SCL, low RSA). Other investigations have shown, in line with Polyvagal Theory, the moderating influence of RSA on sympathetic activity indexed by sAA. For example, Keller and El-Sheikh (2009) found that RSA moderated the association between sAA and externalizing symptoms in a sample of school age children. Low levels of sAA predicted higher externalizing symptoms, but only in the context of RSA augmentation (an increase in RSA) in response to environment challenge. For those children who also had low levels of sAA and greater RSA suppression, fewer externalizing symptoms were present. Taken together, these studies suggest that there is much to be gained by examining distinguishable patterns of physiological response across the PNS and SNS, and how such patterns predict the ability to regulate emotions at the behavioral level.

The present study

The present study aims to explore patterns of ANS functioning indexed by RSA and sAA activity at 7-, 15-, and 24-months of age, and to determine the association of physiological patterns with concurrent emotion regulation behavior. Thus, the first goal of the study is descriptive; to garner a basic understanding of multisystem physiological profiles in a sample of young children living in low-income, non-urban communities. Few studies to date have examined interactions among multiple physiological systems, particularly in the developmental periods of infancy and toddlerhood. Given that individual differences in the pattern of physiological functioning across multiple systems is not directly observable and impossible to estimate a priori, meaningful differences must be inferred by the data. The present study will utilize a person oriented methodological approach, latent profile analysis (LPA), at each assessment point to identify distinct subgroups of children with shared patterns of the following continuous indicators: baseline RSA, RSA suppression, and pre-task sAA levels. LPA is superior to population level aggregate associations, which are faulted in their potential to obscure salient group-based differences. Further, it eliminates the use of arbitrary cutoffs to demarcate population subgroups and classify individuals; a key limitation of variable-centered grouping approaches (Bergman & Magnusson, 1997; Muthén, 2001). Theoretical models of ANS functioning, such as that proposed by Berntson et al. (2004) specify four general patterns of autonomic activity (see Table 1.1). The present study is guided by this model, but given the exploratory nature of the proposed analysis and the inclusion of a novel sympathetic indicator, there are no specific hypotheses about the number of physiological functioning profiles that exist at 7-, 15-, and 24-months of age in this sample. Although, due to known associations between demographic variables like income, race, and sex with physiological indicators (Davis & Emory,

1995; Kristenson, Eriksen, Sluiter, Starke, & Ursin, 2004; DeSantis et al., 2007; Blair et al., 2008; Hill et al., 2008), several demographic variables and a social advantage composite index will be included as indicators of the latent profiles, as well as potential measurement confounds, including infant age at the assessment point and time of day of saliva collection.

The second goal of the study is to utilize the latent physiological profile solution at 7-, 15-, and 24-months of age to predict concurrent emotion regulation behavior. Past research suggests that high baseline RSA and greater RSA suppression are associated with better emotion regulation skills (DeGangi et al., 1991; Porges et al., 1996; Huffman et al., 1998; Stifter & Corey, 2001; Calkins & Keane, 2004; Calkins et al., 2007), whereas sAA activity appears to have a curvilinear relationship with these outcomes where moderate levels are most adaptive (Granger et al., 2007). Low and high levels of SAM markers have been related to emotion dysregulation (Repetti et al., 2002; Boyce & Ellis, 2005; Granger et al., 2007). Taken together, it is hypothesized that latent physiological profiles characterized by high baseline RSA and RSA suppression will be related to better emotion regulation skills, whereas profiles characterized by low baseline RSA and lack of RSA suppression coupled with either low or high sAA activity may be related to increased risk for emotion dysregulation.

Method

Participants

Recruitment. The sample will be drawn from the Family Life Project (FLP), a study of 1,292 children and their families living in the Black South (eastern North Carolina; Sampson,

Wayne, and Wilson counties) and Appalachia (central Pennsylvania; Blair, Cambria, and Huntingdon counties) — two geographical regions of high child poverty. During a 1-year recruitment period, mothers were approached in the hospital after giving birth and asked to participate. Eligibility criteria included English-speaking families, residing in one of the target counties, with no intent to relocate from the area in the next 3 years. A cohort sampling method was used to over sample for low-income families in both states and, specifically in North Carolina, African-American families. African-American families were not over-sampled in Pennsylvania as the target communities were almost exclusively Caucasian ($\geq 95\%$). Over two-thirds of the FLP sample lives below 200% of the poverty line, and approximately 40% are African-American.

For this analysis, the sample is restricted to a random sub-sample of children from whom electrocardiogram (ECG) heart period data was collected ($N=358$, 51.1% female, 37% African-American). Families were visited in the home when children were at 7-, 15-, and 24-months of age. The age range for the 7 month visit ranged from 5 to 10 months ($M = 7.2$ months), 13 to 19 months for the 15 month visit ($M = 15.7$ months), and 22 to 28 months for the 24 month visit ($M = 24.5$ months). Due to the range in age at each of the visits, age-related differences will be controlled for in all analyses. Within this sub-sample, 67% had an income-to-needs ratio less than 200% of the poverty line. Forty-one percent of the families resided in Pennsylvania, and 59% resided in North Carolina. Most of the mothers (76%) had completed a high-school degree or equivalent and 53% were married (see Table 1.2).

Procedure. During the 7-month home visit, mothers completed questionnaires and interviews on family demographics and social risk factors. At the 7-, 15-, and 24-month visits children were presented with a mental development test, and a series of emotional challenge

tasks previously validated to assess the child's regulation of negative reactivity (e.g., Buss & Goldsmith, 1998; Kochanska, Tjebkes, & Forman, 1998; Goldsmith & Rothbart, 1996; Stifter & Braungart, 1995). During these tasks, physiological measurements were collected from children.

To assess RSA, three electrodes were placed on the child's chest, a ground just above the navel, the second below the left breast, and the third above the right breast, resulting in an inverted triangle pattern. Infant electrocardiograms (ECG) were recorded using a Grass preamplifier, and this output was transmitted to laptop computer in the form of a data file containing the interbeat intervals for the two collection periods, during a 5 minute baseline period when children were sitting quietly, and a 15 minute cognitively challenging period when a test of mental development, the Bayley exam (6- and 15-months) / Preschool Language Scale (24-months), was being administered. All ECG heart period data was analyzed off-line for movement artifact using Mindware Technologies.

To elicit fear reactivity and observe how children self-regulated fear, children were presented with four unusual masks in succession (a long-nosed woman, a Frankenstein, a goofy vampire, and a bald conehead) at the 7-, 15-, and 24- month visit. While wearing each mask for 10 seconds, the experimenter repeated the child's name three times while moving from side to side and then leaning towards the child's face.

To elicit frustration reactivity and observe how children self-regulated anger, at the 7-month visit, while the child was seated in a stationary walker, the experimenter gently restrained the child's arms for a period of up to 2 min or 20 s of hard crying. After releasing the child's arms, experimenters and mother remained non-interactive for 1 min. Mothers were then told that they could soothe their infant if necessary as they normally would. At the 15- and 24-month visit, the child was given an attractive Busy Box toy and encouraged to play with it by their

mother for 60s. At 15-months, mothers then removed the toy and set it out of reach, but kept it within the child's sight, and engaged in conversation with the experimenter for up to 2 min. At 24-months, rather than setting the toy out of reach, mothers removed the toy and placed it in a clear plastic container, sealed the container with a lid, and returned the container enclosing the toy back to the child while they engaged in a conversation with the experimenter for up to 2 min. Following this, at both the 15- and 24-month assessment, the mothers were cued to return the toy to the child to play with, but to continue to remain non-interactive for 1 min. During the emotional challenge tasks, mothers were asked to maintain a neutral facial expression and to refrain from verbally interacting with their infants for the duration of the challenge tasks, but were told that they could stop the task at any time if they felt it necessary. All tasks were videotaped in the home for later observational coding of children's emotion regulation behavior.

To assess children's SAM activity, a saliva sample was collected immediately prior to the emotional challenge tasks via a sterile, 6" cotton rope at 7-months, and via a hydrocellulose sponge at 15- and 24-months (Granger et al., 2007). Two additional saliva samples were collected 20-min and 40-min post-peak arousal, which for most infants, occurred at the conclusion of the emotional challenge tasks. The order of the tasks was structured so that the most arousing task was presented last at each age. At 7-months this was the arm restraint, and at 15- and 24-months, this was the mask task. The two post-peak arousal saliva samples were not included in the current analysis as the time course of saliva collection was not designed to capture the fast-acting SAM response, and preliminary investigations using 7-month data suggest that children's sAA levels did not show a response to the emotional challenge tasks (Kivlighan et al., 2005). After collection in the home, all saliva sample were immediately placed on ice, transported to experimenters' homes, and stored frozen (-20°C) before being batched and

overnight shipped on dry ice to the Behavioral Endocrinology Laboratory at Pennsylvania State University. Samples were then stored frozen (-80°C) until assay.

Measures

Demographic and social advantage measures

Income-to-need ratio. At the 7-month home visit, mothers reported on income from all sources and any income from other household members. This figure was used as an estimate of total household income and divided by the federal poverty threshold for 2004 adjusted for number of persons in the home to compute an income-to-need ratio. An income-to-need ratio of 2.00 or below indicates that the family is low-income, and 1.00 or below indicates that the family is living in poverty by federal guidelines, adjusted for family size.

Geographic isolation. To index degree of geographic isolation in these rural communities, address mapping using Global Positioning System (GPS) technology determined the longitude and latitude for the family residence at the 7-month visit. This was used to compute the distance in meters from the family's home to a variety of educational, financial, and social resources including: the nearest gas station, doctor's office, public library, fire station, elementary school, high school, public park, supermarket, county seat, and freeway ramp. The log transformed mean distance to resources was formed to index degree of geographic isolation.

Social advantage. At the 7-month visit, mothers completed questionnaires and interviews on family level social factors. Developed by Blair et al. (2008) in a previous investigation with data from the Family Life Project, an index of social advantage was created by

standardizing and summing six variables: maternal age, years of education, marital status, employment status, economic sufficiency measured using the Conger and Elder (1994) questionnaire, and global rating of social support measured using the Questionnaire of Social Support as adapted by Crnic, Greenberg, Robinson, and Ragozin (1984).

Physiological measures

Respiratory sinus arrhythmia. From electrocardiograms (ECG) recorded in the home when children were approximately 7-, 15-, and 24-months of age, Mindware Technologies software (Gahanna, Ohio) was used to apply an algorithm to the time series of heart period data. The algorithm uses a moving polynomial to de-trend periodicities in heart period that are slower than RSA. A band-pass filter then extracts the heart rate variability within the frequency band of spontaneous respiration in young children, .24 to 1.04 Hz, which is commonly studied to index vagal functioning in infants and young children (see Stifter & Fox, 1990; Porges et al., 1996; Huffman et al., 1998; Stifter & Corey, 2001; Calkins & Keane, 2004). The software then derives a RSA estimate by calculating the natural log of specified heart period variability and is reported in units of $\ln(\text{ms})^2$. Trained coders edited the ECG heart period records for movement artifact where possible, marking the R spike if identifiable on the record. The mean RSA across the baseline minutes is used to index *baseline RSA*. *RSA suppression* is the difference between baseline RSA and mean RSA during the mental development test, such that positive values indicate suppression. A negative value, or increase in RSA from baseline to the mental development test, would indicate RSA augmentation.

Salivary alpha-amylase. Pre-task baseline saliva samples were also assayed for sAA

using a kinetic reaction. A chromagenic substrate, 2-chloro-p-nitrophenol linked with maltotriose, was added to saliva samples to trigger the enzymatic action of α -amylase indexed by the degradation of maltotriose. The amount of α -amylase activity present in the sample is directly proportional to the increase (over a 2- min period) in absorbance (optical density) at 405 nm (Salimetrics, State College, Pennsylvania). The test uses only 10 μ l of saliva (for singlet determinations), with an absolute range from 3.1 - 423.1 U/mL. The lower limit of sensitivity is governed by the change in absorbance. A change in absorbance less than 0.01 does not result in a reliable value. In this case, samples were rerun at a higher concentration. The sAA distributions were square root transformed prior to analysis to correct for positive skew.

Behavioral measure

Emotion regulation of fear and frustration reactivity. Children's behavioral responses to the emotional challenge tasks at 7-, 15-, and 24-months were recorded using digital video, and Better Coding Approach software (Danville, Pennsylvania) was used to code second-by-second displays of child emotion regulation behavior. The presence or absence of select regulatory behaviors were coded including: "orienting to the environment" (e.g. focused gaze on objects or others), "looks to mother" (e.g. gaze at mother's face), "self-comforting" (e.g., sucking thumb), "neutral vocalizations" (e.g., non-negative chatter), "gesture" (e.g. pointing or offering), "avoidance" (e.g., turning away, arching of the back), "tension reduction" (e.g., repeated banging of the hands), and "rejection" (e.g. active refusal). Coders were trained to achieve a minimum reliability of .75 (Cohen's κ), and subsequent interrater reliabilities were established on 15% of the digital videos using kappa coefficients. Across the fear and frustration eliciting challenge

tasks, regulation behaviors averaged a kappa of .91 (range = .82-.99) at 7-months, .92 (range = .86-.97) at 15-months, and .91 (range = .87-.97) at 24-months.

Based on previous research, four regulatory composites were created for data analysis: attentional strategies, self-comforting behavior, communicative behavior, and avoidance (Braungart & Stifter, 1991; Stifter & Braungart, 1995). Attentional strategies represented the combined proportion of time across the task that the child engaged in orienting to the environment or looking at their mother. Self-comforting behavior was simply the proportion of time the child spent self-comforting. Communicative behavior represented the combined proportion of time the child exhibited non-negative vocalizations or used non-verbal gestures. Avoidance represented the combined proportion of time the child tried to actively refuse or ignore the task, escape the restraint of the seat, or reduce tension through repeated banging of arms or legs.

Missing data. Of the subsample of children on whom ECG data was collected to assess RSA there were 358 children with RSA data indexed for at least one of the three assessment points (see Appendix A). Salivary AA was assayed from saliva sample collected on the total sample, and was available on 1171 children for at least one assessment point (see Appendix B). Given the variation in age at which children were assessed, the analysis restricts the sample to 325 children between 5 and 9 months for the 7-month assessment, 341 children between 13 and 19 months for the 15-month assessment, and 330 children between 22 and 28 months for the 24-month assessment. This age restriction resulted in the exclusion of 33 children older than 9 months of age at the 7-month assessment, 17 children older than 19 months of age at the 15-month assessment, and 28 children older than 28 months at the 24-month assessment.

Listwise deletion typically induces bias in parameter estimates by eliminating non-

random cases and reducing error variance. To handle missing data in the present sample, multiple imputation and maximum likelihood approaches were implemented. Multiple imputation (MI) was performed via the SAS MI procedure to generate 40 imputed datasets, recommended for the fraction of missing information in the current sample (see Graham, Olchowski, & Gilreath, 2007); which approximated 40% missing RSA data for some measurement occasions (see Appendix C). In addition, Mplus software has a built-in maximum likelihood (ML) estimation application for handling missing data. In the latent profile analysis using Mplus, multiply imputed datasets on the scale of 40 datasets did not generate a meaningful profile solution as the order of the profiles and distribution was not consistent across each dataset, even with specified model starting values. Therefore, the ML estimation procedure was selected as optimal for these data and utilized to handle missing data in the results presented below (J. Graham, personal communication, April 26, 2010). However, additional data cases were excluded with this method since the program requires that data not be missing across all specified indicators. As a result of this restriction, the latent profile analysis was conducted on 282 children at 7-months of age, 319 children at 15-months of age, and 289 children at 24-months of age.

Plan of analysis

A separate latent profile analysis (LPA) was conducted to examine profiles, or person-oriented subgroups, of infant autonomic and neuroendocrine functioning at the 7-, 15-, and 24-month assessment points. Although theoretical models such as the doctrine of autonomic space would suggest that 4 patterns of autonomic functioning exist (see Table 1.1), the distinct patterns of functioning among the vagal system, SAM system, and L-HPA axis that exist within the

current sample were impossible to estimate a priori. Thus, profile membership was statistically modeled within a separate LPA, based on the observed response pattern of baseline RSA, RSA suppression, and baseline sAA measured when infants were 7-, 15-, and 24-months of age.

To determine the optimal number of profiles that best fits the current data, a model with 2-6 classes was fit at each assessment point, with the goal of identifying mutually exclusive and exhaustive subgroups of children with similar physiological profiles, controlling for age at the time of assessment, and for salivary indicators, time of day of saliva collection. Model goodness of fit was evaluated with the following fit indices: Akaike information criterion (AIC), Bayesian information criterion (BIC), Sample-Size Adjusted BIC, and the *adjusted Lo-Mendell-Rubin likelihood ratio test (LMR-LRT)*. The model with the smallest BIC values, and a significant LMR-LRT test, indicating that the addition of one more profile significantly improves model fit, was selected at each assessment point. Although, similar to exploratory factor analysis, in addition to the fit indices, theoretical justification, model parsimony and interpretability will be considered in model selection (Bauer & Curran, 2003; Muthén, 2004; Jung & Wickrama, 2008). Following the selection of the optimal profile solution, the model was replicated with a minimum of 100 random starting values to test the robustness of model convergence.

Next, observed variables that may define the physiological profile solution were added to LPA models, and model fit indices will be re-evaluated. Profile differences in child sex and race composition was examined, and income-to-need ratio, geographic isolation, and social advantage were included as indicators of the latent profile solution. Further, to assess whether the latent profiles of child physiological functioning at each assessment point are associated with concurrent emotion regulation behavior, class membership was exported from the selected optimal profile solution containing all relevant model indicators. Finally, in separate mixed

linear models, ER composites (attention, self-comforting, communication, avoidance) were regressed on class membership.

Results

Preliminary Analysis

Sample means for the study variables are reported in Table 1.3. On average, children did show RSA suppression to the cognitive challenge at 6 and 15 months, however, this did not hold for the 24-month assessment. At 24-months, the mean RSA suppression value approached a 0 value, indicating no change in RSA between baseline and cognitive challenge, however this estimate is an average, representing some children that suppressed RSA and others that augmented RSA (min = -1.89, max = 1.32). Intercorrelations between child RSA, sAA, observed emotional reactivity, and emotion regulation behaviors were examined separately at each measurement occasion. As shown in Table 1.4, at 7 months of age, a number of bivariate correlations were significant. Of particular interest, there was a positive association between baseline RSA and RSA suppression. In addition, there was a negative association between pre-task sAA and attention regulation behavior during the frustration task such that children with greater levels of pre-task sAA displayed a lower proportion at attention regulation behavior during the frustration task. At 15-months of age, baseline RSA was positively associated with RSA suppression (see Table 1.5). In addition, RSA suppression was negatively correlated with sAA levels, such that children who showed greater suppression of RSA in response to cognitive challenge had higher pre-task levels of sAA. There was also a positive association between pre-task sAA and attention regulation during the fear task such that children with greater levels of sAA displayed a greater proportion of attention regulation during the fear task. At 24-months of

age, baseline RSA was positively associated with RSA suppression (see Table 1.6). In addition, baseline RSA was positively associated with attention regulation behavior during the frustration task, such that higher baseline RSA values were related to a greater proportion of attention behavior during the frustration task. Further, sAA was negatively associated with intensity of negative reactivity during the frustration task, such that children with higher pre-task levels of sAA displayed less intense negative behavioral reactivity in response to a frustration-eliciting task.

Latent Profile Mixture Models of Child ANS Activity

To prepare the physiological variables for the latent profile analysis, baseline RSA, RSA suppression, and pre-task sAA values were standardized at each assessment point. Further, given the positive association between child age and baseline RSA (see Table 1.4, 1.5, 1.6), the effect of child age was regressed out of baseline RSA prior to conducting latent profile modeling. Similarly, saliva collection time of day was positively associated with pre-task sAA levels. Although this correlation was significant at the 15-month assessment ($r = .16, p < .01$), but only approached significance at the 6- and 24-month assessment ($r = .09 - .11, p = .11-.16$), past investigations of sAA activity indicate that sAA exhibits a circadian rhythm, characterized by a continuous increase over the course of a day (Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2005). Therefore, the effect of time of day was regressed out of pre-task sAA levels at each assessment point prior to latent profile modeling.

Latent profile models with two through six profiles were fit at 7-, 15-, and 24- months of age to determine the optimal number of profiles that best describe ANS activity in the current sample of young children living in low-income, rural communities. Model goodness of fit was evaluated using the Bayesian information criterion (BIC), where a smaller value represents a

better fit, and the adjusted Lo-Mendell-Rubin likelihood ratio test (Adj. LMR-LRT), which tests the significance of the difference between a model with k and $k-1$ classes, correcting for the distribution of the likelihood-ratio statistic (Lo, Mendell, & Rubin, 2001).

7-month profiles. At 7-months of age, the three profile solution was selected as optimal for these data based on having the smallest BIC value of the models and a significant adjusted LMR-LRT statistic. The three profile solution was preferred to the four profile solution given the distribution of members across the profile; the four profile solution generated two profiles with less than 5% of the sample in each. For the three profile solution, average posterior probabilities for membership classification ranged from .68 to .97. Fit indices for the 7-month LPA models are reported in Table 1.7. As seen in Figure 1.1, the first profile of the three profile solution, named the “PNS inhibition” profile, comprised 7-month children who exhibited low baseline RSA, lower RSA suppression values, and average sAA values, representing 28% of the sample. The second profile, named the “PNS activation” profile, comprised 7-month children who exhibited high-average baseline RSA, greater RSA suppression values, and average sAA values. The PNS activation profile represented the majority of individuals; 70% of the sample. The third profile, named the “coactivation” profile, comprised 7-month children who exhibited very high baseline RSA and RSA suppression values, and high-average sAA values. The coactivation profile was rare, representing only 2% of the sample. Child sex, $\chi^2(2, 282) = 5.72, p = .06$, and race, $\chi^2(2, 282) = 5.28, p = .07$, were not significantly related to profile membership. Income to needs ratio, geographic isolation, and social advantage were also entered as possible covariates predicting the probability of membership in profiles. There was a significant prediction for income to needs ratio, such that for a one-unit increase in the income to needs ratio there is a corresponding 84% increase in the odds of being in the coactivation profile compared to the PNS

activation profile.

15-month profiles. At 15-months of age, the three profile solution had the smallest BIC value, and represented a significant improvement from the two profile solution, and thus was selected as the optimal solution for the 15-month data (see Table 1.8). Average posterior probabilities for membership classification ranged from .82 to .87. As seen in Figure 1.2, the first profile of the 15- month three profile solution, named the “SNS activation” profile, comprised 15-month children who exhibited low baseline RSA, lower RSA suppression values, and high sAA values, representing 4% of the sample. The second profile, named the “PNS activation” profile, comprised 15-month children who exhibited high baseline RSA, greater RSA suppression values, and average sAA values. The PNS activation profile represented 11% of the sample. The third profile, named the “average” profile, comprised 15-month children who exhibited average baseline RSA and RSA suppression values, and average sAA values relative to the sample means for these variables. The average profile represented 85% of the sample. Although child race, $\chi^2(2, 319) = 4.64, p = .10$ was not significantly related to profile membership, there were significant differences in profile membership by child sex, $\chi^2(2, 319) = 8.75, p < .01$. The SNS activation profile was predominately male relative to the PNS activation and average profiles, which were more evenly split by child sex. Specifically, the 88% of the children in the SNS activation profile were male, 58% of the children in the PN S activation were male, and 45% of the children in the average profile were male. Further, income to needs ratio, geographic isolation, and social advantage were entered as possible covariates predicting the probability of membership in profiles. In the 15-month data, there was no prediction of profile membership by these selected covariates.

24-month profiles. At 24-months of age, none of the multiple profile solutions

represented a significantly better fit than one profile (see Table 1.9 for model fit indices).

Therefore, there was no evidence to suggest that mutually exclusive and exhaustive subgroups of children's ANS functioning modeled with the indicators of RSA and sAA exist at 24-months of age in the current sample. As such, the following post hoc analyses where profile membership is used in the prediction of emotion regulation behavior was tested only for the 7- and 15-month data.

Prediction of Emotion Regulation Behavior by Latent ANS Profile

To test the prediction of emotion regulation behavior by ANS profile at 7- and 15-months, attention, self-soothing, communication, and avoidance regulation behavior composites were regressed on profile membership, controlling for infant age at the time of the assessment. In addition, intensity of negative reactivity was regressed on profile membership for information regarding the average behavioral arousal of each ANS profile. The difference of least-squares means were tested using a Tukey-Kramer adjustment to account for multiple comparisons. As seen in Figure 1.3, at 7 months of age there were significant group differences in self-soothing behavior among the ANS profiles. Children in the coactivation profile exhibited more self-soothing behavior during the fear task, controlling for child age, in comparison to children in the PNS inhibition profile ($t = -4.78, p < .01$) and PNS activation profile ($t = -4.84, p < .01$). A similar pattern emerged during the frustration task; again, children in the coactivation profile exhibited significantly more self-soothing behavior, controlling for child age, in comparison to children in the PNS inhibition profile ($t = -2.58, p < .05$) and PNS activation profile ($t = -2.80, p < .05$). In addition, this finding was replicated when controlling for level of child negative reactivity during the tasks (see Appendix D). There were no significant differences in attention or avoidance regulation, or intensity of behavioral negative reactivity by ANS profile at 7-

months of age.

At 15 months of age there were significant group differences in attention behavior among the ANS profiles, controlling for child age at the time of the assessment. Children in the SNS activation profile exhibited more attention regulation behavior during the fear task, controlling for child age, in comparison to children in the PNS activation ($t = 2.32, p < .05$) and average profile ($t = 3.03, p < .01$) (see Figure 1.4). In addition, this finding was replicated when controlling for level of child negative reactivity during the tasks (see Appendix E). There were no significant differences in self-soothing, communication, and avoidance regulation, or intensity of behavioral negative reactivity by ANS profile at 15-months of age.

Discussion

The primary goal of the current study were to describe latent autonomic nervous system profiles capturing both parasympathetic nervous system activity indexed by RSA and sympathetic nervous system activity indexed by sAA in a sample of young children ages 7-, 15-, and 24- months, living in low-income, non-urban communities. In addition, the current study also aimed to use latent profile membership at each measurement occasion as a predictor of concurrent emotion regulation behavior, including attentional strategies, self-soothing, communication, and avoidance. A growing body of research has examined physiological substrates of emotion regulation, however, the majority of studies have only measured activity of a single system or multiple, independent indicators (Bauer et al., 2002). The results of this study extend the current literature by identifying multi-system patterns of autonomic functioning utilizing a latent profile analytic approach to derive mutually exclusive and exhaustive subgroups of children with unique physiological profiles.

At 7-months of age, the LPA technique yielded three profiles of autonomic functioning: a PNS inhibition profile, characterized by low baseline RSA, lack of RSA suppression to a cognitive challenge, and average levels of sAA; a PNS activation profile, characterized by high-average baseline RSA and RSA suppression, and average levels of sAA; and a coactivation profile, characterized by very high baseline RSA and RSA suppression, and high levels of sAA. From Berntson's doctrine of autonomic space model (1991) four ANS profiles are specified: reciprocal sympathetic, reciprocal parasympathetic, coactivation, and coinhibition (see Table 1.1). The 7-month ANS 3 profile solution did not completely correspond with those specified in the autonomic space model. The PNS inhibition profile, representing 28% of the sample, approximated the reciprocal sympathetic profile, and the PNS activation profile, representing 70% of the sample, approximated the reciprocal parasympathetic profile with respect to PNS activity. A noted difference between the two profiles found with the current data and the autonomic space model was that the SNS indicator, pre-task level of sAA activity, did not differentiate the current profiles; both the PNS inhibition and PNS activation profiles had sAA values near the sample mean. As such, the direction of the net effect of ANS activity on physiological arousal becomes less pronounced without clear SNS activation or inhibition. Children in the PNS inhibition profile likely experienced higher basal physiological arousal having low baseline RSA and average sAA levels; however a lack of RSA suppression or RSA augmentation during the cognitive challenge may result in decreased context-specific physiological arousal. Yet, since only a PNS activity indicator was measured, it is also possible that SNS activation is occurring coupled with lower RSA withdrawal. Conversely, children in the PNS activation profile likely experienced lower basal physiological arousal having high baseline RSA and average sAA levels. Further, these children also showed greater RSA

suppression to a cognitive challenge, representing an increase in physiological arousal via PNS mobilization of cardiac resources. As theorized by Porges' Polyvagal Theory (2007) this flexibility and potential to respond in accord with environmental demands is adaptive, as context-specific PNS activation may interact with, and inhibit SNS activation. Therefore, physiological arousal for each of the 7-month profiles cannot be sufficiently described with a unidimensional net effect, and should instead be interpreted separately as basal level of physiological arousal during the visit, and PNS response to a cognitive challenge. Finally, the coactivation profile was represented in the current sample utilizing RSA and sAA indicators, where basal level of arousal is ambiguous given the opposing effects of high baseline RSA and high-average pre-task sAA. These children showed high levels of RSA suppression in response to the cognitive challenge, resulting in increased physiological arousal. However, it is important to note that this class was rare, comprising only 2% of the sample. Therefore, subsequent findings related to the coactivation class should be interpreted with caution.

At 7-months, mean differences in self-soothing behavior during the fear and frustration tasks were predicted by ANS profile. Specifically, children in the coactivation profile displayed higher levels of self-soothing behavior, including thumb sucking or repetitive rubbing of their hair, clothing, or hands, compared to children in the PNS inhibition and PNS activation profiles. Generally, self-soothing behavior is associated with decreasing negative reactivity at the behavioral level (Stifter & Braungart, 1995), and this is shown by the more pronounced differences in self-soothing when controlling for intensity of child negative emotional reactivity. In the doctrine of autonomic space model, Berntson (1991) suggests that coactivation is maladaptive, exerting an ambiguous net effect on physiological arousal, and El-Sheikh and colleagues (2009) found that children with a coactivation pattern of ANS activity were at-risk for

externalizing outcomes. In the current sample, the coactivation profile appears to represent children from higher income homes that are well regulated behaviorally. The ability to regulate arousal during the challenge tasks may reflect a strong PNS influence coupled with the moderate to moderately high levels of sAA, representing a mid-range in the curvilinear pattern of sAA. However, it is important to reiterate that these differences, although intriguing with respect to the meaning of coactivation with sAA as the SNS indicator, should be interpreted with caution given the small proportion of children in the coactivation class (< 5% of the sample).

At 15-months of age, the LPA technique also yielded three profiles of autonomic functioning: a SNS activation profile, characterized by low-average baseline RSA, lack of RSA suppression to a cognitive challenge, and high levels of pre-task sAA; a PNS activation profile, characterized by high baseline RSA and RSA suppression, and average levels of sAA; and an average profile, characterized by average baseline RSA and RSA suppression, and average levels of sAA. In comparison to the ANS profiles at 7-months, there was no evidence for a coactivation profile at 15-months of age. Children in the SNS activation profile, representing 4% of the sample, with low-average baseline RSA and high pre-task sAA likely had higher basal physiological arousal. Since these children exhibited no RSA suppression or in some cases augmentation to the cognitive challenge, they may have experienced a decrease in context-specific physiological arousal but we do not know their SNS activity during this same period. It is also possible that SNS activation occurred to mobilize physiological resources to support engagement during the cognitive challenge, although this would not be adaptive as the cognitive task does not constitute a threatening situation. Children in the PNS activation profile, representing 11% of the sample, had high baseline RSA and average sAA levels, and therefore, likely had lower basal physiological arousal. These children also had higher RSA suppression

to the cognitive challenge which may result in increased context-specific physiological arousal. Finally, unique to the 15-month data, the majority 85% of children were classified as members of an average profile. The values for baseline RSA, RSA suppression, and pre-task were all within a half standard deviation from the sample means, and thus, with no clear pattern of ANS activation or inhibition, level of physiological arousal is ambiguous for these children.

At 15-months, mean differences in attention behavior during the fear task were predicted by ANS profile such that children in the SNS activation profile displayed more attention regulation behavior compared to children in the PNS activation profile or average profile. In this case, attention regulation behavior included looking away from the mask fear stimulus to orienting towards the environment, or looking to their mother. Shifting attention away from the fearful stimulus appeared to reduce negative reactivity for these children. In addition, just as with the 7-month data, controlling for intensity of negative reactivity increased the degree of difference between the SNS activation profile and the other two profiles. Although SNS activation represents increasing physiological arousal posited to be adaptive within the framework of the doctrine of autonomic space model (Berntson et al., 1991), the finding that children classified as being in the SNS activation profile are displaying more attention regulation behavior is counterintuitive to prior work using sAA as marker for SNS activity which suggest that high sAA levels may place young children at-risk for deficient emotion regulation and externalizing behavior (Keller & El-Sheikh, 2009). It may be that, early in life, in a fear context, these children are experiencing visceral arousal but not displaying high levels of negative reactivity. Alternatively, these children may actually be interested in the novel stimulus, and look away for brief periods to continue attending to the stimulus. These children did show comparable levels of negative reactivity to the other profiles during the frustration task,

suggesting something unique about their response to a fear eliciting task (see Figure 1.4).

However, these differences should be interpreted with caution as the SNS activation profile accounts for less than 5% of the sample.

Several limitations of the present study need to be addressed. First, the generalizability of these findings may be limited to the population sampled, young children living in low-income and non-urban communities. The Family Life Project is a developmental sample, which may have very few children with extreme activation or inhibition of ANS activity and a tendency to derive a majority average ANS profile. Further, the differences in emotion regulation behavior by ANS profile that emerged represent contrasts between the smallest member profile and the majority profiles. LPA is a data driven analytic approach, which may vary depending on sample composition and profile indicators. Related to this, the profile indicator of pre-task sAA did not appear to effectively distinguish the physiological profiles at 7-months of age, and only contributed unique information to the SNS activation profile at 15-months. While the pre-task assay of sAA reflects a basal level of SNS arousal during the course of the visit, it is unlikely a true, resting baseline and at the same time does not reflect an excitatory response to a specific event or emotional challenge. Salivary AA shows a robust response to challenge in older children and adults (Rohleder et al., 2004; Granger et al., 2007), thus, it would be advantageous to collect an additional saliva sample 10-min post exposure to a challenging task, when sAA peaks, to capture SNS responsivity. Another concern regarding the interpretation of findings from this study is that the PNS and SNS indicators were not measured at the same time during the home visit. Baseline RSA was indexed prior to a cognitive challenge, and RSA suppression as the difference in RSA from baseline to challenge. Pre-task sAA was indexed later in the visit, prior to administration of the emotional challenge tasks, designed to elicit fear and frustration

reactivity. Although basal levels of RSA and sAA during the visit were still informative about general ANS activity, it would be ideal to have measured PNS and SNS activity simultaneously to get a full appreciation of inhibition/activation interactions at the physiological level. Fourth, the cognitive challenge presented to children was a different task at 24-months than had previously been administered when children were 7- and 15-months of age. On average, children did not show RSA suppression to the task, as they had previously at earlier assessment points indicating that the task was not comparable at engaging or challenging the 24-month olds, which may have contributed to the lack of a profile solution at this measurement occasion. It would be optimal to utilize the same task stimulus at various assessment points, or to make protocol adjustments for developmental appropriateness but ensure that the task are comparable in eliciting a unidirectional PNS response in the overall sample at each age. Finally, the degree of missing ECG data in the present study is a concern. ECG was collected on a planned subsample of the total sample participating in the Family Life Project. However, of the children who were visited within the restricted age window, and had information for at least one of the indicators to be handled by the maximum likelihood estimation, the sample was reduced to between 282 and 319 cases at each of the assessment points. While physiological indicators at other assessment points and additional correlates were added to strengthen missing data estimation, maximum likelihood assumes all missingness is missing at random. Equipment difficulties limited data collection at some points may have induced random missingness, however, it is also plausible that some children would not cooperate with ECG collection and therefore some data cases are not missing at random. Further, children that would not comply may have been more behaviorally and physiologically aroused and if saliva collection was not completed as well, these children would be excluded from the current analysis.

Taken together, the descriptive nature of the present findings extends the literature examining physiology-behavior links by applying LPA to understand interactions among PNS and SNS indicators of physiological activity, and relating latent ANS profiles to concurrent emotion regulation behavior. Several important ideas may be garnered from these findings to inform future research. First, the inclusion of baseline and challenge-specific indicators of physiological activity may provide unique information about physiological flexibility, broadening the understanding of physiological arousal in context. Past research has often examined these indicators separately, and there is much to be gained by a more dynamic description of physiological responding. Second, it would be optimal to measure PNS and SNS activity during the same task so as to index activity simultaneously, providing the best account of multi-system interactions. Finally, the current study sought to describe cross-sectional patterns of ANS activity at 7-, 15-, and 24-months of age. To extend the current findings, it will be necessary to consider how patterns of ANS activity exhibit stability or change over the first years of life. The current findings suggest the potential for change in ANS activity over time, as different profile solutions were found at 7- and 15-months. The response dynamics of these systems are calibrated early in life, and it would be advantageous to examine the development of PNS and SNS activity in the context of the early environment in future research.

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Table 1.1 *ANS profiles*

Profile	SNS activity	PNS activity	Net effect on ANS arousal
Reciprocal sympathetic	Activation (high sAA)	Inhibition (low RSA)	Increase
Reciprocal parasympathetic	Inhibition (low sAA)	Activation (high RSA)	Decrease
Coactivation	Activation (high sAA)	Activation (high RSA)	Ambiguous
Coinhibition	Inhibition (low sAA)	Inhibition (low RSA)	Ambiguous

Table 1.2
Sample Characteristics

Variable	%	<i>M (SD)</i>
Child sex (Female)	51	
Child age – 7m visit		7.17 (0.96)
Child age – 15m visit		15.76 (0.88)
Child age – 24m visit		24.48 (1.24)
Mother married	53	
Mother employed	48	
Maternal race (African-American)	35	
Maternal age		26.6 (6.15)
Maternal education		14.2 (2.97)

Table 1.3

Descriptive statistics for study variables

<i>Measure</i>	<u>7 months</u>		<u>15 months</u>		<u>24 months</u>	
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
Income-to-needs ratio	1.75	1.46	1.74	1.59	1.71	1.49
Geographic isolation	6.03	4.04	5.88	4.02	6.07	4.04
Index of social advantage	0	.61	0	.59	0	.57
Child physiology						
Baseline RSA	3.87	0.88	4.36	1.05	4.96	1.21
RSA Suppression	0.13	0.69	0.17	0.58	-0.03	0.54
sAA pre-task	44.33	37.35	57.33	48.70	70.29	55.07
Child emotional reactivity						
Fear task negative reactivity	0.15	0.38	0.66	0.65	0.80	0.83
Frustration task negative reactivity	0.82	0.69	0.43	0.28	0.54	0.66
Child emotion regulation behavior						
Fear task						
Attention	0.10	0.13	0.04	0.06	0.06	0.09
Self-comforting	0.02	0.09	0.06	0.17	0.15	0.24
Communication ^a	---	---	0.02	0.04	0.01	0.04
Avoidance	0.03	0.07	0.11	0.17	0.18	0.25
Frustration task						
Attention	0.37	0.27	0.88	0.25	0.21	0.19
Self-comforting	0.04	0.07	0.01	0.07	0.06	0.13
Communication ^a	---	---	0.01	0.04	0.95	0.30
Avoidance	0.18	0.19	0.04	0.06	0.12	0.15

Note. ^a Communication regulation behavior was not coded at the 7 month assessment.

Table 1.4

Intercorrelations of child ANS indicators and observed emotional reactivity and regulation behaviors at 7 months of age

	2	3	4	5	6	7	8	9	10	11	12
1.Child age	.18*	.09	.08	.03	-.31*	-.09	.08	.04	-.13*	-.03	.08
2.Baseline RSA	--	.55*	.03	-.14	-.05	.12	.03	.13	-.03	-.01	-.01
3.RSA Suppression		--	-.02	-.05	.08	.13	.15	-.05	.05	.10	.09
4.Pre-task sAA			--	.03	-.06	.04	.10	.12	-.17*	.08	.10
5.Negative fear reactivity				--	-.02	-.04	.44*	.26*	-.13*	-.01	-.02
6.Attention fear regulation					--	.03	.15*	-.09	.31*	.03	.05
7.Soothing fear regulation						--	-.05	.04	-.02	.22*	-.04
8.Avoidance fear regulation							--	.07	-.03	.02	.05
9.Negative frustration reactivity								--	-.49*	.03	.17*
10.Attention frustration regulation									--	-.12	.04
11.Soothing frustration regulation										--	.05
12.Avoidance frustration regulation											--

Table 1.5

Intercorrelations of child ANS indicators and observed emotional reactivity and regulation behaviors at 15 months of age

	2	3	4	5	6	7	8	9	10	11	12	13	14
1.Child age	.05	-.05	-.01	.09	.01	.07	.13*	.01	.15*	-.02	-.06	.01	-.02
2.Baseline RSA	--	.43*	-.11	-.02	.08	.01	.14	.01	.01	.11	-.04	.04	.04
3.RSA Suppression		--	-.24*	.04	.10	.04	.09	-.04	-.11	.04	.06	.01	-.06
4.Pre-task sAA			--	-.10	.14*	.03	-.02	-.07	-.09	.07	-.01	-.09	.01
5.Negative fear reactivity				--	.17*	-.02	-.20*	.51*	-.01	-.18*	.11	-.05	-.01
6.Attention fear regulation					--	-.02	-.02	.29*	-.05	-.05	.02	.09	.04
7.Soothing fear regulation						--	-.06	-.07	-.02	-.03	.02	-.03	.06
8.Communication fear regulation							--	-.14*	.08	-.03	.04	.03	.02
9.Avoidance fear regulation								--	-.01	-.16*	.08	-.02	-.02
10.Negative frustration reactivity									--	-.06	.01	.11	.02
11.Attention frustration regulation										--	-.54*	-.08	-.03
12.Soothing frustration regulation											--	-.04	-.02
13.Communication frustration regulation												--	-.04
14.Avoidance frustration regulation													--

Table 1.6

Intercorrelations of child ANS indicators and observed emotional reactivity and regulation behaviors at 24 months of age

	2	3	4	5	6	7	8	9	10	11	12	13	14
1.Child age	.17*	.10	-.06	-.12	.04	-.02	.07	-.07	-.07	.02	-.01	.06	-.02
2.Baseline RSA	--	.37*	-.12	-.14	.09	-.12	.08	-.02	-.12	.14*	-.01	.01	.08
3.RSA Suppression		--	.15	-.05	.02	.03	-.01	.09	-.15*	.03	-.11	.08	.08
4.Pre-task sAA			--	.07	-.02	.09	.01	.07	-.07	.02	.02	.10	.11
5.Negative fear reactivity				--	.28*	-.06	-.16*	.46*	.19*	.07	-.06	-.09	.17*
6.Attention fear regulation					--	-.08	-.04	.17*	.03	.22*	-.07	.05	-.06
7.Soothing fear regulation						--	-.03	-.19*	-.01	.05	.31*	-.04	.05
8.Communication fear regulation							--	-.16*	.08	-.06	-.07	.05	-.01
9.Avoidance fear regulation								--	-.01	.02	-.09	.04	.05
10.Negative frustration reactivity									--	-.14*	-.03	-.38*	.24*
11.Attention frustration regulation										--	-.01	.05	-.01
12.Soothing frustration regulation											--	-.12	.06
13.Communication frustration regulation												--	-.26*
14.Avoidance frustration regulation													--

Table 1.7. 7-month latent profiles of child ANS responsivity

	No. of parameters	AIC	BIC	Adj. BIC	Adj. <i>p</i> LMR LRT
1-class	6	1663.47	1685.33	1666.30	---
2-class	10	1625.34	1661.76	1630.05	.0242*
3-class	14	1605.67	1656.66	1612.27	.0003**
4-class	18	1597.17	1662.73	1605.65	.0046**
5-class	22	1588.13	1668.25	1598.49	.3477
6-class	26	1581.99	1676.68	1594.23	.0748

Note. AIC = Akaike information criterion; BIC = Bayesian information criterion; Adj. BIC = Sample-Size Adjusted BIC; Adj. LMR LRT = Adjusted Lo-Mendell-Rubin likelihood-ratio test. * $p < .05$, ** $p < .01$
n=282

Table 1.8. *15-month latent profiles of child ANS responsivity*

	No. of parameters	AIC	BIC	Adj. BIC	Adj. <i>p</i> LMR LRT
1-class	6	1933.07	1955.67	1936.63	---
2-class	10	1907.06	1944.71	1912.99	.2570
3-class	14	1883.40	1936.11	1891.71	.0017**
4-class	18	1874.07	1941.84	1884.75	.1926
5-class	22	1864.61	1947.44	1877.66	.2474
6-class	26	1863.60	1961.49	1879.03	.1829

Note. AIC = Akaike information criterion; BIC = Bayesian information criterion; Adj. BIC = Sample-Size Adjusted BIC; Adj. LMR LRT = Adjusted Lo-Mendell-Rubin likelihood-ratio test. * $p < .05$, ** $p < .01$
n=319

Table 1.9. 24-month latent profiles of child ANS responsivity

	No. of parameters	AIC	BIC	Adj. BIC	Adj. <i>p</i> LMR LRT
1-class	6	1879.15	1901.15	1882.13	---
2-class	10	1863.74	1900.40	1868.69	.1498
3-class	14	1852.72	1904.05	1859.66	.8068
4-class	18	1841.48	1907.47	1850.39	.0784
5-class	22	1839.31	1919.97	1850.21	.1453
6-class	26	1841.50	1936.83	1854.38	.3442

Note. AIC = Akaike information criterion; BIC = Bayesian information criterion; Adj. BIC = Sample-Size Adjusted BIC; Adj. LMR LRT = Adjusted Lo-Mendell-Rubin likelihood-ratio test. **p*<.05, ***p*<.01
n=289

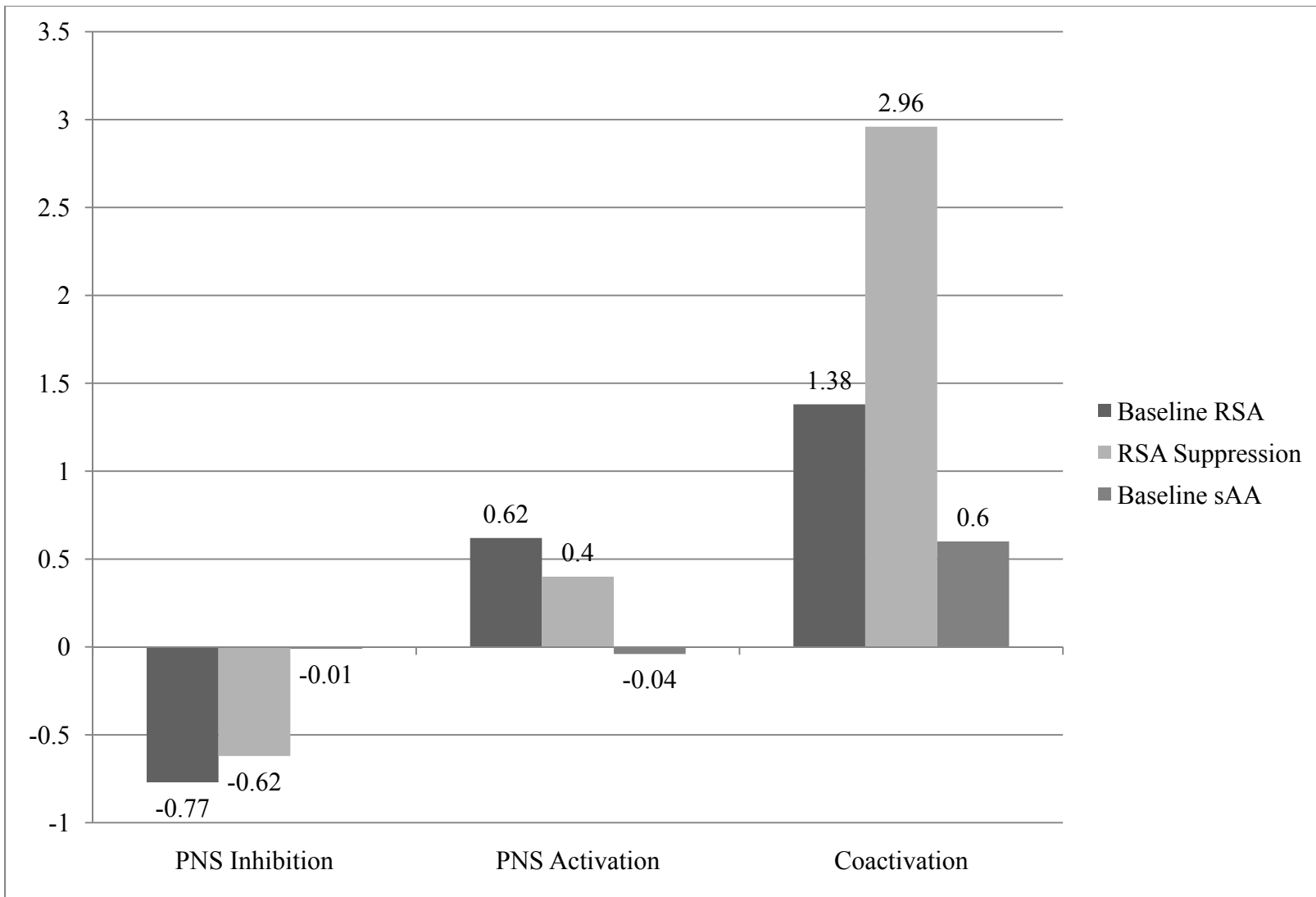


Figure 1.1 ANS profiles at 7-months of age

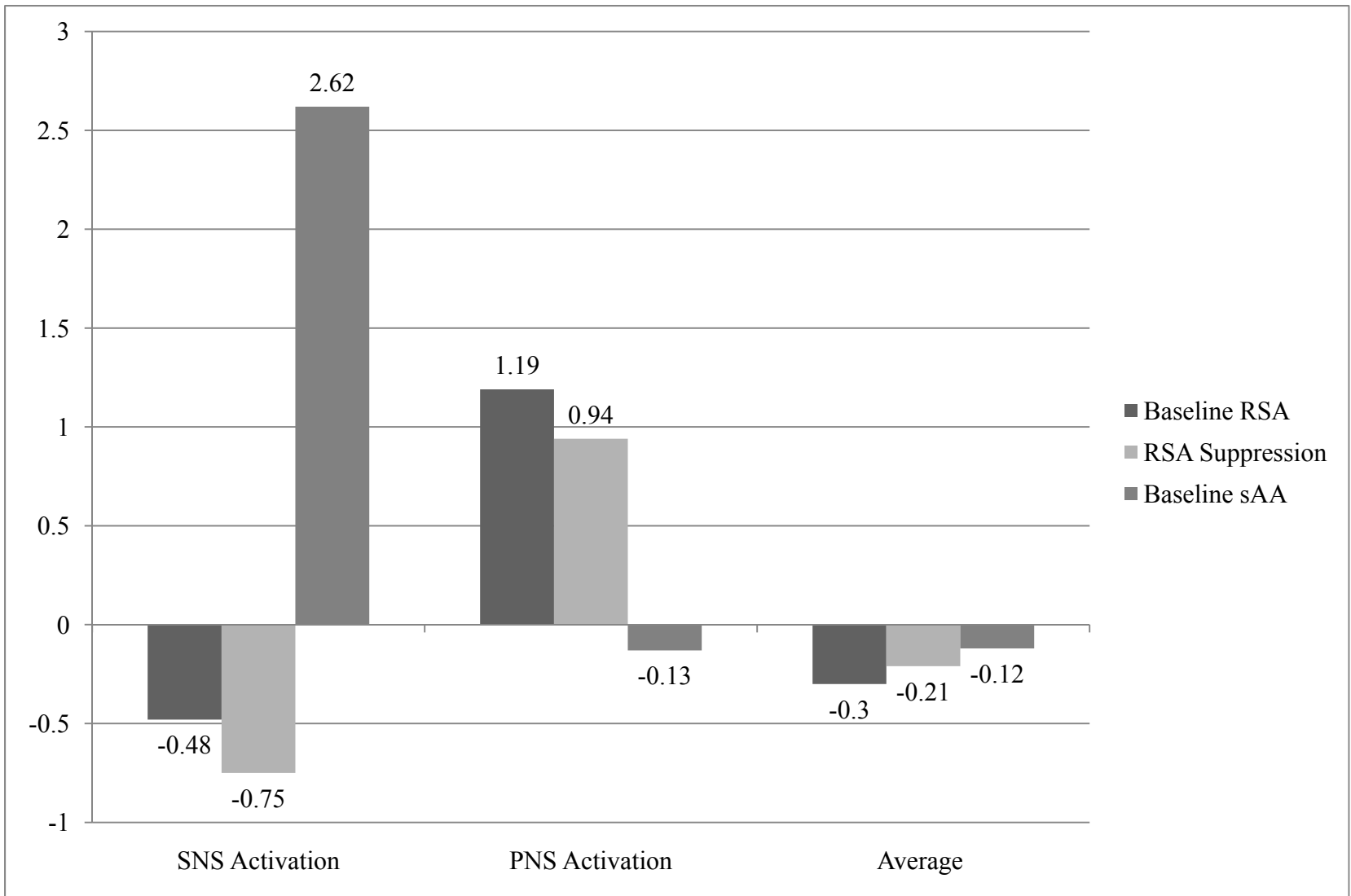


Figure 1.2 ANS profiles at 15-months of age

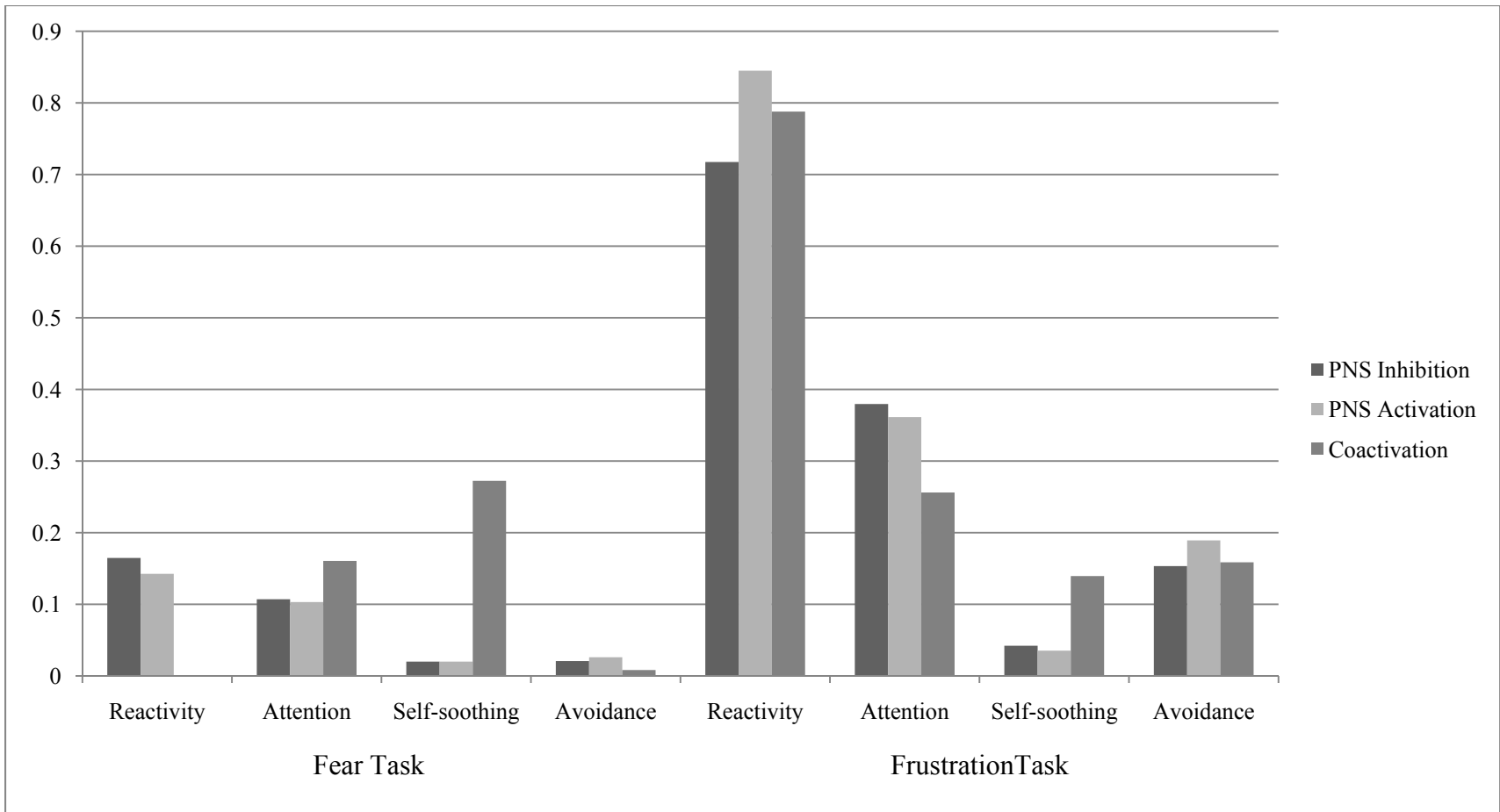


Figure 1.3 Mean differences in emotion negative reactivity and regulation behaviors by 7-month ANS profile

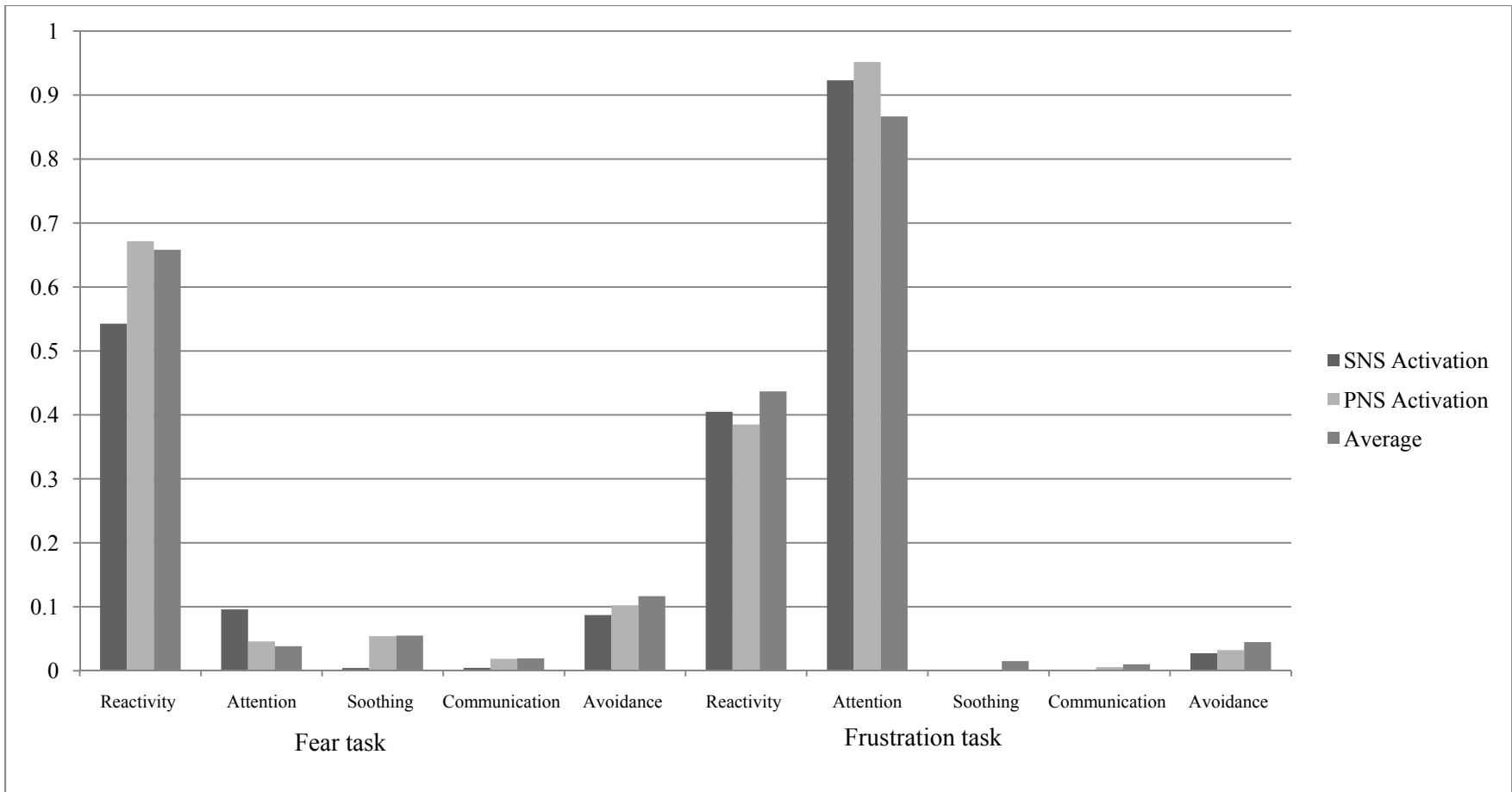


Figure 1.4. Mean differences in emotion negative reactivity and regulation behaviors by 15-month ANS profile

STUDY 2:

The Early Social Environment and Profiles of Infant ANS Activity across the First Two Years in Low-Income Families: Prediction of Behavior Problems in Early Childhood

Physiological arousal and regulation of stress is the product of an integrated network of autonomic responses, indexed by action of the ventral vagal complex (VVC) and sympathetic–adrenomedullary (SAM) system, designed to flexibly meet environmental demands, mobilizing resources to prepare for challenge and regulating visceral state to temper arousal (Boyce & Ellis, 2005). The VVC is a parasympathetic physiological signaling system for inhibiting arousal, promoting calm behavioral states, and engaging in regulatory and social affiliative behaviors (Porges, 1995, 2001, 2007). Individual differences in the functioning of the VVC may be quantified by respiratory sinus arrhythmia (RSA), a measure of heart rate variability (HRV) that varies as a function of environmental demands and occurs at approximately the frequency of spontaneous respiration. The VVC dynamically regulates cardiac output, withdrawing PNS input to rapidly increase cardiac output and promote short-term mobilization of metabolic resources resulting in decreased RSA or RSA suppression, or conversely, increasing PNS input to decrease arousal and support state regulation and calm resulting in RSA augmentation (Porges, 2007). The sympathetic–adrenomedullary (SAM) system guides excitatory processes involved in physiological arousal to stress (Gunnar & Davis, 2003; Chrousos & Gold, 1992). With advancements in the non-invasive collection and assessment of salivary analytes, individual differences in SAM activity can be measured by salivary alpha-amylase (sAA), an enzyme released by glands in the oral cavity (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007). Salivary AA shows a robust response

to physical and psychosocial stressors (10 min for peak sAA arousal; Chatterton et al., 1996; Gunnar & Davis, 2003).

Despite universality in the structure and phylogeny of this autonomic network, considerable individual differences exist in physiological responsivity across the VVC, and SAM system (Gunnar & Davis, 2003; Boyce & Ellis, 2005; Porges, 2007). While some individuals may mount vigorous autonomic responses to a stress perturbation, other individuals may display minimal response to the same event. Early in life, calibration of the response dynamics of the VVC and SAM system are, in part, shaped by early experience (Propper & Moore, 2006; Ellis & Boyce, 2008). These systems are functionally immature at birth, and during the critical periods of infancy and early childhood, exhibit heightened sensitivity to environmental influence (Beauchaine, Gatzke-Kopp, & Mead, 2007; Repetti, Taylor, & Saxbe, 2007). Thus, functioning of the VVC and SAM system provides a biological means for computing environmentally driven trade-offs in physiologic resources (Boyce & Ellis, 2005; Crespi & Denver, 2005).

Context of poverty

Adverse early environments, such as living in poverty, may exacerbate risk for physiological dysregulation, as the VVC and SAM systems may initiate internal changes to adapt accordingly in response to external demands (Hertzman, 1999; Repetti et al., 2007). The context of poverty is characterized by an accumulation of physical and psychosocial stressors, including substandard housing, noise, crowding, family instability and conflict, all of which are significant demands from the environment which augment physiological allostatic load (Evans & English, 2002; Evans, 2003; Evans & Kim, 2007). Allostatic load represents the wear and tear on the body, and the body's ability to withstand an excessive demand placed on the stress response systems (McEwen & Stellar, 1993). When chronic activation threatens homeostasis, allostatic

adjustments are made in attempt to achieve stability through change, particularly early in life when context-sensitive physiological systems are calibrated (McEwen & Stellar, 1993; McEwen, 1998). This continual effort to mount physiological resources may induce permanent alterations in set points of the VVC and SAM response systems (Lupien, King, Meaney, & McEwen, 2000; Evans & English, 2002; Levine, 2005; Evans & Kim, 2007). For example, Evans and English (2002) found elevated systolic blood pressure, diastolic blood pressure, and epinephrine, among low-income elementary-school-age children.

However, a primary mechanism accounting for the effects of poverty on physiological response dynamics is parenting, to the extent that if parent behaviors are statistically controlled, income has negligible effects on children's development, including their physiological responsivity to stress (McLoyd, 1998; Brody et al., 2002; Repetti et al., 2002). Given that parents, and especially mothers, are often the primary source of environmental input and figure prominently as external regulators of arousal in infancy (Kopp, 1982), it is plausible that they may set the tone early in life for the level of allostatic load placed on children's context-sensitive physiological systems. Further, in the context of poverty, maternal behavior, if sensitive and responsive, may serve as a buffer, or if harsh and intrusive, may serve as a conduit, for stress exposures in the home or neighborhood (Repetti et al., 2002).

Parenting and children's inhibitory physiological activity of the VVC

Past research has investigated the effects of maternal parenting behavior on children's VVC functioning, in particular the degree to which infants and young children suppress RSA in response to an environmental challenge. Greater RSA suppression in response to an environmental challenge is positively associated with higher baseline RSA, and indicates

flexibility to withdraw PNS influence on the heart, mobilizing cardiac output (Stifter & Corey, 2001; Porges, 2007). Findings show that maternal sensitivity and engagement is associated with greater RSA suppression, whereas negative control and intrusiveness is associated with less RSA suppression in infancy and toddlerhood (Calkins, Graziano, Berdan, Keane, & Degnan, 2008; Calkins, Smith, Gill, & Johnson, 1998; Haley & Stansbury, 2003; Porter, 2003; Propper & Moore, 2006). For example, in a study of 24-month old children and their mothers, Calkins and colleagues (1998) propose that mothers who use more controlling behavior in managing their children's actions may employ high levels of external regulation and limited parental modeling of effective regulation strategies, such that young children are less able to develop ways to flexibly regulate their physiological arousal and respond to environmental challenges.

Furthermore, a study examining mother-infant co-regulated communication patterns in 3-month old infants found that mothers who are more responsive to their infant's signals, showing a higher proportion of matched affect with their infant and synchrony transitioning between affective states, had infants with greater RSA suppression during a Still-Face paradigm (Moore & Calkins, 2004). Mother-infant dyads that were less synchronized during a normal interaction showed less RSA suppression in response to mother's still face. Taken together, the data suggest that maternal sensitivity, engagement, and attunement with children's emotional state may support better VVC regulation in children in response to an environmental challenge.

Parenting and children's excitatory physiological activity of the SAM system

Studies examining parenting and physiological activity of the SAM system have found that parenting behavior and stress in the family context impact the functioning of the context-sensitive SAM system (Repetti et al., 2002; Kivlighan et al., 2005; Mize, Lisonbee, & Granger,

2005; Evans & Kim, 2007). In a paper by Granger and colleagues (2006) establishing sAA as a viable marker of SAM functioning and reviewing findings from preliminary studies, Kivlighan and colleagues (2005) found evidence for attunement of maternal and infant sAA levels at 7-months of age, particularly for male infants, such that mothers who had a higher sAA level had sons with a similarly high sAA level. This analysis was conducted with a sample of children in the Family Life Project, of which the sample for the current study is also drawn. Further, Granger et al. (2006) presents another study by Mize and colleagues (2005) that investigated sAA levels in 4-year old children, and the association of sAA and the degree of closeness of the teacher-child relationship in a preschool setting. Salivary AA was negatively associated with degree of teacher-child closeness, such that children with high sAA levels had less close relationships with their preschool teachers. Although sAA is a relatively novel indicator of SNS activity, and no studies to date have directly examined how parenting behavior relates to sAA activity in young children, these preliminary investigations offer some evidence to suggest that sAA levels in early childhood are regulated by social relationships (Granger et al., 2006). Further, other indicators of SNS activity have been shown to be sensitive to the family context. For example, Evans and Kim (2007) found that children with higher levels of family stress showed elevated SNS activity, indexed by greater systolic blood pressure. Taken together, it is plausible that parenting behavior may impact the development of sAA early in life (Granger et al., 2006).

RSA, sAA, and child behavior problems

Research has shown that higher baseline RSA and greater RSA suppression is related to better emotional state regulation in infancy (Stifter & Corey, 2001), as well as, fewer behavior

problems in early childhood (Porges, Doussard-Roosevelt, Portales, & Greenspan, 1996; Calkins, 1997; Calkins & Keane, 2004). Generally, although baseline RSA and RSA suppression are positively correlated, studies looking at the association of RSA and behavior problems typically examine RSA suppression to a task in relation to behavior as opposed to basal levels of RSA. For example, Calkins and Dedmon (2000) investigated RSA suppression in a sample of children at high risk for aggressive behavior problems at age 2 years. At-risk children showed significantly lower RSA suppression, or less VVC withdrawal, during a series of challenging tasks than controls. In a separate investigation by Calkins and colleagues (2007) of 5-year old children at risk for externalizing, internalizing, or mixed problems a trend for children at risk for externalizing problems to display less RSA suppression as compared to control children was found. In addition, children at risk for mixed problems, both internalizing and externalizing, actually showed exaggerated RSA suppression, hypothesized to represent excessive physiological regulation. In a longitudinal investigation of RSA stability from 2 to 4.5 years, it was shown that children who displayed greater RSA suppression across the early childhood period had fewer externalizing behavior problems than other children (Calkins & Keane, 2004).

A few studies have examined the relationship between sAA and behavior problems. El-Sheikh and colleagues (2005) found positive associations between sAA and externalizing behavior problems, such as aggression, in middle childhood. In addition, girls with higher sAA increases in response to the stressor also showed greater social problems. More recently, Keller and El-Sheikh (2009) examined the prediction of externalizing problems by sAA, and the moderation of RSA on this association. Findings showed a significant, non-linear association between sAA and externalizing problems in 8 and 10-year olds such that both low and high

levels of sAA were associated with greater externalizing problems. A moderate sAA level was optimal, and associated with fewer externalizing problems. Further, RSA moderated this association such that lower externalizing problems were predicted by low sAA coupled with greater RSA suppression. Although no study to date has examined sAA-behavior problem associations in early childhood, there is evidence to suggest that sAA functioning may predict behavior at younger ages, and the moderation findings by Keller et al. (2009) suggest that it is important to understand VVC and SAM functioning in the investigation of association between physiological activity and problem behavior.

The present study

Response dynamics of the VVC and SAM system may be calibrated during infancy and early childhood when these systems exhibit heightened sensitivity to environmental influence (Boyce & Ellis, 2005; Beauchaine, Gatzke-Kopp, & Mead, 2007; Repetti, Taylor, & Saxbe, 2007). There is evidence that low-income early environments are associated with an accumulation of ecological stress exposures (i.e. single parenthood, higher rates of unemployment, less education, limited social support, etc.) which tax physiological functioning, possibly potentiating prolonged or exaggerated physiological arousal (Aber, Jones, & Cohen, 2000; Evans & English, 2002; Raver, 2004). Further, little is known about low-income environments in non-urban areas, but it is plausible that geographic isolation associated with non-urban areas may serve as an additional ecological risk. However, the effects of income, social risk, and isolation on children's physiological functioning may be accounted by parental factors, to the extent that income has been found to have negligible effects on child development after parenting behavior has been added to the model (McLoyd, 1998; Brody et al., 2002; Blair et al., 2008). Early in life, mothers are often the primary source of environmental input (Kopp,

1982). Thus, maternal parenting behavior may be an important determinant of allostatic load on children's physiological systems, either acting as a buffer, or conduit for stress exposures present in the distal context. Past research suggests that maternal engagement and control impacts responsivity of single physiological systems (e.g., Calkins et al., 1998). Further, physiological responsivity across these systems is associated with the capacity to regulate emotional arousal at the behavioral level (Calkins, 1997; Huffman et al., 1998; Stifter & Corey, 2001; Repetti, Taylor, & Seeman, 2002). However, no study to date has considered how the early environment predicts interrelated functioning across multiple physiological systems which are developing over time. This is a significant gap in the literature as functioning of the VVC and SAM systems do not operate in isolation, but rather, these physiological systems form an integrated autonomic network, exerting coordinated responding in accordance with environmental demands (Bauer, Quas, & Boyce, 2002; El-Sheikh et al., 2009).

To understand developmental patterns of physiological functioning over time among the VVC and SAM systems that predict early behavior problems, a prospective longitudinal investigation is essential. The present study will explore the application of latent profile analysis to classify systematic variation in the nature and course of multisystem physiological functioning across 7-, 15-, and 24-months of age, and to assess whether these classifications are associated with behavior problems at 3-years. Further, based on past findings which suggest physiological sensitivity to context early in life, to better specify profiles in the latent mixture models, demographic risk factors of income, geographic isolation, and social advantage, as well as, maternal parenting behavior will be incorporated as predictors of profile membership.

Method

Participants

Recruitment. The sample will be drawn from the Family Life Project (FLP), a study of 1,292 children and their families living in the Black South (eastern North Carolina; Sampson, Wayne, and Wilson counties) and Appalachia (central Pennsylvania; Blair, Cambria, and Huntingdon counties) — two geographical regions of high child poverty. During a 1-year recruitment period, mothers were approached in the hospital after giving birth and asked to participate. Eligibility criteria included English-speaking families, residing in one of the target counties, with no intent to relocate from the area in the next 3 years. A cohort sampling method was used to over sample for low-income families in both states and, specifically in North Carolina, African-American families. African-American families were not over-sampled in Pennsylvania as the target communities were almost exclusively Caucasian ($\geq 95\%$). Over two-thirds of the FLP sample lives below 200% of the poverty line, and approximately 40% are African-American.

For this analysis, the sample is restricted to a random sub-sample of children from whom electrocardiogram (ECG) heart period data was collected ($N=309$, 50% female, 34% African-American). Families were visited in the home when children were at 7-, 15-, 24-, and 35-months of age. The age range for the 7 month visit ranged from 5 to 10 months ($M = 7.2$ months), 13 to 19 months for the 15 month visit ($M = 15.7$ months), 22 to 28 months for the 24 month visit ($M = 24.4$ months), and 34 to 40 months for the 35 month visit ($M = 36.7$ months). Due to the range in age at each of the visits, age-related differences will be controlled for in all analyses. Within this sub-sample, 66% had an income-to-needs ratio less than 200% of the poverty line. Forty-eight percent of the families resided in Pennsylvania, and 52% resided in North Carolina. Most

of the mothers (76%) had completed a high-school degree or equivalent and 56% were married (see Table 2.1).

Procedure

During the 7-month home visit, mothers completed questionnaires and interviews on family demographics and social risk factors. At the 7-, 15-, and 24-month visits mothers engaged in a 10 min free play with their child, which was later coded for parenting behavior. Baseline physiological measurements (i.e., ECG, saliva) were collected from children. At the 35-month visit, mothers rated their children's early problem behaviors.

Measures

Demographic and social advantage measures

Income-to-need ratio. At the 7-, 15-, and 24-month visits, mothers reported on income from all sources and any income from other household members. This figure was used as an estimate of total household income and divided by the federal poverty threshold for the current year (2004, 2005) adjusted for number of persons in the home to compute an income-to-need ratio. An income-to-need ratio of 2.00 or below indicates that the family is low-income and 1.00 or below indicates that the family is living in poverty by federal guidelines, adjusted for family size. The mean income-to-needs ratio across the three assessment points was created for data analysis.

Geographic isolation. To index degree of geographic isolation in these rural communities, address mapping using Global Positioning System (GPS) technology determined the longitude and latitude for the family residence at the 7-, 15-, and 24-month visits. This was

used to compute the distance in meters from the family's home to a variety of educational, financial, and social resources including: the nearest gas station, doctor's office, public library, fire station, elementary school, high school, public park, supermarket, county seat, and freeway ramp. The log transformed mean distance to resources was formed to index degree of geographic isolation. The mean geographic isolation across the three assessment points was created for data analysis.

Social advantage. At the 7-, 15-, and 24-month visit, mothers completed questionnaires and interviews on family level social factors. Developed by Blair et al. (2008) in a previous investigation with data from the Family Life Project, an index of social advantage was created by standardizing and summing six variables: maternal age, years of education, marital status, employment status, economic sufficiency measured using the Conger and Elder (1994) questionnaire, and global rating of social support measured using the Questionnaire of Social Support as adapted by Crnic, Greenberg, Robinson, and Ragozin (1984). The mean social advantage across the three assessment points was created for data analysis.

Maternal Parenting Behavior

Observed parenting during free-play interaction. At the 7-, 15-, and 24-month home visit, mothers were videotaped while engaging in a 10 min free-play with their infant (Cox, Paley, Burchinal, & Payne, 1999). Each time, mothers were given a set of developmentally appropriate toys, and asked to play with their infant as they normally would if they had free time at home. Mother-child interactions were coded to assess levels of mothers' sensitivity, detachment, intrusiveness, positive regard, negative regard, and animation in interacting with the

child. Each dimension of maternal behavior was rated across the duration of the 10 min interaction on a 5-point Likert scale ranging from 1 (*not at all characteristic*) to 5 (*highly characteristic*) in a single global rating. Based on the results of factor analyses conducted with an oblique rotation (i.e., Promax), two parenting factors emerged: maternal positive engagement and maternal intrusiveness. Maternal positive engagement ($\alpha = .89$) was comprised of four maternal ratings: detachment (level of emotional involvement; reverse scored), positive regard (positive feelings expressed toward child), animation (level of energy), and stimulation for development (appropriate level of scaffolding of activities with child). Maternal intrusiveness ($\alpha = .69$) was comprised of three maternal ratings: sensitivity (level of responsiveness to child's needs, gestures, and expressions; reverse scored), intrusiveness (degree to which the mother ignored child's signals, and imposed her own agenda on the interaction), and negative regard (level of harsh, negative feelings expressed toward child). Reliability was determined by calculating the intraclass correlation coefficient for ratings made by two coders to approximately 30% of the tapes randomly drawn at each assessment point. Reliability was acceptable for both intrusiveness ($r = .88$) and engagement ($r = .80$).

Physiological measures

Respiratory sinus arrhythmia. To assess RSA, infant electrocardiograms (ECG) were recorded using a Grass preamplifier, and this output was transmitted to laptop computer in the form of a data file containing the interbeat intervals for the collection period, during a 5 minute baseline period when children were sitting quietly. All ECG heart period data was analyzed off-line using Mindware Technologies. From electrocardiograms (ECG) recorded in the home when children were approximately 7-, 15-, and 24-months of age, Mindware Technologies software

(Gahanna, Ohio) was used to apply an algorithm to the time series of heart period data. The algorithm uses a moving polynomial to de-trend periodicities in heart period that are slower than RSA. A band-pass filter then extracts the heart rate variability within the frequency band of spontaneous respiration in young children, .24 to 1.04 Hz, which is commonly studied to index vagal functioning in infants and young children (see Stifter & Fox, 1990; Porges et al., 1996; Huffman et al., 1998; Stifter & Corey, 2001; Calkins & Keane, 2004). The software then derives a RSA estimate by calculating the natural log of specified heart period variability and is reported in units of $\ln(\text{ms})^2$. Trained coders edited the ECG heart period records for movement artifact where possible, marking the R spike if identifiable on the record. The mean RSA across the baseline minutes is used to index *baseline RSA*.

Salivary alpha-amylase. Saliva samples collection during the visit, prior to the administration of a series of emotionally challenging tasks, were assayed for salivary α -amylase using a kinetic reaction. A chromagenic substrate, 2-chloro-p-nitrophenol linked with maltotriose, was added to saliva samples to trigger the enzymatic action of α -amylase indexed by the degradation of maltotriose. The amount of α -amylase activity present in the sample is directly proportional to the increase (over a 2- min period) in absorbance (optical density) at 405 nm (Salimetrics, State College, Pennsylvania). The test uses only 10 μl of saliva (for singlet determinations), with an absolute range from 3.1 - 423.1 U/mL. The lower limit of sensitivity is governed by the change in absorbance. A change in absorbance less than 0.01 does not result in a reliable value. In this case, samples were rerun at a higher concentration. The salivary α -amylase distributions were square root transformed prior to analysis to correct for positive skew.

Behavioral measure

Early Behavior Problems. To assess children's early problem behaviors at 35-months of age, mothers' completed the Strengths and Difficulties Questionnaire (SDQ; Goodman, 1997). The SDQ has been shown to demonstrate predictive validity for later indices of internalizing and externalizing problems, as well as problems with inattention and hyperactivity (Goodman & Scott, 1999). Four scales index early problem behaviors. The emotional symptoms scale consists of 5 items (e.g., "Often unhappy, depressed or tearful", "Nervous or clingy in new situations"; $\alpha = .65$). The conduct problems scale consists of 5 items (e.g., "Often fights with other children or bullies them", "Often argumentative with adults"; $\alpha = .66$). The hyperactivity scale consists of 5 items (e.g., "Restless, cannot stay still for long", "Easily distracted, concentration wanders"; $\alpha = .68$). The peer problems scale also consists of 5 items (e.g., "Rather solitary, prefers to play alone", "Picked on or bullied by other children"; $\alpha = .5$). Items are rated from 0 ("Not true") to 2 ("Certainly true"). A mean summary score of total problem behaviors has acceptable reliability in the current sample ($\alpha = .8$).

Missing data. ECG data was collected on a subsample of children participating in the Family Life Project at 7-, 15-, and 24-months ($n=358$). Given the variation in age at which children were assessed, the analysis restricted the sample to children between 5 and 10 months for the 7-month assessment, 13 and 19 months for the 15-month assessment, and 22 and 28 months for the 24-month assessment. This resulted in the exclusion of 10 children older than 10 months of age at the 7-month assessment, 16 children older than 19 months of age at the 15-month assessment, and 23 children older than 28 months at the 24-month assessment. The remaining sample of 309 children within the specified age windows across the three

measurement occasions was used in data analysis. Of these 309, all had cortisol data and 8 were missing sAA data on at least one of the three measurement occasion. Listwise deletion typically induces bias in parameter estimates by eliminating non-random cases and reducing error variance. To handle missing data in the present sample, a maximum likelihood estimation application in the Mplus software used to conduct latent profile analysis was implemented. The inclusion of repeated physiological indicator measurement at 7-, 15- and 24- months bolstered ML estimation given the correlation of the RSA and sAA indicators over time.

Plan of analysis

To detect developmental patterns of multisystem physiological functioning across the first two years, latent profile analysis (LPA) was employed to examine profiles, or person-oriented subgroups, of infant autonomic functioning at the 7-, 15-, and 24-month assessment points. Due to the fact that only 3 measurement occasions were included in the current analysis, growth trajectories could not be estimated. As such, classes are referred to as *longitudinal profiles* rather than *trajectories* throughout the paper to indicate that classes are compared on the basis of mean levels across the host of ANS indicators over time and not by growth factors.

To determine the optimal number of profiles that best fit the current data, a model with 2-6 classes was fit, with the goal of identifying mutually exclusive and exhaustive subgroups of children with similar physiological profiles, controlling for age at the time of assessment, and for salivary indicators, time of day of saliva collection. Model goodness of fit was evaluated with the following fit indices: Akaike information criterion (AIC), Bayesian information criterion (BIC), Sample-Size Adjusted BIC, and the *adjusted Lo-Mendell-Rubin likelihood ratio test (LMR-LRT)*. The model with the smallest BIC values, and a significant LMR-LRT test, indicating that the

addition of one more profile significantly improves model fit, was selected at each assessment point. Although, similar to exploratory factor analysis, in addition to the fit indices, theoretical justification, model parsimony and interpretability were considered in model selection (Bauer & Curran, 2003; Muthén, 2004; Jung & Wickrama, 2008). Following the selection of the optimal profile solution, the model was replicated with a minimum of 100 random starting values to test the robustness of model convergence.

Next, observed variables that may define the longitudinal ANS profile solution were added to the selected LPA model in a step-wise manner. Variables that did not predict profile membership were excluded in subsequent tests. First, child sex and race was entered as covariates. Second, social environment variables including income-to-needs ratio, geographic isolation, and social advantage were added. Third, maternal parenting behavior, including maternal positive engagement and maternal intrusiveness, was added to the model. Variables that are non-significant were removed from subsequent models. Further, to assess whether the longitudinal profiles of child physiological functioning were associated with behavior problems at 3-years, class membership posterior probabilities were exported from the selected optimal class solution containing covariates. In a separate general linear model, behavior problem scales were regressed on class membership.

Results

Mean values for the social environment variables, child RSA and sAA levels, 2-year emotion reactivity and regulation behavior, and 3-year problem behaviors are presented in Table 2.2. In line with past research, mean RSA increased with age across the 3 measurement occasions indexed during the infancy and toddler developmental periods (Porter, Bryan, & Hsu,

1995; Stifter & Jain, 1996). Paired t-tests showed a significant increase in RSA from 7- to 15-months ($t = 5.36, p < .01$), and 15- to 24-months of age ($t = 4.39, p < .01$). Although sAA levels were not significantly correlated to variation in child age at the time of the visit, sAA levels also increased across the 3 measurement occasions. Paired t-tests showed a significant increase in sAA from 7- to 15-months ($t = 3.95, p < .01$), and 15- to 24-months of age ($t = 3.01, p < .01$).

Prior to conducting any further analysis of the data, mean composites were created for each of the social environment variables. The income-to-needs ratio ($r = .73-.79$), geographic isolation ($r = .87-.95$), index of social advantage ($r = .82-.86$), maternal engagement parenting behavior ($r = .49-.64$), and maternal intrusiveness parenting behavior ($r = .39-.43$) exhibited considerable stability across the three measurement occasions. In addition, given that RSA and sAA increased with child age in the current study, the bivariate correlations between child age, RSA, and sAA were examined. As expected, child age was positively associated with RSA at 7- and 24-months of age ($r = .24, p < .01$; $r = .16, p < .01$). Child age at the time of the visit was not related to sAA levels. In addition, past investigations of sAA activity indicate that sAA exhibits a circadian rhythm, characterized by a continuous increase over the course of a day (Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2005). Consequently, the bivariate correlations between time of day the saliva sample was collected and sAA were examined. Time of day was positively associated with 15-month sAA levels ($r = .17, p < .01$). Based on past research and the significant association found with the current sample at 15-months of age, the effect of child age was regressed out of RSA and the effect of time of day was regressed out of sAA level at each measurement occasion.

Table 2.3 presents the bivariate correlations between the study variables, using mean composites of the social environment variables, and controlling for the effect of child age on

RSA and the effect of time of day on pre-task sAA levels. RSA at 15- and 24-months of age was negatively associated with income-to-needs ratio and social advantage, such that greater income and social advantage were significantly related to lower child RSA. Salivary AA level at 24-months of age was positively associated to maternal engagement, such that increasing displays of engaged parenting behavior were related to greater sAA level. Further, sAA level at 24-months was negatively associated with maternal intrusiveness, such that increasing displays of intrusive parenting behavior were related to lower sAA level. There were no significant bivariate associations between RSA or sAA level and 3-year behavior problems.

Longitudinal Latent Profile Mixture Models of Child ANS Activity

Latent profile models with two through six profiles were fit with the 7-, 15-, and 24-month physiological indicators to determine the optimal number of profiles that best described ANS activity in the current sample. Model goodness of fit was evaluated using the Bayesian information criterion (BIC), where a smaller value represents a better fit, and the adjusted Lo-Mendell-Rubin likelihood ratio test (Adj. LMR-LRT), which tests the significance of the difference between a model with k and $k-1$ classes, correcting for the distribution of the likelihood-ratio statistic (Lo, Mendell, & Rubin, 2001). Fit indices for all models are reported in Table 2.4. The four profile model was selected as optimal for these data based on having the smallest BIC value of the models that also had a significant adjusted LMR-LRT statistic. In addition, the four profile model solution derived profiles with a sufficient number of individuals classified as being members of a given profile (e.g., no profiles with $< 5\%$ of the sample) and mutually exclusive profiles on a conceptual level. Further, the average latent profiles probabilities for classification were acceptable ($range = .80-.97$).

Figure 2.1 and 2.2 present the mean physiological indicator estimates for the four profile solution for 7-, 15-, and 24-month ANS activity. PNS patterns of RSA (see Figure 2.1), and SNS patterns of sAA (see Figure 2.2) across the three measurement occasions are presented in separate figures so as to generate a relative comparison of indicator level to the sample mean value. To indicate overall profile classification based on indicators shown in Figures 2.1 and 2.2, the naming convention and marker shape are identical for both figures. In the four profile longitudinal model, the first profile representing 43% of the sample, comprised children who exhibited average initial RSA and average increase in RSA over time. Children in Profile 1 also exhibited average initial sAA level, an average increase in sAA from 7- to 15-months, and a greater than average increase in sAA from 15- to 24-months. The second profile, representing 20% of the sample, comprised children who exhibited average initial RSA, a greater than average increase in RSA from 7- to 15-months, and a lower than average increase in RSA from 15- to 24-months. Children in Profile 2 also exhibited average initial sAA level, an average increase in sAA from 7- to 15-months, and a lower than average, minimal increase in sAA from 15- to 24-months. The third profile, representing 30% of the sample, comprised children who exhibited average initial RSA, an average increase in RSA from 7- to 15-months, and a lower than average increase in RSA from 15- to 24-months. Children in Profile 3 also exhibited average initial sAA level, an average increase in sAA over time. The fourth profile, representing 7% of the sample, comprised children who exhibited average initial RSA, a lower than average increase in RSA from 7- to 15-months, and a greater than average, steep increase in RSA from 15- to 24-months. Children in the Profile 4 exhibited lower than average sAA level, and an average increase in sAA over time.

Prediction of Membership in Longitudinal ANS Profiles: Demographic and Social Environment Variables

Next, the prediction of membership in the longitudinal profiles by selected demographic and social environment variables were tested. Child sex, race, income-to-needs ratio, and degree of geographic isolation were not significantly related to profile membership. However, as seen in Table 2.5, mean social advantage did significantly predict profile membership, such that for every 1 unit increase in social advantage the odds of being in Profile 2 decreased 2.20-fold, the odds of being in Profile 3 decreased 2.15-fold, and the odds of being in Profile 4 decreased 4.35-fold relative to Profile 1. Therefore, children with higher social advantage had greater odds of exhibiting the longitudinal RSA and sAA pattern of Profile 1, where both RSA and sAA showed an average increasing pattern over time. Conversely, children with less social advantage had greater odds of being classified in Profile 2, 3, or 4, which each exhibit some physiological deviation from the average increasing Profile 1 pattern. Neither the addition of maternal engagement nor maternal intrusiveness parenting behavior was significant in predicting profile membership after accounting for the significant effect of social advantage (see Table 2.5). Post-hoc comparisons of the least-square means for parenting behaviors corroborated this finding. Although there were significant differences in maternal engagement behavior, such that mothers of children in Profile 1 displayed more engagement behavior than mothers of children in Profile 2 ($t = 3.07, p < .05$), this difference was non-significant after including the effect of social advantage. The maternal intrusiveness least-square means difference only approached significance for the comparison of Profile 1 and 2, where mothers of children in Profile 1 displayed less intrusive behavior than mothers of children in Profile 2 ($t = -2.60, p = .05$). However, similarly, this difference was non-significant after accounting for social advantage.

Prediction of 3-Year Behavior Problems by Longitudinal ANS Profile

To test the prediction of 3-year problem behaviors as rated by parents on the SDQ by longitudinal ANS profile, the emotional symptoms subscale, the conduct problems subscale, the hyperactivity subscale, the peer problems subscale, and the total problems composite were regressed on profile membership. Children in Profile 2 were rated as having significantly more emotional symptoms in comparison to children in Profile 1 ($t = 3.78, p < .01$), children in Profile 3 ($t = 2.57, p < .05$), and children in Profile 4 ($t = 2.73, p < .01$). In addition, children in Profile 2 were rated as having significantly more overall behavior problems in comparison to children in Profile 1 ($t = 2.98, p < .05$), and children in Profile 4 ($t = 3.51, p < .01$). Further, children in Profile 3 were rated as having significantly more overall behavior problems in comparison to children in Profile 4 ($t = 2.67, p < .05$).

Discussion

The central goal of the current study was to classify systematic variation in the longitudinal pattern of child autonomic nervous system development, using RSA as an indicator of parasympathetic nervous system activity and sAA level as an indicator of sympathetic nervous system activity, across 3 measurement occasions, when children were 7-, 15-, and 24-months of age. The second goal was to examine how several demographic and social environment variables predicted membership status within the optimal profile solution. Finally, the third goal was to utilize class membership as a predictor of problem behaviors at 3-years of age. The model containing 4 longitudinal profiles was identified as optimal to describe mutually exclusive and exhaustive ANS patterns of development across 7-, 15-, and 24-months of age in the current sample. The latent profile analytic approach is advantageous as it supports the examination of

subpopulations with distinct developmental patterns of combined PNS and SNS activity. Importantly, at the level of a single physiological system, or measurement occasion, bivariate associations between physiology and behavior in the present study were non-significant (see Table 2.3).

In examining possible predictors of longitudinal profile membership, the current findings highlight the importance of early social environment for children's physiological development. Specifically, greater social advantage was associated with increased odds of classification in Profile 1 versus all others (see Table 2.5). Profile 1 represented 43% of the sample, and was characterized by a steady increase in both RSA and sAA which was above the sample average increasing pattern over time. In contrast, Profile 2, 3, and 4 all showed some deviation from the average developmental pattern between 15- and 24-months of age. Profile 2 tracked at an average increase from 7- to 15-months of age for RSA and sAA but later diverged from the average sAA pattern, showing minimal increase in sAA from 15- to 24-months. Profile 3 showed a similar average increasing pattern from 7- to 15- months but diverged from the average RSA pattern, showing less increase in RSA from 15- to 24-months. Profile 4 had lower initial RSA and sAA values, showed average increase in sAA but tracked at a lower level than average at all measurement occasions, and showed an average RSA increasing pattern from 7- to 15-months and a greater than average increase in RSA from 15- to 24-months. Further, accounting for the effect of social advantage, maternal engagement and intrusive parenting behavior did not predict membership in the longitudinal profiles. Taken together, these findings suggest that the early social environment does impact children's ANS development over the first 2-years of life. Children of mothers who have less social advantage, indexed by maternal age, years of education, marital status, employment status, economic sufficiency, and social support may

experience a greater number of stressors through, for example, being unemployed and having difficulty making ends meet, or not having support of a marital partner, or perceiving limited social support. In turn, these social factors may influence mothers' parenting behavior towards their young child. To this end, there was a significant positive association between social advantage and maternal positive engagement parenting behavior, and significant negative association between social advantage and maternal intrusiveness parenting behavior. These associations may explain, in part, why maternal parenting behavior was not a significant predictor of profile membership, when controlling for the impact of social advantage. However, this finding contradicts the expectation from prior work that parenting behavior in the proximal, caregiving environment may be the mechanism through which distal social advantage impacts children's ANS development (Brody et al., 2002; Repetti et al., 2002). Past research shows that the effects of social advantage, and income level in particular, have minimal effect on child outcomes after parenting has been entered into the model (McLoyd, 1998;). Reasons for why the effects of social advantage remained significant with the addition of maternal engagement and intrusiveness behavior in the current sample may be related to the level of information garnered from the composite variables used in analysis. The social advantage index was highly correlated over time ($r = .82-.86$), and thus may have yielded more information about stable features of the social environment across 7- to 24-months of age. In comparison, the intercorrelation for maternal positive engagement behaviors ($r = .49-.64$) and intrusive behaviors ($r = .39-.43$) over time were moderate, indicating less stability. Therefore, it is possible that the maternal parenting composite variables provided less information, and thus, predictive power for classifying children to longitudinal ANS profiles.

The current findings also demonstrate the advantage of looking at ANS development across multiple interacting physiological systems indexing PNS and SNS activity. At the bivariate level, RSA and sAA were not associated with 3-year problem behavior at each measurement occasion (see Table 2.3). When these indicators are used to create 4 longitudinal profiles representing distinct physiological activity patterns across repeated RSA and sAA indicators, differences in 3-year problem behaviors by physiological profile emerged. In particular, children in Profile 2 presented more emotional symptoms in comparison to children in Profile 1, 3, and 4. Further, children in Profile 2 presented more overall behavior problems than children in Profile 1 and 4. And, children in Profile 3 also presented more overall behavior problems compared to children in Profile 4. From these findings, it appears that children classified as being members of Profile 2, characterized by increasing RSA in accord with sample average values, and increasing sAA from 7- to 15-months in accord with sample average values, but a deviation in this pattern where only a minimal increase in sAA is shown from 15- to 24-months, are rated as exhibiting the most problem behaviors at 3-years of age. Thus, in terms of physiological activity at the final measurement occasion, these children have average baseline RSA and below-average sAA level. This, in combination with the altered developmental pattern of sAA from 15- to 24-months may support the conclusion that lower levels of sAA, and sAA development predicts more behavior problems in the context of average RSA. Here, it is interesting to draw the comparison of Profile 4, who also has below-average sAA levels at 24-months. Children in Profile 4 have fewer behavior problems than children in Profile 2. This may be better understood in light of the differing RSA development patterns. Although children in Profile 4 have initial RSA values lower than the sample mean, they experienced a rapid increase in RSA from 15- to 24-months. At the 24-month assessment, children in Profile 4 have

the highest mean RSA value. Therefore, for Profile 4, high RSA may buffer the association of low sAA and 3-year behavior problems. Indeed, previous work of Keller and El-Sheikh (2009) has found evidence to suggest that RSA may moderate the relationship between sAA levels and externalizing problems in childhood. It is also of interest to compare patterns of ANS development between Profiles 3 and 4, where children in Profile 3 showed more overall behavior problems than children in Profile 4. Children in Profile 3 had average initial values for both RSA and sAA, and showed an increasing sAA pattern tracking just above the sample mean. However, their RSA pattern increased less than the sample mean, and by the last measurement occasion, they had the lowest RSA values.

Although the current findings offer insight to the development of ANS activity across PNS and SNS systems, how the early social environment impacts ANS development, and how longitudinal profiles of ANS activity may predict later behavior problems, there are several limitations of the current study that should be noted. First, the generalizability of these findings is limited to low-income, non-urban families as these variables characterized the current sample drawn from the Family Life Project. Second, the longitudinal profiles derived in the current analysis used baseline or pre-task physiology indicators. Future studies may benefit from additional indicators capturing change in ANS arousal in relation to a specific psychosocial challenge. Related to this, although both baseline RSA and sAA were measured during the course of a single home visit, they were not measured at the same time. To fully appreciate the interactive effect of PNS and SNS, it would be necessary to have a contemporaneous assessment of RSA and sAA activity. Third, the current analysis is limited in only having 3 measurement occasions, which does not support a test of latent growth modeling. A minimum of four measurement occasions are recommended for growth models to have sufficient model power and

flexibility to free selected model parameters (Muthén, 2004). Future studies including more occasions may statistically test the difference in trajectory slopes between the profiles, an analysis not supported within the latent profile modeling technique used in the current study. Fourth, and related to the number of measurement occasions, maternal parenting behavior was averaged across the three occasions to form a composite variable for the current analysis. Although there was a moderate positive association within measures of positive engagement and intrusiveness across the three occasions, it may be of interest in future studies employing a growth mixture model to utilize maternal parenting behavior as a time-varying predictor of ANS development. It is likely that parenting behaviors are not entirely static over time, and change with children's increased capacity to reciprocate, communicate, and actively participate in social interaction from infancy to toddlerhood. Thus, parenting behavior at individual measurement occasions may provide more detailed information, and predict shifts in ANS development over time.

In sum, the current findings highlight the importance of examining patterns of both PNS and SNS development in prediction of early behavior problems and provide some evidence for physiological sensitivity to the early social environment. Across the four longitudinal ANS profiles, there appears to be an overall increasing pattern for RSA and sAA from 7- to 15-months, but differentiation in the developmental pattern for RSA and sAA from 15- to 24-months. Future research may extend the current findings by including more measurement occasions and time-varying predictors to better understand trajectories of child ANS activity.

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Table 2.1
Sample Characteristics

Variable	%	<i>M (SD)</i>
Child sex (Female)	50	
Child age – 7m visit		7.15 (0.97)
Child age – 15m visit		15.74 (0.91)
Child age – 24m visit		24.44 (1.23)
Child age – 35m visit		36.72 (1.10)
Mother married	56	
Mother employed	49	
Maternal race (African-American)	32	
Maternal age		26.3 (6.13)
Maternal education		14.3 (2.98)

Table 2.2
Descriptive statistics for study variables

<i>Measure</i>	<u>7 months</u>		<u>15 months</u>		<u>24 months</u>		<u>35 months</u>	
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
Income-to-needs ratio	1.78	1.47	1.81	1.64	1.71	1.51	--	--
Geographic isolation	6.14	4.19	6.00	4.09	6.06	4.07	--	--
Index of social advantage	0	.61	0	.60	0	.57	--	--
Maternal engagement	3.05	0.83	2.86	0.82	2.90	0.85	--	--
Maternal intrusiveness	2.67	0.71	2.61	0.70	2.71	0.87	--	--
Child physiology								
Baseline RSA	3.87	0.88	4.37	1.05	4.96	1.20	--	--
sAA pre-task	44.34	37.47	57.03	49.61	70.07	54.94	--	--
3-year SDQ total early behavior problems								
Emotional symptoms	--	--	--	--	--	--	0.32	0.33
Conduct problems	--	--	--	--	--	--	0.64	0.41
Hyperactivity	--	--	--	--	--	--	0.97	0.44
Peer problems	--	--	--	--	--	--	0.45	0.34
Total problems	--	--	--	--	--	--	0.59	0.28

Table 2.3

Intercorrelations of social environment variables, child ANS activity, and behavior problems

	2	3	4	5	6	7	8	9	10	11	12
1. Mean income-to-needs ratio ^a	.04	.69*	.49*	-.46*	-.13	-.16*	-.16*	-.07	.01	.05	-.34*
2. Mean geographic isolation ^a	--	.01	-.03	-.05	-.12	-.09	-.08	.12	.05	.05	.04
3. Mean index of social advantage ^a		--	.47*	-.50*	-.06	-.19*	-.18*	-.01	.09	.10	-.40*
4. Mean maternal engagement ^a			--	-.55*	.01	-.03	-.09	-.01	.06	.21*	-.36*
5. Mean maternal intrusiveness ^a				--	.03	.12	.13	.06	-.08	-.16*	.36*
6. 7M baseline RSA ^b					--	.38*	.44*	-.01	.08	.02	.11
7. 15M baseline RSA ^b						--	.45*	.01	-.07	.02	.10
8. 24M baseline RSA ^b							--	.09	-.01	-.05	-.02
9. 7M pre-task sAA ^c								--	.43*	.36*	.02
10. 15M pre-task sAA ^c									--	.48*	-.05
11. 24M pre-task sAA ^c										--	-.10
12. 36M SDQ total problem behaviors											--

^a Social environment variables are mean values across the 7-, 15-, and 24-month assessments.

^b Baseline RSA values controlling for effect of child age.

^c Pre-task sAA values controlling for effect of time of day.

* $p < .05$

Table 2.4. *Longitudinal profiles of child ANS activity across infancy and toddlerhood*

	No. of parameters	AIC	BIC	Adj. BIC	Adj. <i>p</i> LMR LRT
1-class	12	4087.77	4132.57	4094.51	---
2-class	19	3975.93	4046.87	3986.61	.0246*
3-class	26	3921.04	4018.10	3935.64	.0267*
4-class	33	3829.68	3952.88	3848.22	.0246*
5-class	40	3800.42	3949.76	3822.89	.0959
6-class	47	3773.45	3948.92	3799.85	.2728

Note. AIC = Akaike information criterion; BIC = Bayesian information criterion; Adj. BIC = Sample-Size Adjusted BIC; Adj. LMR LRT = Adjusted Lo-Mendell-Rubin likelihood-ratio test. * $p < .05$
n=309

Table 2.5. Odds ratios for predictors of ANS profile: Profile 1 as comparison

Measure	β	SE	z	Odds (Inv)
Profile 2				
Social advantage	-.787	.454	-1.73†	.45 (2.20)
Maternal engagement	.140	.199	0.70	1.15 (0.87)
Maternal intrusiveness	-.536	.329	-1.63	.58 (1.71)
Profile 3				
Social advantage	-.769	.418	-1.84†	.46 (2.15)
Maternal engagement	-.158	.167	-0.95	.85 (1.17)
Maternal intrusiveness	-.140	.333	-0.42	.87 (1.15)
Profile 4				
Social advantage	-1.453	.579	-2.51*	.23 (4.35)
Maternal engagement	-.447	.287	-1.56	.64 (1.56)
Maternal intrusiveness	-.528	.374	-1.41	.59 (1.69)

Note. Odds ratio in parentheses represents the odds of membership in Profile 1.

*p<.05, †p<.10

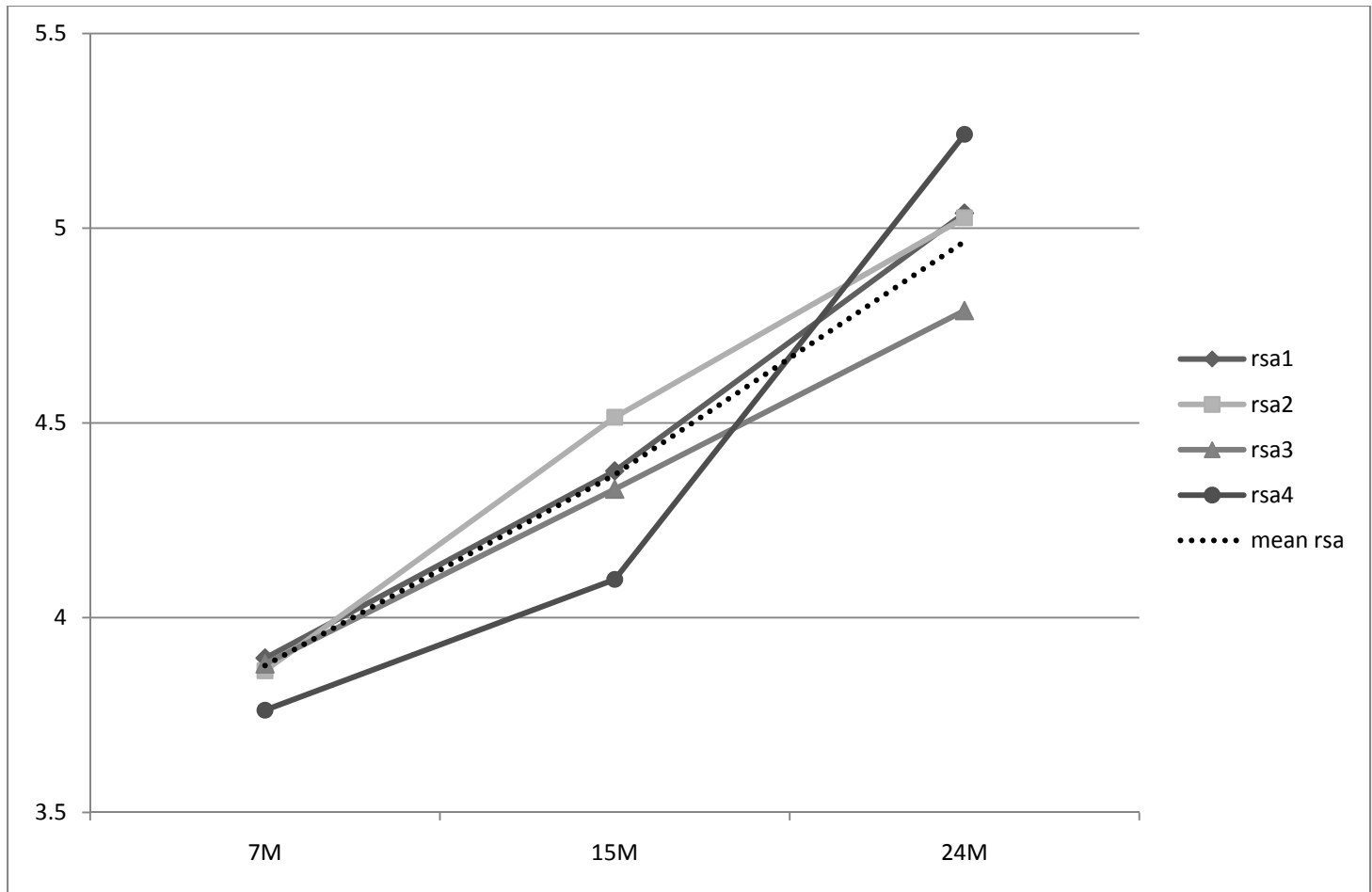


Figure 2.1. Longitudinal profiles of PNS activity in infancy and toddlerhood.

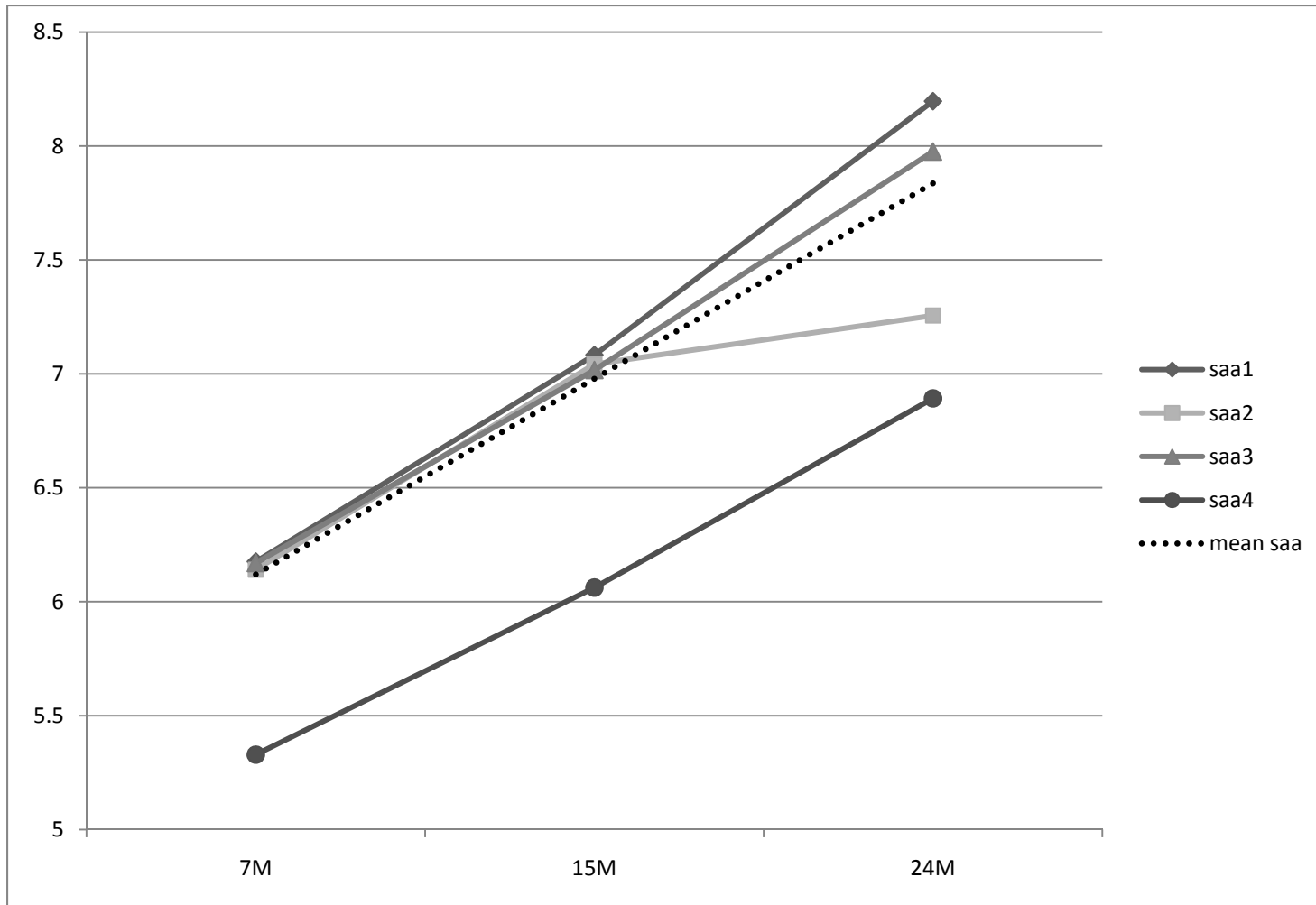


Figure 2.2. Longitudinal profiles of SNS activity in infancy and toddlerhood.

STUDY 3:

Profiles of Infant Autonomic and Neuroendocrine Functioning: Contributions of Prenatal Exposure to Cigarette Smoking

Data from the National Pregnancy and Health Survey (2004) estimates that approximately 20% of women in the United States smoke during pregnancy. Cigarettes deliver chemical toxins to the fetus via the maternal bloodstream including nicotine, carbon monoxide, ammonia, nitrogen oxide, lead, and other metals (Huisink & Mulder, 2006; Schuetze & Eiden, 2006). During pregnancy, nicotine compromises placental function by reducing uterine blood flow, and thus the delivery of essential nutrients and oxygen necessary for normal fetal growth, which may result in episodic periods of fetal hypoxia-ischemia (Morrow, Ritchie, & Bull, 1988; Huisink & Mulder, 2006). The teratogenic effects of nicotine are evident in the increased risk of infants born to women who have smoked during their pregnancy to be born low birth weight, small for gestational age, and to present other perinatal morbidities (Willoughby et al, 2007; Higgins, 2002). However, in doses lower than required to restrict physical growth, nicotine is a neurotoxin that readily passes through the placental barrier to reach fetal circulation, targeting the developing fetal brain and central nervous system (CNS) (Levin & Slotkin, 1998; Lichtensteiger et al., 1988). Nicotine binds optimally with nicotinic cholinergic receptors in the CNS, located in central catecholaminergic regions of the brain directly implicated in physiological responsivity to stress, exerting behavioral effects noted far beyond the fetal period (Schuetze & Eiden, 2006; Oliff & Gallardo, 1999; Levin & Slotkin, 1998; Lichtensteiger et al., 1988).

Numerous studies have found behavioral effects of prenatal exposure to cigarette smoking, which show a dose dependent relationship with the number of cigarettes smoked during pregnancy (Brennan, Grekin, Mortensen, & Mednick, 2002; Wakschlag, Pickett, Cook,

Benowitz, & Leventhal, 2002; DiFranza, Aligne, & Weitzman, 2004). Neonates of women who smoked during pregnancy present altered neurobehavioral functioning in the form of increased tremors and startling, heightened auditory responsiveness, and rapid transitioning between behavioral states in response to mild stimulation, such as administration of the neonatal behavioral assessment scale (NBAS) (Schuetze & Zeskind, 2001; Law et al., 2003). Later in infancy, the effects of prenatal exposure to cigarette smoking are apparent in diminished positive affect, lower sociability, and poor sustained attention (Willoughby et al., 2007). Although prenatal exposure to cigarette smoking is generally associated with an infant behavioral profile characterized by underarousal (e.g., Franco et al., 1999), in some cases, compared to non-exposed infants, increased irritability is shown in the first year of life (Willoughby et al., 2007). Behavioral effects persist into childhood and adolescence, where prenatal exposure to cigarette smoking is associated with externalizing behavior problems including aggression in preschool, attention deficit/hyperactivity disorder in childhood, and conduct disorder and delinquency in adolescence (Wakschlag, Leventhal, Pine, Pickett, & Carter, 2006; Kopp & Beauchaine, 2007).

Taken together, these studies suggest that the behavioral effects of prenatal exposure to cigarette smoking are not transient. Given that women who smoke during their pregnancy are expected to differ on a number of demographic and psychosocial variables from women who do not smoke, it is likely that factors including socioeconomic status, maternal internalizing problems, and social support impact children's behavioral outcomes (Willoughby et al., 2007). However, the fetus appears to be particularly vulnerable to the neurotoxic effects of nicotine during the second and third trimester, when the calibration of physiological stress responsivity occurs (Slotkin, Lappi, & Seidler, 1993; Kopp & Beauchaine, 2007). Therefore, another possible mechanism for the effects of prenatal exposure to cigarette smoking on postnatal child

behavior is through dysregulated physiological functioning. There is biological plausibility for fetal programming of physiological systems involved in stress arousal and regulation, including the parasympathetic nervous system (PNS), sympathetic nervous system (SNS), and neuroendocrine processes, by nicotine exposure.

Individual differences in the functioning of the PNS may be quantified by respiratory sinus arrhythmia (RSA), a measure of heart rate variability (HRV). RSA is a naturally occurring rhythm in heart rate (HR) pattern that occurs at approximately the frequency of spontaneous respiration and varies as a function of environmental demands. Thus, RSA represents the PNS influence on the heart via the vagus nerve (Porges, 1995). In utero, fetuses exposed to maternal cigarette smoking had significantly lower HRV during late gestation compared to nonexposed fetuses, and also presented disrupted temporal rhythms of HR (Zeskind & Gingras, 2006). Spectrum analysis of HRV showed that fetuses exposed to maternal smoking had lower powered heart rate rhythms and lower overall power. Interestingly, Zeskind & Gingras (2006) also showed a significant negative correlation between the frequency of maternal cigarette smoking during the first trimester of pregnancy and fetal HRV. There is also some evidence to suggest that prenatal exposure to cigarette smoking is associated with higher HR and lower RSA in infancy (Schuetze & Eiden, 2006; Schuetze & Zeskind, 2001; Franco, Chabanski, Szliwowski, Dramaix, & Kahn, 2000). In a study examining HR and RSA of newborns assessed at 2-4 weeks of age, Schuetze and Eiden (2006) compared infants of mothers who smoked during pregnancy, infants of mothers who did not smoke but were exposed to second-hand tobacco smoke in the prenatal environment, and infants of mothers who did not smoke and were not exposed to any second-hand smoke. Compared to mothers who had no exposure during pregnancy, infants of mothers who had any exposure had a higher HR and lower RSA in the first month of life. These

effects were most pronounced among infants whose mothers smoked, showing a linear dose-response with the number of cigarettes, although the group differences between smoke-exposed infants were non-significant. This study also presents evidence that gender may moderate the relationship between prenatal exposure to cigarette smoking and indicators of infant PNS functioning. Males, in both exposure groups had higher HR and lower RSA than girls. This suggests that male fetuses may be especially vulnerable to the neurotoxic effects of nicotine during pregnancy.

Prenatal exposure to cigarette smoking may also increase fetal SNS activation. SNS activity expressed in actions of the sympathetic–adrenomedullary (SAM) system is traditionally indexed by plasma catecholamines, systolic blood pressure, skin conductance level, and pre-ejection period (PEP). However, with recent advancements in the non-invasive collection and assessment of salivary analytes, individual differences in SAM activity can be measured by salivary alpha-amylase (sAA), an enzyme released by glands in the oral cavity (Chatterton, Vogelsson, Lu, Ellman, & Hudgens, 1996; Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007). Even though no study to date has examined prenatal exposure to cigarette smoking and children’s sAA levels, there is reason to pursue this linkage, as other related indicators of SNS activity appear to be altered by nicotine exposure. In a sample of infants assessed during the first week of life, and at 3-months of age, infants of smokers had a higher mean resting systolic blood pressure compared to control nonexposed infants (Browne, Colditz, & Dunster, 2000). Browne and colleagues (2000) hypothesize that prenatal smoking repeatedly induces fetal hypoxia which may increase vascular resistance, increasing systolic blood pressure in infants. Further, in a sample of 3864 5-year old children, the systolic blood pressure of children whose mothers had

smoked during pregnancy was significantly higher than for children who were not exposed (Lawlor et al., 2004).

Other studies suggest that prenatal exposure to cigarette smoking induces dysregulation of the fetal limbic hypothalamic-pituitary-adrenal (L-HPA) axis (e.g., Jacobson, Bihun, & Chiodo, 1999; Ramsay, Bendersky, & Lewis, 1996). The fetal L-HPA axis is structurally developed and functional by the second half of gestation and shows responsivity to stressors including hypoxia and nutrient restriction (Phillips & Jones, 2006). Therefore, factors that induce these conditions, namely prenatal exposure to cigarette smoking, have the potential to initiate a fetal stress response and condition reactivity of the fetal L-HPA. Furthermore, in samples of umbilical blood drawn immediately after the birth of the baby, higher levels of adrenocorticotropin hormone (ACTH) was found in samples where the mother smoked during pregnancy. Since ACTH stimulates the release of cortisol, the end-product of the L-HPA axis, into the bloodstream by the adrenal cortex (Gunnar & Davis, 2003; Chrousos & Gold, 1992), prenatal smoking may up-regulate maternal cortisol levels in circulation, impede functioning of the placenta, and alter fetal stress responsivity. Salivary cortisol levels have been extensively studied as a reliable marker of the physiological stress response, and show a robust response to physical and psychosocial stressors, including inoculation and emotional challenge (Gunnar & Davis, 2003), and several studies have explored cortisol levels in infants prenatally exposed to cigarette smoking. Ramsay and colleagues (1996) found that infants whose mothers smoked during pregnancy had higher basal cortisol at 2- and 6-months, and attenuated cortisol reactivity, quantified by relatively small increases in cortisol levels or no increase at all in response to an inoculation procedure, at 2-months. However, other studies have found exaggerated cortisol reactivity patterns among prenatally exposed to cigarette smoking. In a sample of 7-month old

infants, Schuetze et al. (2008) assayed salivary cortisol multiple times, both prior to and following a series of negative emotion eliciting tasks, including a gentle arm restraint (Stifter & Braungart, 1995) and a presentation of four novel masks (Goldsmith & Rothbart, 1996). Infants that were exposed to cigarette smoking prenatally had higher cortisol reactivity than nonexposed infants, although no pre-task differences were supported. However, there was also evidence for gender differences in cortisol levels such that exposed boys had significantly higher cortisol reactivity than nonexposed infants or exposed girls (Schuetze, Lopez, Granger, & Eiden, 2008).

Given that the functioning of the two branches of the ANS and related L-HPA processes do not operate in isolation, but exert coordinated and flexible responding in response to environmental demands (Bauer, Quas, & Boyce, 2002; El-Sheikh et al., 2009), it is possible that RSA, sAA, and cortisol levels are altered in infants exposed to prenatal cigarette smoking. No study to date has examined patterns of physiological functioning across these systems in response to the neurotoxic effects of nicotine. Thus, the first goal of the present study is to employ a person-oriented analytic approach to examine patterns of physiological functioning in 7-month old infants, and to examine the effects of prenatal exposure to cigarette smoking on these patterns. However, women that continue to smoke prenatally are more likely to be low-income, experience greater internalizing symptoms, less social support, lower educational attainment, and be single, among other things (Al-Sahab, Saqib, Hauser, & Tamim, 2010; Higgins et al., 2009; Willoughby et al., 2007). Since women who smoke during pregnancy are expected to differ on a number of demographic and psychosocial variables from women who do not smoke, propensity score matching (PSM; Parsons, 2001) was used to create a matched sample of two groups of infants whose mothers differ with respect to prenatal smoking, but are equivalent with regard to a large number of potential confounder variables (see Table 3.1). In

addition, increased pregnancy and delivery complications (i.e., maternal hypertension, low birth weight, etc.) may be related to prenatal smoking and infant physiological functioning (Gibson & Tibbets, 1998). Therefore, the total number of pregnancy and delivery complications was entered as a covariate in latent profile models. The second goal of the present study was to examine gender differences in the association between prenatal exposure to cigarette smoking, and physiological functioning profile as indexed by patterns of functioning across the PNS, SNS, and L-HPA. Accumulating evidence suggests that the male fetus may be more susceptible to the neurotoxic effect of nicotine on developing physiological systems than the female fetus (Hans, 1994; Schuetze et al., 2008). Exposed males have significantly lower RSA and higher cortisol reactivity compared to nonexposed infants and exposed females (Schuetze & Eiden, 2006; Schuetze et al., 2008).

Method

Participants

Recruitment. The sample will be drawn from the Family Life Project (FLP), a study of 1,292 children and their families living in the Black South (eastern North Carolina; Sampson, Wayne, and Wilson counties) and Appalachia (central Pennsylvania; Blair, Cambria, and Huntingdon counties) — two geographical regions of high child poverty. During a 1-year recruitment period, mothers were approached in the hospital after giving birth and asked to participate. Eligibility criteria included English-speaking families, residing in one of the target counties, with no intent to relocate from the area in the next 3 years. A cohort sampling method

was used to over sample for low-income families in both states and, specifically in North Carolina, African-American families. African-American families were not over-sampled in Pennsylvania as the target communities were almost exclusively Caucasian ($\geq 95\%$). Over two-thirds of the FLP sample lives below 200% of the poverty line, and approximately 40% are African-American.

For this analysis, the sample is restricted to a random sub-sample of children from whom electrocardiogram (ECG) heart period data was collected ($N=325$, 51.5% female, 37% African-American). Families were visited in the home when children were at 2-, and 7-months of age. The age range for the 7 month visit ranged from 5 to 9 months ($M = 7.1$ months). Due to the range in age at each of the visits, age-related differences were controlled for in all analyses.

Within this sub-sample, 67% had an income-to-needs ratio less than 200% of the poverty line. Forty-one percent of the families resided in Pennsylvania, and 59% resided in North Carolina. Most of the mothers (76%) had completed a high-school degree or equivalent and 53% were married. Of the 325 families, there were 86 families in which the mother reported prenatal smoking (see Table 3.1). Given that women who smoke during their pregnancy are expected to differ on a number of demographic and psychosocial variables from women who do not smoke, rather than trying to statistically control for these differences, a SAS macro developed by Parsons (Parsons, 2001) was implemented to conduct propensity score matching (PSM). The PSM resulted in a matched sample of 144 infants (72 per group) whose mothers did not differ on any of the 18 potential confounders but did differ with respect to prenatal smoking (see Table 3.1).

Procedure. During the 2-month home visit, mothers were interviewed about family demographics, their pregnancy and birth experience including prenatal substance use, and completed questionnaires on negative life events, mastery, symptomatology, and social support. At the 7-month home visit, mothers reported on household income, and physiological measurements were collected from children.

To assess RSA, infant electrocardiograms (ECG) were recorded using a Grass preamplifier, and this output was transmitted to laptop computer in the form of a data file containing the interbeat intervals for the two collection periods, during a 5 minute baseline period when children were sitting quietly, and a 15 minute cognitively challenging period when a test of mental development, the Bayley exam, was being administered. All ECG heart period data was analyzed off-line using Mindware Technologies.

To assess changes in cortisol indicative of the child's L-HPA response to the emotional challenge tasks previously validated to elicit fear (presentation of masks) and frustration reactivity (gentle arm restraint) in infants (e.g., Buss & Goldsmith, 1998; Kochanska Tjebkes, & Forman, 1998; Goldsmith & Rothbart, 1996; Stifter & Braungart, 1995), three saliva samples were collected around the tasks via a sterile, 6" cotton rope (Granger et al., 2007). One saliva sample was taken immediately prior to the start of the emotional challenge tasks (pre-task), and two samples were taken after administration of the tasks: 1) 20 min post-peak arousal (reactivity), and 2) 40 min post-peak arousal (recovery). The task order was structured so that the most arousing task was last, which at 7-months was the frustration-eliciting task. Thus, for most infants, peak arousal occurred at the conclusion of the emotional challenge tasks.

The pre-task saliva sample at 7-months was also used to assess salivary α -amylase levels indicative of the child's SAM activity. The two post-peak arousal saliva samples were not used

as the time course of collection was not designed to capture a more fast-acting SAM response, and preliminary investigations using these data suggest that, at least at the 7-month visit, children's salivary α -amylase levels did not show a response to the emotional challenge tasks (Kivlighan et al., 2005).

After collection in the home, all saliva samples were immediately placed on ice, transported to experimenters' homes, and stored frozen (-20°C) before being batched and overnight shipped on dry ice to the Behavioral Endocrinology Laboratory at Pennsylvania State University. Samples were then stored frozen (-80°C) until assay.

Measures

Variables for Propensity Score Matching

Propensity score matching (PSM; Parsons, 2001) was used to create a matched sample of two groups of infants whose mothers differ with respect to prenatal smoking, but are equivalent with regard to a large number of potential confounder variables. To create the matches, a propensity score is obtained from a multivariate logistic regression model where prenatal smoking status is regressed on all selected confounders. From this, each infant of a mother who smoked prenatally is matched to a non-smoking counterpart using a nearest-neighbor approach, first matching on propensity score by 5 decimal digits, then 4, and so on until all possible matches have been made. This approach is much more precise than categorical matching and can accommodate multiple confounder variables. The following variables were selected based on past research which shows that women that continue to smoke prenatally are more likely to be low-income, experience greater internalizing symptoms, less social support,

lower educational attainment, and be single, among other things (Al-Sahab, Saqib, Hauser, & Tamim, 2010; Higgins et al., 2009). In addition, the matching approach used in the current study is modeled from a previous investigation of prenatal exposure to cigarette smoking with a larger subsample of children participating in the Family Life Project dataset conducted by Willoughby, Greenberg, Blair, Stifter and the FLP Investigators (2007). The same 18 demographic, psychosocial risk, and prenatal exposure variables used in the study by Willoughby et al. (2007) were selected for propensity score matching in the current study to be consistent with previous investigations from this research project (see Table 3.1).

Demographic data. The demographic variables used in the present study were primarily collected from primary caregivers at the 2-month home visit. Mothers reported on site, race, age, education, marital and employment status, and household composition.

Income-to-need ratio. At the 7-month visit, mothers reported on income from all sources and any income from other household members. This figure was used as an estimate of total household income and divided by the federal poverty threshold for 2004 adjusted for number of persons in the home to compute an income-to-need ratio. An income-to-need ratio of 2.00 or below indicates that the family is low-income, and 1.00 or below indicates that the family is living in poverty by federal guidelines, adjusted for family size.

Mastery Scale. The Mastery Scale (Pearlin & Schooler, 1978) is a 7-item scale that mothers completed at the 2-month visit which measures the extent to which one regards one's life-chances as being under one's own control in contrast to being fatalistically ruled (e.g., "I

have little control over the things that happen to me”). Items are scored on a 4-point Likert scale, ranging from 1 (*strongly disagree*) to 4 (*strongly agree*).

Brief Symptom Index 18. At the 2-month visit, mothers’ psychological state was assessed using the Brief Symptom Inventory 18 (BSI-18; Derogatis, 2000). The BSI-18 is a short, yet highly sensitive self-report screening index of psychological distress derived from the long form of the BSI (BSI; Derogatis, 1983). The BSI-18 includes 18 items that are divided evenly across three subscales: depression, anxiety, and somatization. The Global Symptom Index, which is the mean score across all 18 items, was used to index maternal internalizing problems. Further, a 5-item Hostility subscale from the original BSI was also added, and the mean score across these items was used to index maternal hostility.

Negative Life Experiences. A life experience survey (Sarason & Siegel, 1978) was completed by mothers at the 2-month visit. The survey included 46 events, covering a broad spectrum of daily living (e.g., major changes in family income, household structure, sleeping habits, serious illness). For each event, mothers indicated if it had occurred with the previous 6 months, and, if so, whether the event was perceived to be positive or negative. To measure negative life experiences, the total number of events perceived to be negative was summed.

Questionnaire of Social Support. A modified 16-item version of the Questionnaire of Social Support (QSS; Sarason, Levine, Basham, & Sarason, 1983) was collected at the 2-month visit. Modifications included collapsing sources of support and satisfaction (e.g., How satisfied are you with...”), whereas in the original QSS presence of support and satisfaction with the

support were asked separately. For each item, mothers rated their satisfaction on a 4-point Likert scale ranging from 1 to 4 about perceived support from community involvement (mean of 2 items), friendships (mean of 4 items), family (mean of 6 items), and intimate relationships (mean of 3 items). In addition, mothers were asked in a single item to make a global rating of their satisfaction with their social support from all sources. This global rating correlated between .3 and .5 with the four subscales, and was used to index social support.

Prenatal alcohol and illicit drug use. At the 2-month visit, mothers completed the pregnancy and delivery module of the Missouri Assessment of Genetics Interview (MAGIC; Reich, Todd, Joyner, Neuman, & Heath, 2003), reporting whether they drank any alcohol during pregnancy (“How frequently did you drink anything with alcohol in it when you were pregnant?” [if response was No, follow up query:] “So you never had even one drink when you were pregnant?”). Mothers also reported on illicit drug use during pregnancy (“Were you taking any street drugs like marijuana or cocaine? Were you taking any other hard drugs?”). In the current sample, the endorsement rates of prenatal alcohol and illicit drug use were low, therefore a dichotomous variable for both were retained. Reich et al. (2003) reported good short- and long-term reliability, especially within the first year post term, for self reports of maternal smoking, alcohol, and drug use during pregnancy.

Independent Variables

Prenatal exposure to cigarette smoking. The pregnancy and delivery module of the MAGIC interview also included separate questions about the presence and frequency of prenatal

smoking for each of the three trimesters of pregnancy. The number of cigarettes smoked that was reported by mothers who indicated smoking during their pregnancy was highly correlated across the three trimesters, which may, in part, be due to the retrospective report (see Table 3.2). A composite variable was created, to reflect the mean number of cigarettes smoked daily during pregnancy. The mean number of cigarettes smoked daily during pregnancy and when infants were 7-months of age, assessed in the 24-hour period preceding the home visit, was nearly identical ($M = 9$ cigarettes/day), however these values were only moderately correlated ($r = .49$). For comparison, a second composite variable was created, reflecting the maximum cigarettes smoked daily during pregnancy, which showed a stronger, but still moderate correlation with mean number of cigarettes smoked daily at the 7-month visit ($r = .57$). Further, exposure to cigarette smoking was highly stable in the current sample, with 92% of prenatal smokers reporting continued smoking at the 7-month visit. Taken together, this is interpreted as an indication that women did differentiate their responses to pre- and postnatal smoking, however, the recall for smoking in the past 24-hours at 7-months may be a better representation of true smoking level compared to the retrospective account of prenatal smoking reported at the 2-month home visit. A categorical variable of prenatal smoking/non-smoking was retained for data analysis.

Pregnancy and delivery complications. As mothers who smoke may have difficulty with pregnancy and delivery, pregnancy and delivery complications (PDCs) were used as a covariate in the analyses. These data were drawn from the pregnancy and delivery module of the MAGIC interview. Mothers were asked if they experienced any of the following pregnancy complications: heavy bleeding, excess vomiting, weight loss over 10 pounds, infection requiring medical care, high blood pressure, water retention, convulsions, serious accidents, emotional

problems, family problems, and other illness; and any of the following delivery complications for themselves or their baby: induced labor, C-section, breach baby, fetal distress, baby breathing difficulty, congenital malformation, baby required surgery, baby stayed in hospital, and baby stayed in the neonatal intensive care unit. A sum of all possible pregnancy and delivery problems was used to index PDCs. Reich et al. (2003) reported good reliability (Kappa range = .5-.8) for individual PDCs following the birth of their baby and up until 18 months post term.

Outcome variables

Respiratory sinus arrhythmia. From electrocardiograms (ECG) recorded in the home when children were approximately 7-, 15-, and 24-months of age, Mindware Technologies software (Gahanna, Ohio) was used to apply an algorithm to the time series of heart period data. The algorithm uses a moving polynomial to de-trend periodicities in heart period that are slower than RSA. A band-pass filter then extracts the heart rate variability within the frequency band of spontaneous respiration in young children, .24 to 1.04 Hz, which is commonly studied to index vagal functioning in infants and young children (see Stifter & Fox, 1990; Porges et al., 1996; Huffman et al., 1998; Stifter & Corey, 2001; Calkins & Keane, 2004). The software then derives a RSA estimate by calculating the natural log of specified heart period variability and is reported in units of $\ln(\text{ms})^2$. Trained coders edited the ECG heart period records for movement artifact where possible, marking the R spike if identifiable on the record. The mean RSA across the baseline minutes is used to index *baseline RSA*. *RSA suppression* is the difference between baseline RSA and mean RSA during the mental development test, such that positive values

indicate suppression. A negative value, or increase in RSA from baseline to the mental development test, would indicate RSA augmentation.

Salivary cortisol. All samples were assayed for salivary cortisol using an FDA 510K cleared, sensitive enzyme immunoassay (Salimetrics, State College, Pennsylvania). The test uses 25 μ l of saliva (for singlet determinations), had a range of sensitivity from 0.007 to 1.8 μ g/dl and an average intra and inter-assay coefficients of variation of less than 10% and 15%, respectively. Performance is robust for samples with pHs ranging from 4.0 to 9.0. All samples were assayed in duplicate, and the criterion for repeat testing was variation between duplicates greater than 20%. In these cases, the average of the duplicates was used in all analyses.

In addition to the three raw measures of cortisol (pre-task baseline, 20 min post-peak arousal, 40 min post-peak arousal), cortisol reactivity and regulation were calculated. The difference between the 20 min post-peak arousal cortisol level and the pre-task *baseline cortisol level*, divided by initial pre-task baseline level is used to index *cortisol reactivity*. Similarly, the difference between the 40 min and the 20 min post-peak arousal, divided by the 20 min post-peak arousal is used to index *cortisol regulation*. The cortisol distributions were log transformed prior to analysis to correct for positive skew. Further, given special characteristics of the sample (single-parent household, economic disadvantage) and the time necessary for completion of all in-home assessments, there was variability in the time of day when saliva samples were collected. As such, time of day is included as a covariate in all analyses involving cortisol.

Salivary alpha-amylase. Pre-task baseline saliva samples were also assayed for salivary α -amylase using a kinetic reaction. A chromagenic substrate, 2-chloro-p-nitrophenol linked with

maltotriose, was added to saliva samples to trigger the enzymatic action of α -amylase indexed by the degradation of maltotriose. The amount of α -amylase activity present in the sample is directly proportional to the increase (over a 2- min period) in absorbance (optical density) at 405 nm (Salimetrics, State College, Pennsylvania). The test uses only 10 μ l of saliva (for singlet determinations), with an absolute range from 3.1 - 423.1 U/mL. The lower limit of sensitivity is governed by the change in absorbance. A change in absorbance less than 0.01 does not result in a reliable value. In this case, samples were rerun at a higher concentration. The *baseline salivary α -amylase* distributions were square root transformed prior to analysis to correct for positive skew.

Missing data. ECG data was collected on a subsample of children participating in the Family Life Project at 7-, 15-, and 24-months (n=358). Given the variation in age at which children were assessed, the analysis restricted the sample to 325 children between 5 and 9 months for the 7-month assessment on whom RSA was measured on at least one of the three occasions, resulting in the exclusion of 33 children older than 9 months of age at the 7-month assessment. Of the 325 children retained based on age restrictions, all had cortisol data and 10 were missing sAA data on at least one of the three measurement occasion. Listwise deletion typically induces bias in parameter estimates by eliminating non-random cases and reducing error variance. To handle missing data in the present sample, a maximum likelihood estimation application in the Mplus software used to conduct latent profile analysis was implemented. However, additional data cases were excluded in the identification of prenatal smokers and creation of the propensity score matched sample. As a result of this restriction, the latent profile analysis was conducted on 144 children at 7-months of age.

Plan of analysis

Utilizing a matched sample of infants not exposed and exposed to prenatal smoking, a latent profile analysis (LPA) was conducted to examine profiles, or person-oriented subgroups, of infant autonomic and neuroendocrine functioning. Profile membership was statistically modeled within LPA, based on the observed response pattern of baseline RSA, RSA suppression, baseline sAA, baseline cortisol, cortisol reactivity, and cortisol recovery measured when infants were 7-months of age. To determine the optimal number of profiles that best fit the current data, models with 2-6 classes were fit, with the goal of identifying mutually exclusive and exhaustive subgroups of children with similar physiological profiles. Model goodness of fit was evaluated with the following fit indices: Akaike information criterion (AIC), Bayesian information criterion (BIC), Sample-Size Adjusted BIC, and the *Lo-Mendell-Rubin likelihood ratio test (LMR-LRT)*. The model with the smallest BIC values was selected. Although, similar to exploratory factor analysis, in addition to the fit indices, theoretical justification, model parsimony and interpretability were considered in model selection (Muthén, 2001; Bauer & Curran, 2003; Jung & Wickrama, 2008). Next, observed variables that may define the physiological profile solution were added to LPA models. Prenatal smoking and pregnancy and delivery complications were added as predictors of latent profile membership. Given that past research suggests that the effect of prenatal smoking on infant physiological functioning differs by infant sex, child sex was added as a known class, and the analysis was re-run as a multiple group model where class structure was held equivalent for males and females to examine the interactive effect of prenatal smoking status and infant sex on physiological activity.

Results

Mean values in the total matched sample for the independent and outcome variables by prenatal smoking group are presented in Table 3.3. Table 3.4 reports these same variables by prenatal smoking group and child sex. Further, Table 3.5 presents the bivariate correlations between measures of infant physiological functioning. There was a significant positive association between baseline RSA and RSA suppression, such that children with higher baseline RSA showed greater suppression of RSA during a cognitive challenge. In addition, the negative association between pre-task cortisol level and cortisol reactivity was significant, such that children who had higher pre-task cortisol levels showed less reactivity, or increase in cortisol level in response to emotion challenge ($r = -.34, p < .01$). Cortisol recovery was significantly positively associated with pre-task sAA levels, such that children who showed higher pre-task levels exhibited greater declines in cortisol level from 20-min to 40-min post peak emotional arousal ($r = .24, p < .05$). Further, there was also a significant positive association between cortisol recovery and cortisol reactivity, such that children who showed greater increases in cortisol in response to emotional arousal by 20-min post peak emotional arousal also showed greater decreases in cortisol by 40-min post peak emotional arousal ($r = .24, p < .05$).

Latent Profile Mixture Models of Child ANS and L-HPA Activity

To prepare the physiological variables for the latent profile analysis, given ascending values of baseline child RSA across the first year of life, the effect of child age was regressed out of baseline RSA (Porter, Bryan, & Hsu, 1995; Porges, 1995). Similarly, given the circadian rhythm of cortisol activity and sAA characterized by increasing levels over the course of a day, the effect of time of day was regressed out from the sAA and cortisol variables (Gunnar & Davis,

2003; Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2005). Further, all variables were standardized prior to latent profile modeling.

Latent profile models with two through six profiles were fit with the 7-month physiological indicators to determine the optimal number of profiles that best described ANS and L-HPA activity in the total matched sample by prenatal smoking group. Model goodness of fit was evaluated using the Bayesian information criterion (BIC), where a smaller value represents a better fit, and the adjusted Lo-Mendell-Rubin likelihood ratio test (Adj. LMR-LRT), which tests the significance of the difference between a model with k and $k-1$ classes, correcting for the distribution of the likelihood-ratio statistic (Lo, Mendell, & Rubin, 2001). Fit indices for all models are reported in Table 3.6. Although the adjusted LMR-LRT test did not indicate that two profiles was a significant improvement from one, the two profile model was selected as optimal for these data based on having the smallest BIC value, a sizeable proportion of members in each profile, and represented the most parsimonious, interpretable model based on the selected indicators. Further, the average latent profiles probabilities for classification were acceptable ($range = .83-.86$). Subsequent models with a greater number of profiles were rejected based on interpretability of the pattern of physiological indicators and the uneven distribution of members in each profile. For example, the four profile solution generated two profiles with only 14 members in each, which would not permit the exploration of sex differences in the effect of smoking on physiological profile. Figure 3.1 presents the mean physiological indicator estimates for the two profile solution for 7-month ANS and L-HPA activity patterns in the matched sample. In this base model, the first profile named the “ANS coinhibition, L-HPA non-reactor”, representing 60% of the sample, comprised infants who exhibited low baseline RSA and less RSA suppression, low-average pre-task sAA level, high-average pre-task cortisol level, and low

cortisol reactivity and recovery. The second profile named the “ANS coactivation, L-HPA reactor”, representing 40% of the sample, comprised infants who exhibited high baseline RSA and RSA suppression, high-average pre-task sAA level, low pre-task cortisol level, and high cortisol reactivity and recovery.

In this base model, prenatal smoking group was entered as a predictor of the probability of membership in the profiles. This is tested by means of an odds ratio for likelihood of classification in a specified reference profile relative to all other profiles in the model. The effect of prenatal smoking status significantly predicted profile membership such that for infants exposed to cigarette smoking in utero there was a 3.68 increase in the odds of being in the ANS coinhibition, L-HPA non-reactor profile relative to the ANS coactivation, L-HPA reactor profile ($p < .05$). Next, the total number of pregnancy and delivery complications was added as a covariate predicting latent ANS and L-HPA activity profile in the selected two-profile model. PDCs did not predict latent profile membership (OR = 1.03, $p = .91$) (see Table 3.7). However, as presented in Table 3.7, prenatal smoking continued to have an impact on membership classification when controlling for PDC's such that there was a 3.67 increase in the odds of being in the ANS coinhibition, L-HPA non-reactor profile relative to the ANS coactivation, L-HPA reactor profile ($p < .05$).

Planned comparison results for effect of prenatal smoking by infant sex. A multiple group mixture model for infant sex was estimated using the two-profile solution with covariates from the previous analysis, where the indicator mean levels for the ANS coinhibition, L-HPA non-reactor profile and the ANS coactivation, L-HPA reactor profile were constrained to be equal across sex. For females, 62% of female infants in the sample were classified as members of the ANS coinhibition, L-HPA non-reactor profile and 38% were classified as members of the

ANS coactivation, L-HPA reactor profile. For males, 56% of male infants in the sample were classified as members of the ANS coinhibition, L-HPA non-reactor profile and 44% were classified as members of the ANS coactivation, L-HPA reactor profile. In the multiple group model, total number of PDCs did not predict profile membership for males ($OR = 1.30, p = .41$) or females ($OR = 0.83, p = .62$) (see Table 3.8). Planned comparisons testing the effect of smoking on profile classification by infant sex revealed no significant differences, although the pattern of findings was in the expected direction. As shown in Table 3.8, exposure to prenatal smoking was associated with increased odds of being in the ANS coinhibition, L-HPA non-reactor profile for females ($OR = 2.55, p = .16$) and males ($OR = 5.65, p = .21$). However, in examining the probability of prenatal smoking by profile, for males, the probability of being exposed to prenatal smoking in the ANS coinhibition, L-HPA non-reactor class was .72 versus .31 for the ANS coactivation, L-HPA reactor profile. In females, the probability of being exposed to prenatal smoking was .55 for the ANS coinhibition, L-HPA non-reactor profile and .32 for the ANS coactivation, L-HPA reactor profile.

Discussion

The goals of the current study were, first, to identify latent profiles of infant autonomic and neuroendocrine functioning in a matched sample of 7-month old infants exposed and not exposed to maternal prenatal smoking. The second goal was to examine the prediction of profile membership by smoking group and pregnancy and delivery complications. Finally, the third goal was to conduct a multiple group latent profile model by infant sex, to test sex differences in the effect of smoking on latent ANS and L-HPA activity. Latent profile modeling results indicate that a two-profile solution, consisting of an ANS coinhibition, L-HPA non-reactor

profile and an ANS coactivation, L-HPA reactor profile, best fits these data with the total matched sample. The primary advantage of using a latent profile approach for this analysis is the ability to identify data driven, mutually exclusive, and exhaustive subgroups of infants who share a similar pattern of physiological activity across the PNS, SNS, and L-HPA systems. This eliminates any arbitrary splits in the sample and supports an interpretation of multi-system physiology functioning with uncorrelated indicators (see Table 3.5).

For the two profile solution, although the total number of pregnancy and delivery complications did not predict profile membership, infants of mothers who smoked during pregnancy had significantly higher odds of being classified into the ANS coinhibition, L-HPA non-reactor profile compared to the ANS coactivation, L-HPA profile. Based on the profile indicator estimates for the ANS coinhibition, L-HPA non-reactor profile, prenatal smoke exposure may lower baseline RSA and the ability to suppress RSA for the purpose of mobilizing cardiac output to mount arousal for engagement with a cognitive challenge. Further, it appears that prenatal smoking may induce higher basal cortisol in infancy and an altered response pattern to emotional challenge characterized by attenuated or flattened cortisol reactivity and recovery. This finding corroborates the past work of Ramsay and colleagues (1996) who found the same pattern of cortisol activity in response to a 2-month inoculation procedure among infants of mothers who smoked during the prenatal period as found in the current study. Although other studies have shown that infants exposed to prenatal cigarette smoking show exaggerated cortisol reactivity, mixed findings in the literature may be attributed to variations in the level and timing of maternal smoking.

Prenatal smoking effects on pre-task levels of sAA were less pronounced, but may be slightly below average. This constellation of physiological functioning may represent a more

maladaptive response to environmental demands and perhaps less engagement with tasks requiring sustained attention. Conversely, the ANS coactivation, L-HPA reactor profile represents infants who are able to draw on PNS activation, and who show an adaptive cortisol pattern of lower basal levels, followed by reactivity to a challenge coupled with recovery. It is interesting to note that 60% of infants in the sample were classified as members of the ANS coinhibition, L-HPA non-reactor profile. This profile may be overrepresented given that half of infants in the current sample had mothers that smoked cigarettes during the prenatal period. To this end, the matched sample ensures that mothers of infants in the sample did not differ on numerous demographic and psychosocial variables previously established as being related to prenatal smoking, therefore, the current sample may represent one of higher demographic and social risk.

The multiple group model, which added infant sex to the two-profile latent solution, indicated that, although non-significant, infants in the prenatal smoking group had an increased odds of being in the ANS coinhibition, L-HPA non-reactor profile for both females and males. As expected, the odds ratio of classification in the first profile was greater for males than for females. Further, the results in probability scale show that among children classified in profile 1, the ANS coinhibition, L-HPA non-reactor profile, the probability that the males in this profile were exposed to cigarette smoking prenatally was .72. In comparison, the probability that the females in this profile were exposed to cigarette smoking prenatally was .55. This pattern of findings should be interpreted with caution as the odds ratio was not significant, but it does suggest that a greater proportion of males than females exhibit physiological adaptations to the fetal exposure to cigarette smoking manifested through a pattern of low ANS arousal, high baseline cortisol, and attenuated cortisol reactivity, which may add further support to previous

findings that suggest that the male fetus is more susceptible to the neurotoxic effects of nicotine compared to the female fetus (Schuetze et al., 2008; Hans, 1994).

There are several limitations of the current study that should be noted. First, the size of the current matched sample (N=144, 72 prenatal smokers) may have limited the ability to detect effects, specifically in the case of sex differences. When the two-profile solution was split by infant sex, the number of infants in each profile ranged from 52 to only 20. When these four-profile were further split by prenatal smoking group, it is likely that the groups became too small to detect significant differences. Second, the pre-task indicator of sAA may not be an optimal indicator for SNS activity as it corresponds to general arousal during the home visit. It does not index responsiveness to a specific challenge. To appropriately capture SNS activation or inhibition, and to get a better estimation of the net effect of coordinated PNS and SNS activity on ANS arousal, it would be necessary to have an additional measurement of sAA 10-min post peak arousal to index sAA reactivity and 20-min post peak arousal to index sAA recovery. Third, although the goal of the current study was specifically to examine the effects of prenatal cigarette exposure on infant ANS and L-HPA functioning, we were unable to control for the effect of postnatal smoking due to the stability of smoking in the current sample. Nearly all women that reported smoking prenatally were also smoking at the 7-month postnatal period. Thus, there may be compound effects on infant physiology of continued smoke exposure in the postnatal environment that is not detected by the current findings. Prenatal smoking was also a retrospective report at 2-months post birth, and as a result, may have been underreported. Further, second-hand smoke exposure during pregnancy was not assessed, and may also be an important factor for fetal physiological development (Schuetze & Eiden, 2006). Future studies would benefit from a larger sample size where comparisons between no smoke exposure,

prenatal smoking only, postnatal smoking only, and pre-post natal continued smoking effects on infant physiology may be drawn. This could not be examined in the current sample due to very small sample size of these groups, as just 6 women indicated smoking during only the prenatal period, and 8 women indicated smoking only during the postnatal period. And, as mentioned, it would be important to also consider second-hand smoke effects on the fetus and young infant. Finally, the generalizability of the current findings may be limited to low-income, rural families that may present higher demographic and psychosocial risks. Although created a matched sample for the current analysis removed the influence of these potential confounding variables, the total matched sample may be a low-income, rural population.

In sum, latent profile analysis is a useful analytic technique for describing multi-system patterns of infant ANS and L-HPA functioning, and identifying unique subgroups of infants with shared patterns. The findings from the current study suggest that exposure to prenatal cigarette smoke may have important ramifications for the calibration of fetal PNS and L-HPA responsivity, with effects persisting through early infancy, in the form of decreased potential for RSA withdrawal, higher basal cortisol, and flattened cortisol reactivity at 7-months of age. Although sex differences were non-significant in the current sample, there is evidence to suggest they should be pursued in future studies with larger sample sizes capable of detecting multiple group model effects. In addition, future studies should attempt to isolate the effects of pre- from postnatal smoking. Finally, the current findings may be extended by an examination of how latent physiological functioning predicts concurrent or later behavioral outcomes.

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Table 3.1

Characteristics of Prenatal Smoking Groups Prior to and After Propensity Score Matching

	Before Matching					After Matching				
	Nonsmokers ^a		Prenatal Smokers ^b		Difference	Nonsmokers ^c		Prenatal Smokers ^c		Difference
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>Probability</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>Probability</i>
Maternal age	26.44	6.45	25.66	5.03	.274	25.22	5.58	25.91	5.11	.459
Children <5 yrs in household (No.)	1.62	0.74	1.51	.76	.294	1.62	0.78	1.53	.79	.507
Income/need ratio	1.87	1.50	1.49	1.37	.054	1.55	1.11	1.55	1.40	.980
Internalizing symptoms	45.56	8.52	48.56	10.39	.024	47.83	9.12	48.03	10.39	.908
Hostility	50.62	9.92	53.28	10.32	.045	53.20	9.66	52.05	10.13	.505
Mastery (locus of control)	3.15	0.45	3.13	0.46	.763	3.06	0.43	3.12	0.47	.490
Negative life events (No.)	2.91	2.86	4.25	4.17	.011	3.82	3.58	4.01	4.10	.769
Social support	4.26	0.77	4.01	0.84	.019	4.21	0.81	3.95	0.85	.078
	%		%		<i>Probability</i>	%		%		<i>Probability</i>
White	66.09		75.00		.147	66.67		74.24		.340
High school (GED)	78.11		69.74		.137	72.73		69.70		.701
College (4-year degree)	18.88		2.63		< .001	3.03		3.03		1.00
Employed (2-month visit)	42.06		32.89		.156	40.91		31.82		.278
Residential spouse	55.79		30.26		< .001	40.91		34.85		.473
Residential unmarried partner	16.31		44.74		< .001	37.88		39.39		.858
Poor (recruitment screen)	70.82		89.47		.001	78.79		87.88		.161
Reside in Pennsylvania	44.64		59.21		.027	45.45		56.06		.223
Any prenatal alcohol	7.76		21.05		.001	13.64		10.61		.594
Any prenatal illicit drug use	0.43		5.26		.004	0.00		3.03		.154

Note. ^a*n*=239, ^b*n*=86, ^c*n*=72

Table 3.2

Cigarette dose information for maternal prenatal smoking

	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>
1. Cigarettes per day, T1	1.0					
2. Cigarettes per day, T2	.97	1.0				
3. Cigarettes per day, T3	.85	.91	1.0			
4. Mean cigarettes per day (T1-T3)	.98	.99	.95	1.0		
5. Max. cigarettes per day (T1-T3)	.97	.95	.92	.97	1.0	
5. Cigarettes in last 24 hr	.50	.50	.56	.49	.57	1.0

Note. T1= first trimester, T2= second trimester, T3= third trimester, last 24 hr refers to measurement during the 7-month visit.

Table 3.3

Descriptive statistics for study variables by prenatal smoking group

<i>Measure</i>	<u>Nonsmokers^a</u>		<u>Prenatal smokers^b</u>	
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
Pregnancy and delivery complications	2.97	2.12	2.92	2.23
Infant physiology at 7-months				
Baseline RSA	4.04	0.89	3.69	0.87
RSA Suppression	0.16	0.61	0.07	0.69
sAA pre-task	44.82	37.71	48.09	48.56
Cortisol pre-task	0.21	0.21	0.22	0.24
Cortisol 20-min postpeak	0.25	0.25	0.21	0.18
Cortisol 40-min postpeak	0.22	0.21	0.20	0.13

Note. ^a*n*=72, ^b*n*=72

Table 3.4

Descriptive statistics for study variables by prenatal smoking group and infant sex

<i>Measure</i>	Female				Male			
	<u>Nonsmokers^a</u>		<u>Prenatal smokers^b</u>		<u>Nonsmokers^c</u>		<u>Prenatal smokers^d</u>	
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
Pregnancy and delivery complications	3.05	2.27	3.18	2.38	2.88	1.98	2.69	2.10
Infant physiology at 7-months								
Baseline RSA	3.97	0.96	3.59	0.76	4.13	0.80	3.81	0.98
RSA Suppression	0.06	0.67	0.08	0.68	0.29	0.52	0.06	0.73
sAA pre-task	49.26	45.24	49.61	64.74	40.84	29.60	47.03	34.68
Cortisol pre-task	0.23	0.27	0.21	0.22	0.19	0.11	0.24	0.26
Cortisol 20-min postpeak	0.27	0.29	0.24	0.21	0.23	0.18	0.18	0.16
Cortisol 40-min postpeak	0.21	0.26	0.19	0.13	0.23	0.13	0.20	0.14

Note. ^a*n*=39, ^b*n*=33, ^c*n*=33, ^d*n*=39

Table 3.5

Intercorrelations of infant physiological variables at 7-months of age

	1	2	3	4	5	6
1. Baseline RSA	1.0					
2. RSA suppression	.59**	1.0				
3. Pre-task salivary AA	.06	.05	1.0			
4. Pre-task cortisol level	-.01	.01	-.08	1.0		
5. Cortisol reactivity	.05	.12	.11	-.34**	1.0	
5. Cortisol recovery	.18	.13	.24*	.12	.29**	1.0

Note. Cortisol reactivity= the difference between 20-min post peak arousal and pre-task divided by pre-task cortisol level.

Cortisol recovery = the difference between 40-min and 20-min divided by 20-min post peak arousal cortisol level.

Table 3.6. 7-month latent profiles of child ANS and L-HPA responsivity

	No. of parameters	AIC	BIC	Adj. BIC	Adj. <i>p</i> LMR LRT
1-class	13	1964.89	2003.50	1962.36	---
2-class	21	1936.53	1998.89	1932.44	.2714
3-class	29	1915.78	2001.90	1910.14	.5186
4-class	37	1896.57	2006.45	1889.37	.0549
5-class	45	1878.31	2011.95	1869.56	.1782
6-class	53	1868.87	2026.27	1858.57	.2398

Note. AIC = Akaike information criterion; BIC = Bayesian information criterion; Adj. BIC = Sample-Size Adjusted BIC; Adj. LMR LRT = Adjusted Lo-Mendell-Rubin likelihood-ratio test.

Table 3.7 Odds ratios for predictors of 7-month profile: ANS coactivation, L-HPA reactor profile as comparison

Measure	β	SE	z	Odds (Inv)
ANS coinhibition, L-HPA non-reactor				
Pregnancy and delivery complications	.026	.249	0.10	1.03 (0.97)
Prenatal smoking	1.301	.527	2.47*	3.67 (0.27)

Note. Odds ratio in parentheses represents the odds of membership in the ANS coactivation, L-HPA reactor profile.

*p<.05

Table 3.8. Odds ratios for predictors of 7-month profile by child sex: ANS coactivation, L-HPA reactor profile as comparison

Measure	Females				Males			
	β	<i>SE</i>	<i>t</i>	Odds (Inv)	β	<i>SE</i>	<i>t</i>	Odds (Inv)
ANS coinhibition, L-HPA non-reactor								
Pregnancy and delivery complications	-0.19	0.39	-0.50	0.83 (1.21)	0.26	0.32	0.83	1.30 (0.77)
Prenatal smoking	--	1.80	1.41	2.55 (0.39)	--	4.52	1.25	5.65 (0.18)

Note. Odds ratio in parentheses represents the odds of membership in the ANS coactivation, L-HPA reactor profile.

* $p < .05$

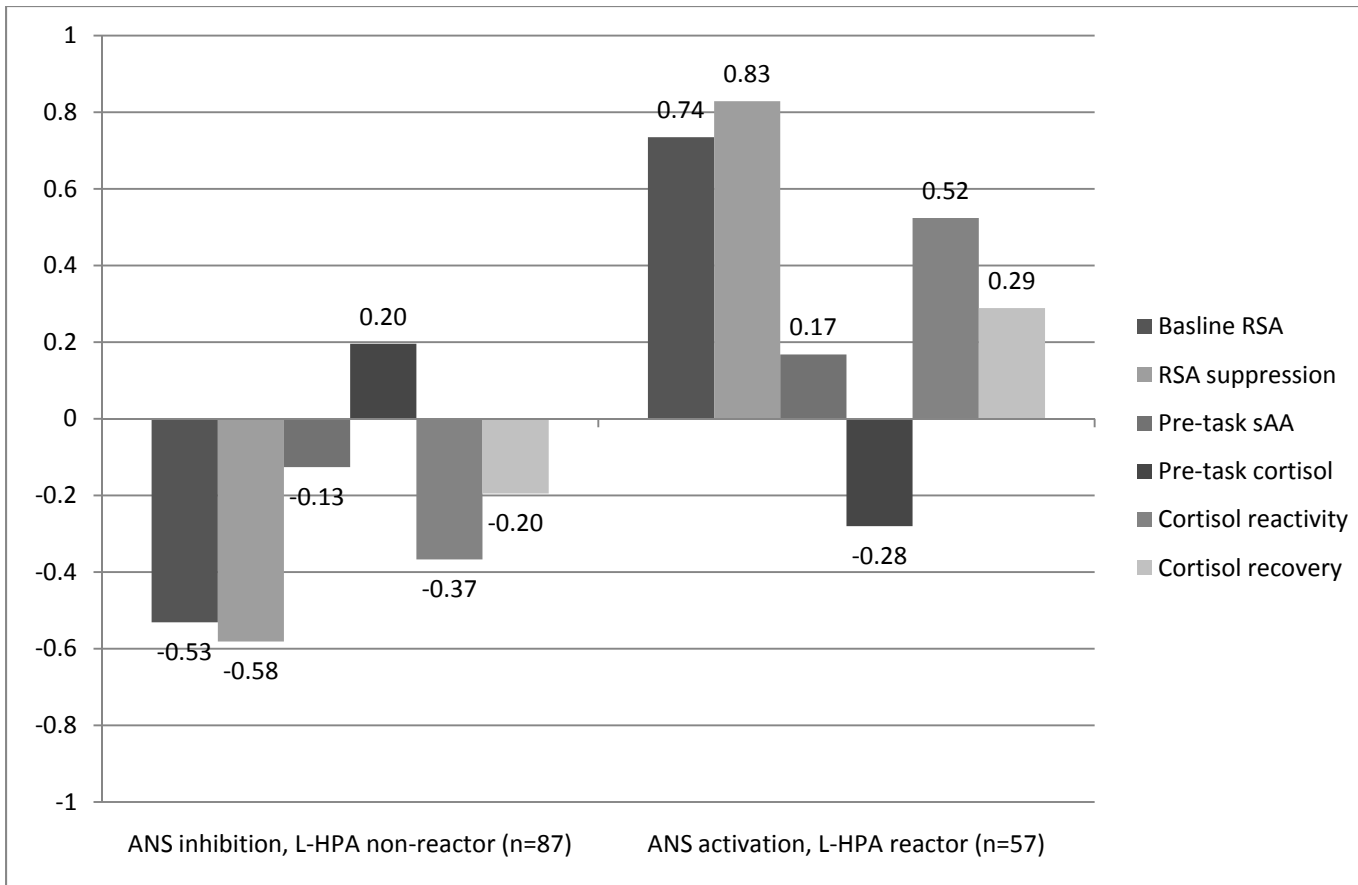


Figure 3.1. ANS and L-HPA activity profiles at 7-months of age

CONCLUDING REMARKS

The current dissertation project advances the literature on autonomic nervous system (ANS) development in infancy and toddlerhood and ANS-behavior associations by investigating patterns of parasympathetic and sympathetic nervous system activity with a latent profile modeling approach, at a single measurement occasion and over time, and the prediction of emotion regulation and early childhood behavior problems by ANS profile. Although the idea is generally conceived that individual differences in the expression and regulation of emotion are functionally dependent on the state of the autonomic nervous system (Porges, Doussard-Roosevelt, & Maiti, 1994), past research has been limited in examining inhibitory mechanisms of the parasympathetic functioning, or excitatory mechanisms of sympathetic functioning, separately.

Findings across the three studies demonstrate the advantage of measuring the response dynamics of the parasympathetic nervous system, indexed by respiratory sinus arrhythmia (RSA), and sympathetic nervous system, indexed by salivary alpha-amylase (sAA). At the bivariate level, single system functioning was not related to concurrent emotion regulation behavior or later behavior problems (Study 1, 2). However the multi-system latent physiological profile predicted differences in self-soothing and attention regulation behavior, controlling for intensity of negative emotional reactivity (Study 1). Further, longitudinal patterns of RSA and sAA development from infancy to 2-years of age predicted differences in emotional symptoms and total behavior problems at 3-years of age (Study 2). Taken together, these findings emphasize the salience of investigating interactions among multiple physiological systems implicated in autonomic control. Studies employing a multi-system approach to understanding physiological arousal and flexibility have been termed a ‘new generation of studies’ better poised

to elucidate physiology-behavior links, re-conceptualizing risk and protective factors for early mental health problems (El-Sheikh et al., 2009).

Physiological sensitivity to context during the prenatal period (Study 3) and early postnatal social environment (Study 2) was also supported by findings from the dissertation project. In Study 2, prenatal exposure to cigarette smoking was associated with increased odds of being classified in a 7-month physiological profile characterized by ANS coinhibition, low basal cortisol, and attenuated cortisol reactivity in comparison to the ANS coactivation, L-HPA reactor profile (Study 2). In Study 3, higher social advantage in the early environment was associated with increased odds of being classified in a longitudinal profile characterized by normative increasing RSA and sAA from 7- to 24-months in comparison to longitudinal profiles that represented some deviation in this increasing pattern from 15- to 24-months. These findings from Study 2 and 3 suggest that environmental factors impact ANS and L-HPA activity early in life.

In addition, these findings highlight the benefits of employing latent profile analysis to explore meaningful differences in patterns of physiological functioning that are not directly observable and must be inferred from the data. Latent profile analysis is a strong, person-oriented methodological tool for researchers interested in identifying distinct subgroups of children with similar patterns of physiological arousal and regulation. This method is superior to population level aggregate associations, which are faulted in their potential to obscure group-based differences, and variable-centered grouping techniques, which are limited by using arbitrary cutoffs to demarcate population subgroups (Bergman & Magnusson, 1997). Latent profile analysis improves upon these approaches by utilizing continuous indicators to generate the latent profile solutions within a formal statistical model, therefore, not requiring any

classification decisions be made by the investigator where individuals may be forced into groups in error (Muthén, 2001). Further, it also utilizes a maximum likelihood estimation procedure to handle missing data, avoiding typical listwise deletion (Little & Rubin, 1987).

The findings from this dissertation project offer several suggestions for future research. First, although RSA and sAA activity during a single measurement occasion was informative, it would be ideal to have a contemporaneous assessment of these indicators to fully appreciate the interactive influence on net physiological arousal, both at a basal state and in response to specific challenge perturbations. Second, it would be advantageous to include more measurement occasions for examining RSA and sAA development early in life. Study 2 of the dissertation project represents the first study to date examining longitudinal profiles of RSA and sAA in infancy and toddlerhood in a developmental, and low-income, non-urban sample. While the examination of longitudinal patterns of RSA and sAA contribute to the literature, the addition of more measurement occasions would support a growth model analysis and the ability to examine profile growth factors in relation to child behavioral outcomes. Finally, with regard to physiological sensitivity to context, future studies should consider the inclusion of time-varying environmental effects during the prenatal period (Study 3) and early postnatal social environment (Study 2) to garner a better understanding of environmental effects on the calibration of these developing systems.

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Appendix A

Available RSA data cases

6-month RSA	15-month RSA	24-month RSA	Frequency	Percent	Cumulative Frequency	Cumulative Percent
0	0	0	934	72.29	934	72.29
1	0	0	44	3.41	978	75.70
0	1	0	43	3.33	1021	79.02
0	0	1	60	4.64	1081	83.67
1	1	0	34	2.63	1115	86.30
1	0	1	48	3.72	1163	90.02
0	1	1	60	4.64	1223	94.66
1	1	1	69	5.34	1292	100.00

RSA data cases	Frequency	Percent	Cumulative Frequency	Cumulative Percent
0	934	72.29	934	72.29
1	358	27.71	1292	100.00

Appendix B

Available sAA data cases

6-month sAA	15-month sAA	24-month sAA	Frequency	Percent	Cumulative Frequency	Cumulative Percent
0	0	0	121	9.37	121	9.37
1	0	0	95	7.35	216	16.73
0	1	0	83	6.42	299	23.14
0	0	1	51	3.95	350	27.09
1	1	0	166	12.85	516	39.94
1	0	1	109	8.44	625	48.38
0	1	1	203	15.71	828	64.09
1	1	1	464	35.91	1292	100.00

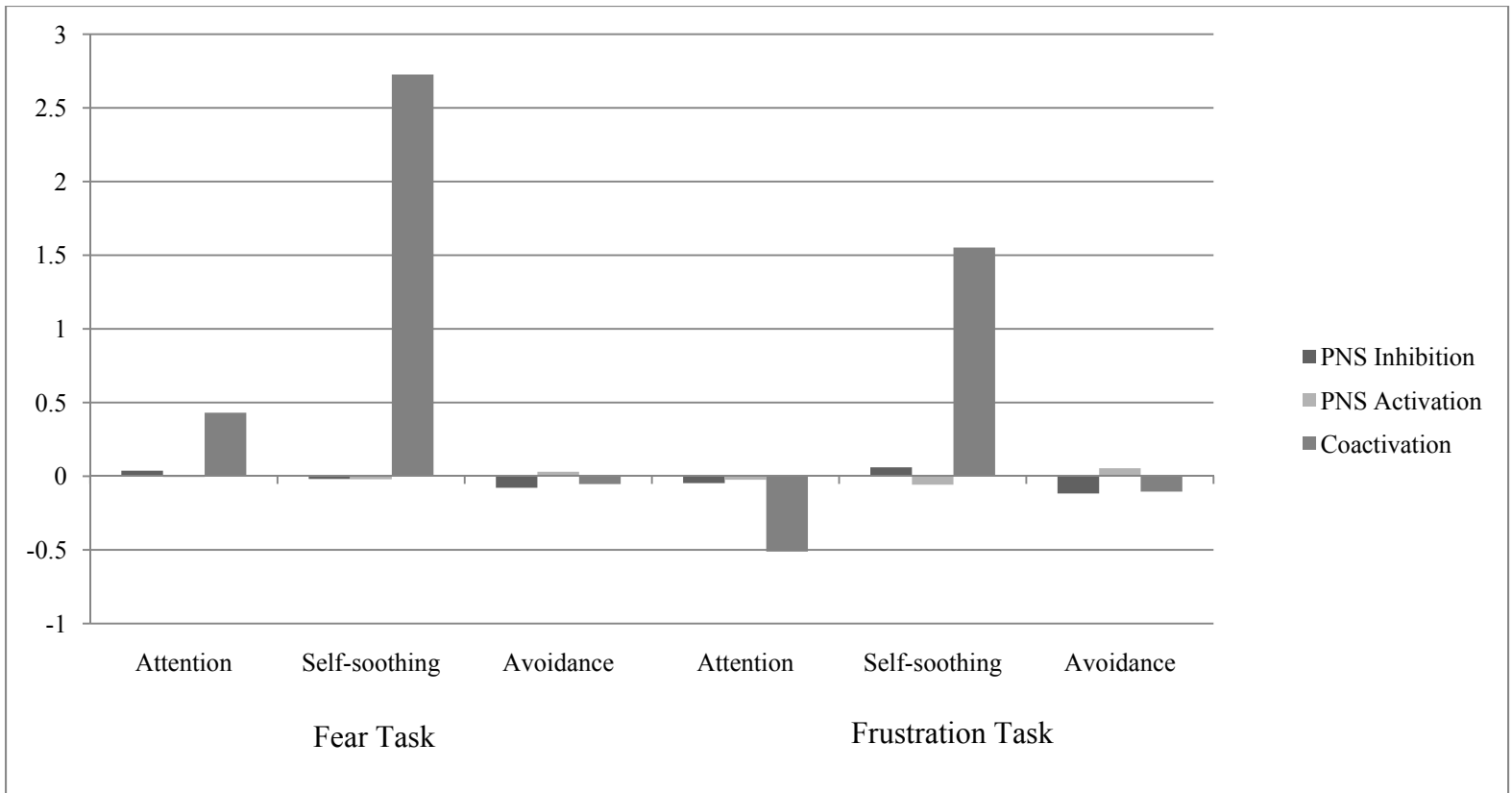
sAA data cases	Frequency	Percent	Cumulative Frequency	Cumulative Percent
0	121	9.37	121	9.37
1	1171	90.63	1292	100.00

Appendix C

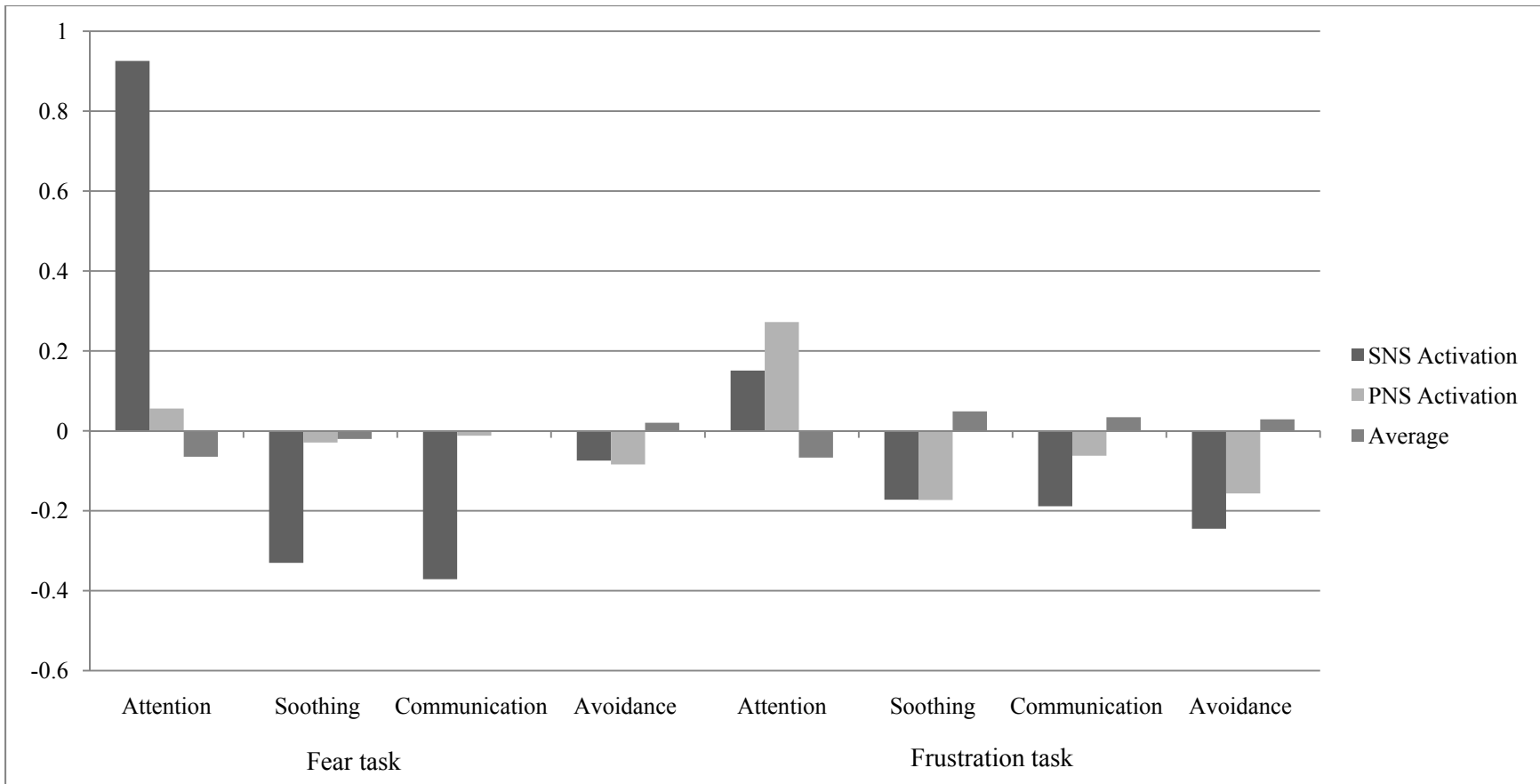
Complete data cases and percent missingness for child physiology and behavior

<i>Measure</i>	<u>7 months</u>		<u>15 months</u>		<u>24 months</u>	
	<i>cases</i>	<i>% missing</i>	<i>cases</i>	<i>% missing</i>	<i>cases</i>	<i>% missing</i>
Income-to-needs ratio	325	0.00 %	341	0.00 %	330	0.00 %
Geographic isolation	309	4.92 %	317	7.04 %	301	8.79 %
Index of social advantage	325	0.00 %	341	0.00 %	330	0.00 %
Child physiology						
Baseline RSA	178	45.23 %	204	40.18 %	227	31.21 %
RSA Suppression	175	46.15 %	200	41.35 %	214	35.15 %
sAA pre-task	230	29.23 %	274	19.65 %	218	33.94 %
Child emotion reactivity						
Fear task negative reactivity	291	10.46%	267	21.70%	238	27.88%
Frustration task negative reactivity	251	22.77%	300	12.02%	254	23.03%
Child emotion regulation behavior						
Fear task						
Attention	293	9.85 %	269	21.11 %	246	25.45 %
Self-comforting	293	9.85 %	269	21.11 %	246	25.45 %
Communication ^a	---	---	269	21.11 %	246	25.45 %
Avoidance	293	9.85%	269	21.11 %	246	25.45 %
Frustration task						
Attention	253	22.15 %	261	23.46 %	262	20.61 %
Self-comforting	253	22.15 %	261	23.46 %	262	20.61 %
Communication ^a	---	---	261	23.46 %	262	20.61 %
Avoidance	253	22.15 %	307	9.97 %	262	20.61 %

Note. ^a Communication regulation behavior was not coded at the 7 month assessment.



Appendix D. Group differences in emotion regulation behaviors controlling for reactivity intensity by 7-month ANS profile.



Appendix E. Group differences in emotion regulation behaviors controlling for reactivity intensity by 15-month ANS profile.

VITA
Kristin M. Voegtline

EDUCATION

- Ph.D. Human Development & Family Studies, 2010
The Pennsylvania State University
Dissertation: “*Physiological bases of emotion regulation in young children living in low-income, rural communities*”, Chair: *Dr. Cynthia A. Stifter*
- M.S. Human Development & Family Studies, 2007
The Pennsylvania State University
Thesis: “*Preterm infant temperament and maternal symptomatology in the context of rural poverty*”, Chair: *Dr. Cynthia A. Stifter*
- B.S. Human Development & Family Studies, 2004
Graduation with distinction, emphasis in Child Development
University of Wisconsin-Madison

AWARDS AND HONORS

- 2009 – 2010 Joachim Wohlwill Endowment in Human Development and Family Studies
The Pennsylvania State University
- 2008 – 2009 Prevention Research Center Graduate Fellow
The Pennsylvania State University
- 2005 College of Health and Human Development Scholarship
The Pennsylvania State University

RESEARCH POSITIONS

- 2009 – 2010 **Graduate Research Assistant:** The Back to Baby Basics Project
- 2004 – 2009 **Graduate Research Assistant:** The Family Life Project
- 2002 – 2004 **Undergraduate Research Assistant:** The Development of Self-Regulation in High-Risk Infants Project

PUBLICATIONS

- Voegtline, K. M.,** Stifter, C. A., & the Family Life Project Key Investigators (in press). Preterm birth, maternal symptomatology, and infant negativity in the context of rural poverty. *Infant Behavior & Development*.
- Voegtline, K.M.,** Stifter, C.A., Blair, C.B., Granger, D.A., & the FLP Investigators (manuscript in preparation). Latent profiles of autonomic and neuroendocrine functioning and emotion regulation in young children living in low-income communities.
- Voegtline, K.M.,** Stifter, C.A., Blair, C.B., Granger, D.A., & the FLP Investigators (manuscript in preparation). Profiles of infant autonomic and neuroendocrine functioning: Contributions of prenatal exposure to cigarette smoking.
- Voegtline, K.M.,** Kivlighan, K.T., Granger, D.A., Blair, C. B., & Stifter, C.A. (manuscript in preparation). Adrenocortical sensitivity of preterm infants to maternal depression in a rural, low-income sample at 6, 15, and 24 months.