The Pennsylvania State University

The Graduate School

College of Health and Human Development

DYADIC STRESS VULNERABILITY IN MOTHER-INFANT DYADS: SYMPATHETIC NERVOUS SYSTEM AND ADRENOCORTICAL REACTIVITY TO INFANT CHALLENGE

A Thesis in

Biobehavioral Health

by

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Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

December 2006

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ABSTRACT

The mother-infant relationship is bi-directional with implications for both infant and maternal health, behavior, and physiology. The current study examined the impact of the mother-infant relationship on infant and maternal stress physiology through examination of sympathetic and adrenocortical responses to infant challenge. A sample of 284 mother-infant dyads was selected from families participating in a large, multi-site study of child development under conditions of rural poverty. During a home visit, mothers and infants participated in a free-play session and a series of infant challenge tasks to elicit distress to novelty and frustration. Observational coding of maternal sensitivity, intrusiveness, and detachment were conducted from digital recordings of the free-play and infant behavioral reactivity and regulation were coded from DVDs of the challenge tasks. Saliva samples were collected before, 20-, and 40-minutes after the challenge tasks and assayed for salivary α-amylase (sAA) and cortisol. Symptoms of maternal depression and anxiety were assessed using the Brief Symptom Index (BSI). A growth mixture modeling technique was applied to sAA and cortisol data. Analyses were controlled for sampling time of day, demographic factors, health behaviors, and medication use. In general, there were more associations with infant and maternal behaviors for infant physiology than for maternal stress physiology. Infant reactivity and regulatory behaviors interacted to predict infant sAA and cortisol response to challenge. Infants displaying both high behavioral reactivity and regulatory behavior were more likely to have low, non-reactive sAA levels and cortisol reactivity with recovery. In contrast, low reactive infants (i.e., little fussing) displaying few regulatory behaviors tended to show sAA increases and were less likely to display cortisol reactivity. Infants of highly detached mothers tended to display low and non-reactive sAA levels, while infants of highly anxious mothers more often showed sAA increases. Mothers of boys were more likely to have higher baseline cortisol levels than mothers of girls. Physiological attunement in mother-infant dyads was found for baseline levels of sAA and cortisol, but not between profiles of reactivity to the challenge. The present study has implications for understanding how early mother-infant relationships in high-risk environments can influence the development of stress physiological systems.

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ABBREVIATIONS

ACTH – adrenocorticotrophic hormone

ANS – autonomic nervous system

AvePP – average posterior probabilities

BIC – Bayesian Information Criteria

CBG – cortisol binding globulin

CRH – corticotropin releasing hormone

HPA – hypothalamic-pituitary-adrenal

NBAS – Neonatal Behavioral Assessment

NSAID – nonsteroidal anti-inflammatory drug

OTC – over-the-counter

PEP – pre-ejection period

PNS – parasympathetic nervous system

PVN – paraventricular nucleus

PVZ – paraventricular zone

sAA – salivary α-amylase

SIDS – sudden infant death syndrome

SNS – sympathetic nervous system

ACKNOWLEDGEMENTS

I would like to acknowledge the many individuals without whom this work would not be possible. In particular, I would like to extend my gratitude to my graduate advisor Douglas Granger for the time and care he took with guiding my professional development. I also extend my gratitude to my committee members Elizabeth Susman, John Graham, Clancy Blair, and Laura Klein for their guidance in the development of this project over the last 3 years. Thanks also to the Family Life Project Investigators for a career-shaping experience, to the NICHD and the Penn State Children, Youth, and Families Consortium for support of this research, to Salimetrics for contributions of funds, reagents, and for welcoming me into your family, to my fellow graduate students Birdie Shirtcliff, Nancy Dreschel, and Leah Hibel for sharing this experience with me, and to the many undergraduate students it was my pleasure to work with, especially Michael Coccia and Seon Claire Kang.

DEDICATION

For my mother and father, in thanks for their many years of love, encouragement, and help with homework. You always believed I would reach this goal.

For Mark, in thanks for your love and for being my greatest friend and supporter during my years in graduate school.

Chapter 1

OVERVIEW OF THE PROBLEM

The mother-infant relationship is bi-directional in nature with implications for both infant and maternal health, behavior, and physiology (Field, 1992, Papousek & von Hofacker, 1998; Stern, 1985; for review see Harrist & Waugh, 2002). In particular, the influence of maternal caregiving behavior and mother-infant attachment on the development of infant stress responsive physiology has been a key area of study for the last 15 to 20 years (for review, see Gunnar & Donzella, 2002). Less work has examined the implications of this primary social relationship for maternal physiological responses to stress. In addition, much of the research on stress physiology during infancy has focused exclusively on the activity of the hypothalamic-pituitary-adrenal (HPA) axis, whereas concurrent examination of both sympathetic nervous system (SNS) and HPA axis responses to stress might provide a more complete representation of individual differences in physiological responsivity to stress in early development (Henry, 1992). The current study attempts to address these gaps in our knowledge through examination of the impact of key aspects of the mother-infant relationship on both sympathetic and adrenocortical reactivity to infant challenge in a sample of 6 month-old infants and their mothers. In addition, the possibility of "attunement" or an association between infant and maternal physiological stress reactivity will also be explored. Such an analysis will enhance our understanding of how early social environments shape the development of stress physiological systems.

Developmental science has long recognized the importance of context for human development. This phenomenon was central to the work of some of the most well known theorists of the last century (Bowlby, 1969; Erikson, 1950; Freud, 1905) and continues to be relevant to contemporary developmental research. Individuals are viewed as being embedded in a complex, person-environment system (Cairns, Elder & Costello, 1996; Magnusson & Cairns, 1996) and development is believed to be directional, such that interactions with the early environment have implications for all later development (Goldhaber, 2000). As such, modern developmental science emphasizes the need for longitudinal designs, examination of multiple levels of analysis, and the importance of context (Cairns et al.1996). Each level of analysis within a person, whether biological, behavioral, or social, is interdependent and interacts with every other level (Magnusson, 1999).

This ideology has lead researchers to examine the impact of environmental context on the development of stress responsive physiological systems, particularly the HPA axis. Levine and Meaney have demonstrated the importance of early experience (e.g., handling, maternal behavior) for the development of HPA axis function in rat pups (for reviews, see Champagne & Meaney, 2001; Levine, 2000). Several others have extended this work into primate models, demonstrating that both rearing conditions (Higley, Suomi, & Linnoila, 1992) and foraging demands (Rosenblum & Paully, 1984) can result in heightened reactivity to stress in primate infants. Sterling and Eyer (1988) formulated their principle of allostasis based on observations that social forces, such as socioeconomic status, are closely tied to physical health outcomes in human populations. Allostasis allows an organism to adjust its physiology to its environment, as well as to

anticipate necessary changes that allow it to adapt to or modify its environmental context. McEwen and Stellar (1993) built upon this work with their theory of allostatic load, which suggests that wear-and-tear on an organism's physiology is due to repeated activation or inefficient regulation of physiological systems, such as a lack of down-regulation following the abatement of a stressor. These theories integrate evidence from both animal and human studies that early context can "get under the skin" and have long-term effects on both physical and mental health.

Some empirical support for these theories has come from research on the consequences of poverty for child development. Children living under conditions of poverty are more likely to encounter polluted water and air and their homes are often more noisy, crowded, and of poorer quality than are those of wealthier children (Evans, Gonnella, Marcynyszcyn, Gentile, & Salpekar, 2005). These children are also more likely to be reared in chaotic and unstable family environments, to be separated from their families of origin, and to receive unresponsive and authoritarian parenting than more economically advantaged children (Evans, 2004; McLoyd, 1998). In addition, children living under conditions of rural poverty experience the same, if not greater, risks as their urban counterparts (Hernandez, 1993; Sherman, 1992). Over time, the experience of poverty appears to influence the functioning of stress physiological systems. Elevated levels of cortisol, epinephrine, norepinephrine, and resting blood pressure have been observed among children living under conditions of poverty (Evans & English, 2002; Evans, 2003; Lupien, King, Meaney, & McEwen, 2001) and the accumulation of poverty related risks was found to be linked to the altered functioning in these stress physiological systems (Evans, 2003). While these studies provide evidence that the long-term effects

of poverty on stress physiology can be observed in middle childhood, few studies have examined the impact of impoverished environments on the development of stress physiology during infancy.

Additional evidence of the importance of early context has come from research focused on the effects of the family environment on HPA axis function. For example, Cicchetti and Rogosh (2001) have observed altered diurnal cortisol patterns in maltreated boys as compared to control subjects. Bugental, Martorell, and Barraza, (2003) also found greater cortisol reactivity to maternal separation in infants who received regular corporal punishment and elevated baseline cortisol levels for infants of emotionally withdrawn mothers. Theoretically, healthy and supportive early environments provide a context in which children can develop emotional security and trust in their physical safety and well-being. In contrast, "risky" or unhealthy contexts undermine feelings of safety or security, are characterized by conflict or cold, unsupportive, or neglectful relationships, and may also include abuse or violence (Repetti, Taylor, & Seeman, 2002; Taylor, Repetti & Seeman, 1997). These family environments are believed to disrupt both psychosocial functioning and regulation of stress responsive physiological systems leaving children vulnerable to the development of both mental and physical health disorders later in life (Repetti et al., 2002; Taylor et al., 2004). Thus, there is mounting evidence that early social contexts can have long-term implications for the functioning of stress physiological systems.

Given the heightened risk for unstable, unsupportive family environments and the observed physiological costs for children of living under conditions of poverty, a better understanding of how the mother-infant relationship confers risk or resilience on both

members of the dyad is needed. During infancy, a child's relationship with the primary caregiver is the core of the early social environment (Hofer, 1994) and maternal parenting behavior may act as a filter, either buffering or magnifying the stresses of the larger social context, such as the stress of living in poverty, on a child (Belsky, 1984). Much work has already investigated the importance of the mother-infant relationship for the development of stress responsive physiology. Researchers have previously examined the influence of maternal caregiving behavior and attachment on the HPA axis activity in young children (for review, see Gunnar & Donzella, 2002). However, this work often focuses exclusively on the effects of the mother-infant relationship on infant stress reactivity. To our knowledge, few studies have examined the impact of this relationship on maternal stress physiology. While it is true that the relationship with the primary caregiver dominates the infant's social world, the mother-infant dyad represents a dynamic or ever-changing system with bi-directional influences on the development of the dyadic relationship (Field, 1992). Thus, this relationship may also have important implications for maternal stress physiology and subsequently, the capacity of the mother to provide future sensitive care to her child.

Each member of the dyad possesses unique characteristics and experiences, which may influence the development of mother-infant relationship quality and stress physiology. Previous research has identified infant temperament, maternal sensitivity, and psychopathology as important contributors to mother-infant relationship quality (Beckwith, Rozga, & Siman, 2002; Ijzendoorn & Bakermans-Kranenburg, 2004). Many of these factors also influence the development of infant stress physiology. In particular, the complex link between infant temperament and physiological reactivity has been well

studied (Kagan, Reznick, & Snidman, 1987; Lewis & Ramsay, 2005; Stansbury & Gunnar, 1994). Warm and sensitive maternal care has been found to be supportive of the development of both stress responsive and emotional regulatory systems in children (for review, see Gunnar & Donzella, 2002). There is also a large body of literature addressing the impact of maternal psychopathology, especially depression, on the development of physiological responses to stress in mother-infant dyads (Field et al., 2000; Goodman & Gotlib, 1999). There is some evidence that infant behavior can also influence maternal responses to stress. Persistent crying has been found to increase maternal stress and depression (Papousek & von Hofacker, 1998).

Theoretically, the shared experiences of the daily challenges faced by the mother-infant dyad are believed to shape the development of stress responsive physiological systems in mother and infant simultaneously (Field, 1992). Behavioral and physiological synchrony might be the outcome of these mutual influences. The importance of behavioral synchrony, characterized by mutual regulation and reciprocity, for the mother-infant relationship has been well established (for review, see Harrist & Waugh, 2002). A similar attunement in physiological responses to stress in mother-infant dyads has also been suggested (Field, 1992; Sethre-Hofstad, Stansbury, & Rice, 2002). According to Field (1992), the behavioral and physiological systems of each dyad member should become "attuned" over time as each partner continually modulates the other's level of arousal. Thus, the multiple harmonious interactions and shared affective states that occur during daily activities form the basis for mother-infant dyadic relationship (Field, 1987; Tronick & Giannino, 1987). The current study will focus on determining which aspects (i.e., infant or maternal behaviors or characteristics) of this bi-directional relationship are

most important for the co-development of the stress responsive physiology in mother and child.

Finally, most of the work examining the impact of the mother-infant relationship on the development of stress physiology has focused on the activity the HPA axis (Granger, Kivlighan, et al., 2006). However, the psychobiology of the physiological stress response includes both the sympathetic branch of the autonomic nervous system (SNS) and the hypothalamic-pituitary adrenal (HPA) axis (Chrousos & Gold, 1992). Research has shown that individual interpretation of stressors has the capacity to differentially influence the responsiveness of these two physiological systems (Frankenhaueuser, Lundberg, & Forman, 1980; Lundberg & Frankenhaueuser, 1980; Henry, 1992). Thus, focus on the activity of the HPA axis in isolation may provide only partial information about an individual's response to stress. While the SNS and the HPA axis do not always act in concert, there are physiological connections between these systems at the level of the hypothalamus (Chrousos & Gold, 1992). Therefore, the activity of each system provides context for the other, and examining the activity of both will expand our understanding of stress and its underlying physiology. Considering the physiological context, as well as the psychological, and social contexts of the HPA axis is responsive to the recent arguments that our developmental models of biosocial phenomena need to include multiple measurements of biological processes (Donzella, Gunnar, Krueger, & Alwin, 2000; Granger & Kivlighan, 2003; Repetti et al., 2002).

The purpose of the current project is to build upon existing knowledge regarding the influence of the mother-infant relationship on the development of stress physiology in mother-infant dyads. In particular, this study will contribute uniquely to the literature

through examination of the impact of mother-infant relationships on both infant and maternal stress physiology and through concurrent examination of sympathetic and adrenocortical responses to dyadic challenge in a sample of low-income mothers and their 6-month old infants. Specifically, the goals of this project are 1) to determine if infant behaviors (i.e., behavioral reactivity and emotion regulation) and characteristics (i.e. infant gender) are associated with sympathetic and adrenocortical responses to challenge in mother-infant dyads early in infancy, 2) to determine if maternal behaviors (i.e., maternal caregiving behavior) and symptoms of psychopathology (i.e., depression or anxiety) influence sympathetic and adrenocortical responses to challenge in mother-infant dyads, and 3) to determine if there is attunement in mothers' and their infants' physiological (i.e., sympathetic or adrenocortical) responses to child challenge early in infancy.

Chapter 2

STRESS: PHYSIOLOGY AND DEVELOPMENT

Evolution has equipped the human body to deal with challenge, increasing our chances of survival. The challenges we face today differ greatly from those that confronted our ancestors. Rather than relying on strength and speed to survive acute threats to our survival, stress in developed countries today is chronic, whether it is the challenge of being cared for by a depressed mother, succeeding in a demanding workplace, or dealing with the challenges of living in poverty (Sapolsky, 1998). Modern society and medicine have greatly extended the lifespan. This is a relatively new development in evolutionary history and while evolution has equipped us to deal with stress in the short term by capitalizing on ensuring survival until we reach reproductive age, our adaptations to challenges in early life are not without costs to health later in life (Sapolsky, 1998). In other words, influences early in development, even during the prenatal period, may prepare our physiological systems to deal with a difficult and challenging environment, but early stress may also result in a reorganization of our physiology leading to a host of health problems, including cardiovascular disease, cancer, and depression (McEwen, 2003; McEwen & Seeman, 1999). As such, it becomes important to conceptualize stress physiological systems as developmental in nature and to begin to understand the myriad contextual influences that shape their development with consequences across the lifespan. Specifically, aspects of the mother-infant relationship may be especially salient for the development of stress physiological systems during early childhood.

Our understanding of the nature and purpose of the stress response has continually evolved since the inception stress research. The field of stress research began with Cannon's work on the concept of homeostasis. Homeostasis is achieved within an organism through maintenance of a constant internal environment (Cannon, 1932). Yet, in times of emergency, the body must take more extreme measures to maintain stability. Cannon labeled the behavioral and physiological responses to threat, including elevations in heart rate and respiration, the "flight or flight" response (Cannon, 1929). Hans Selye continued to refine the concept of stress with the suggestion that physiological responses to all "noxious agents" or stress are non-specific and proceed through a series of stages depending on the duration of the stressor and the resources available to the organism. Specifically, he theorized that acute stressors produce distinct physiological patterns from chronic stressors (Selye, 1950).

The concept of stress continues to be refined by contemporary researchers today. According to Sapolsky (1998), the working definition of a stressor is "anything that throws your body out of homeostatic balance." Examples of stressors include physical challenges such as injury or extreme temperatures, and psychological stressors, such as test anxiety or social rejection. Chrousos, Torpy, and Gold (1998) add that "stress is the recognition by the body of a stressor and therefore, the state of threatened homeostasis; and adaptive responses are the body's attempt to counteract the stressor and reestablish homeostasis." These definitions of stress emphasize that stress results in disruption of homeostasis, but this concept of stress is mechanistic in nature. Regulation of homeostasis in this sense depends upon maintaining pre-established parameters solely through the use of negative feedback. However, to resist change, especially in the face of

an ever-changing environment, is untenable. Instead, Sterling and Eyer (1988) have suggested that an alternative conceptualization of stress and our physiological regulation of stress may be in order. Allostasis refers to an organism's maintenance of stability through adjustment of internal physiological parameters (e.g. hormones) in response to the demands of the organism's environment (Sterling & Eyer, 1988). It is through the process of allostasis that development and change can occur in stress responsive physiological systems across the lifespan. McEwen and Seeman (1999) have laid out key features for this process. The brain coordinates both the behavioral (e.g., health behaviors) and the physiological responses when a person is challenged. However, this integrative process does not occur outside of the personal history of the individual. A myriad of influences, such as genetic predisposition and early developmental experiences contribute to individual differences in an person's ability to cope with challenges, predisposing an individual to display a specific pattern of both behavioral and physiological responses to stress.

Allostatic load refers to wear-and-tear on an organism's physiology due to repeated physiological adaptation to environmental stress (i.e. cycles of allostasis) or inefficient self-regulation of these systems (i.e., over or under-activation; McEwen & Stellar, 1993). Such damage can result in an accelerated aging process, as well as many of the major diseases of western society including cardiovascular disease, cancer, and diabetes (McEwen & Seeman, 1999). Thus, allostasis and allostatic load are the products of the interplay of influences at multiple levels of organization, including genetic, physiological, psychological, and social-environmental phenomena over time.

These continuous processes occur across our entire lives, from birth to death, and it is through these means that our environmental contexts, especially our close social relationships, can shape physiological responses to challenge across the lifespan. Thus, stress responsive physiological systems do not mechanistically and non-specifically respond to stressors in order to rigidly maintain a narrow homeostatic set point. Instead, these systems are developmental in nature and due to the rapid changes occurring during the first years of life, early environmental influences may have a greater impact on the functioning of these systems than at any other time in the lifespan (Dawson, Ashman, & Carver, 2000). Therefore, in order to understand how early social influences, such as the mother-infant relationship, can have long-term implications for stress responsive physiological systems, it is essential that the structure, function, and early developmental processes of the stress responsive physiological systems are well understood. In addition, while methodological advances enhance our ability to study these systems under a variety of conditions, stress researchers should be also aware of potential limitations to these technologies as we continue to push the boundaries of stress research.

Stress Responsive Physiological Systems

When confronted with challenge, a host of physiological changes occur, including shifts in attention, elevated metabolic rate, and decreased vegetative activity (Cannon, 1929; for review, see Nelson, 2000). The majority of these changes are largely coordinated by the activity of the two major stress responsive systems, the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis. The ANS is further divided into two branches, the sympathetic nervous system (SNS) and the

parasympathetic nervous system (PNS; Thompson, 2000). Knowledge of the structure and function of both stress responsive systems may result in a more complete understanding of the role of sympathetic and adrenocortical responses to stress in mother-infant dyads.

Structure and Function of the Autonomic Nervous System

The ANS monitors and controls the functioning of the internal environment via the antagonistic actions of its sympathetic and parasympathetic branches (Thompson, 2000). The SNS is responsible for the familiar physiological changes associated with the classic "fight-or-flight" response, including elevated heart rate, blood pressure, increased perspiration, and reduced salivary production (Cannon, 1932). These changes are mediated through direct sympathetic innervation of target organs and by the release of the catecholamines, epinephrine and norepinephrine, from the adrenal medulla into the bloodstream (Nelson, 2000; Thompson, 2000). Spillover of norepinephrine from synaptic sites with target tissues constitutes an additional major source of this neurohormone in the bloodstream (Griffin & Ojeda, 2000). Both epinephrine and norepinephrine are sympathomimetics and binding to receptors on the target tissues results in the same effects as direct innervation, such as increased heart rate and blood pressure (Thompson, 2000). Finally, given the speed of nerve conduction, the time course of sympathetic activation occurs on the order of seconds, and the effects subside relatively quickly due to the short half-life of catecholamines (minutes; Sapolsky, 1998).

In contrast, the activity of the PNS is counter-regulatory to that of the SNS. The role of the PNS is to regulate vegetative functions, such as digestion, as well as to reduce metabolic strain on the body (for reviews, see Thompson, 2000). As such, the PNS

reduces heart rate, dilates blood vessels to reduce blood pressure, redirects blood flow to the extremities and gastrointestinal tract, increases the production of saliva and peristalsis, and regulates sexual function (Nelson, 2000). During times of stress, the PNS will also withdraw its influence over organs, such as the heart, in order to prepare the organism to deal with the challenge at hand (Porges, 1998).

Structure and Function of the Hypothalamic-Pituitary-Adrenal Axis

Slower, long-term responses to challenge are mediated via the activity of the HPA axis (Chrousos & Gold, 1992). Corticotrophin-releasing hormone (CRH) produced by the paraventricular nucleus (PVN) of the hypothalamus is released into the portal blood supply between the hypothalamus and the anterior pituitary gland. CRH stimulates the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary into the general circulation. Cortisol, the major end product of the HPA axis, is then released from the adrenal cortex in response to ACTH (Chrousos & Gold, 1992; Thompson, 2000). The HPA axis is a self-regulatory system and a negative feedback loop maintains cortisol levels within a specific range. Receptors for cortisol are located in the hippocampus and at the level of the hypothalamus and pituitary. Stimulation of these receptors down-regulates the activity of the HPA axis, resulting in lower cortisol levels downstream (Nelson, 2000; Thompson, 2000).

Like many other physiological systems, the HPA axis displays a characteristic diurnal rhythm (Thompson, 2000). Cortisol levels are high upon waking, reach their peak approximately 30-minutes after waking, and then gradually decrease until they reach their nadir in the late evening. While sleeping, cortisol levels begin to rise again a few hours before waking (Griffin & Ojeda, 2000). The HPA axis is also reactive to

perceived environmental challenges. However, given that cortisol has a longer half-life (70-90 minutes) than the catecholamines, the time course of HPA axis activation is longer than that of the SNS (Griffin & Ojeda, 2000). Cortisol levels peak at approximately 15 to 20-minutes post stressor (Sapolsky, 1998), and return to baseline around 40-60 minutes (Dickerson & Kemeny, 2004). It has been suggested that the purpose of this extended response to stress is to aid recovery from stress or to prepare the body for the next stressor (Sapolsky, 1998).

The primary function of cortisol is to elevate glucose levels in the blood, making energy stores available so that an organism can deal with the challenges at hand. It also stimulates the breakdown of proteins into amino acids, facilitates tissue repair, enhances memory formation, and regulates blood pressure (McEwen & Seeman, 1999; Thompson, 2000). Thus, cortisol has protective effects for an organism in the short term, but can cause damage with repeated exposure or chronically elevated levels. Chronically high levels of cortisol can lead to abdominal obesity, neuronal atrophy, and immunosuppression (McEwen & Seeman, 1999). Early in development, cortisol may also have the propensity to alter brain development. During infancy, there is a great deal of plasticity in developing nervous system resulting from rapid synapse formation during the first 2 to 3.5 years of life (Huttenlocher, 1994; Huttenlocher & Dabholkar, 1997; Rakic, Bourgeois, & Goldman-Rakic, 1994; for review, see Dawson et al., 2000). Glucocorticoids regulate metabolism, physiology, and gene expression in several brain regions, but especially in areas of the hippocampus linked to memory formation (Heffelfinger & Newcomer, 2001). These effects appear to be curvilinear with both extremely low and high levels of glucocorticoids suppressing neuronal activity (Joels &

de Kloet, 1994) and resulting in permanent negative consequences for brain functions, such as memory formation (Heffelginfer & Newcomer, 2001). Thus, the influence of early environments on the function of the HPA axis may have especially serious implications for future behavioral and cognitive development.

Interactions Between the ANS and the HPA Axis

While the autonomic nervous system and the HPA axis do not always act in concert, both are directly controlled by the same region of the hypothalamus, the paraventricular zone (PVZ). As mentioned previously, CRH originates in the paraventricular nucleus (PVN) located in the center the PVZ. Descending axons from the PVN also synapse with motor neurons of the ANS in the spinal cord as well as with brainstem nuclei that coordinate the activity of both branches of the ANS (Thompson, 2000). The vagus nerve, a major parasympathetic fiber also projects back to the PVN and is hypothesized to provide feedback about autonomic activity. Taken together with the negative feedback from cortisol receptors in the hypothalamus, it is clear that this brain region controls and monitors the activity of both major stress responsive systems. The PVN also receives input from higher brain centers, such as the hippocampus, amygdala, and prefrontal cerebral cortex. Thus, information about perceptions of stress or challenge is also received by the hypothalamus (Thompson, 2000). With so many physical connections between these physiological systems, it is not surprising the activity of one system can affect the activity of the other. There appears to be a functional positive feedback loop between the SNS and the HPA axis such that activation of one will promote the activation of the other (Chrousos & Gold, 1992). It has also been suggested that the role of glucocorticoids, such as cortisol, may be to suppress the activity

of the SNS rather than to augment it (Munck, Guyre, & Holbrook, 1984). Thus, the HPA axis may also protect the body from the long-term damage due to stress. In summary, there appears to be multiple connections between the SNS and the HPA axis and these connections appear to influence the functioning of these systems. While the physiological links between these systems are fairly well understood, the functional interaction of these systems, whether coordinated or dissociated, is less well understood (Bauer, Boyce, & Quas, 2002). The implications of these interactions will be discussed further in a later section of this review.

Development of Stress Responsive Physiological Systems

The activity of the ANS and the HPA axis show change and development across the human lifespan. However the most obvious shifts in the functioning of these systems mostly occur early in childhood. It is because of the rapid development and plasticity in these systems early in life, that early social and environmental influences have the greatest propensity for permanent reorganization of their functioning at this time (Dawson et al., 2000; Susman, 2006). The age-related trends in the functioning of these stress responsive physiological systems from birth are described below.

Development of the SNS in Childhood

The development of the ANS begins during fetal life and continues beyond birth. As such, early postnatal experiences have the potential to impact the development of the SNS and permanently alter the functioning of this system (Young, 2002). Research on the development on autonomic activity in humans is limited, but suggests this system does not reach adult levels of activity until adolescence or adulthood. While heart rate is

not a pure measure of sympathetic activity, several studies have observed decreases in heart rate over the first year of life and into adulthood (Bar-Haim, Marshall, & Fox, 2000; Porges, Doussard-Roosevelt, Portales, & Suess, 1994; Stifter & Jain, 1996). In contrast, parasympathetic activity, as measured by respiratory sinus arrhythmia (RSA), increases from birth until middle childhood (Bar-Haim et al., 2000; Marshall & Stevenson-Hinde, 1998). There have been very few studies of the ontogeny of sympathetic activity in humans, especially during infancy. Very recently, Alkon and colleagues (2006) validated a protocol for the measurement of pre-ejection period (PEP), a measure of sympathetic regulation of heart rate, in 6 and 12 month-old infants. They observed a trend for an increase in mean PEP (i.e., reduction in sympathetic arousal) from 6 to 12 months as well as moderate intra-individual stability in PEP over the 6-month time frame. Similarly, in a study of 3 to 8 year-old children, younger children were found to have higher sympathetic activation (lower PEP) as compared to older children (Alkon et al., 2003). For both studies, PEP reactivity to a stressor was not related to age. Thus, the limited evidence to date suggests that in parallel with the decrease in heart rate with age, resting sympathetic activity may also show age-related decreases and reach adult levels by early adolescence. However, sympathetic reactivity to stress does not appear to be age-related.

Development of the HPA Axis in Childhood

The function of the HPA axis in the newborn differs greatly from that typically observed in the adult. While the free, unbound fraction of cortisol in circulation is similar to that observed in adults (Gunnar, 1992), the total amount of cortisol in the plasma of newborns is much lower. This is because the production of cortisol binding globulin (CBG) is also lower, resulting in more rapid breakdown of cortisol in circulation

(Stahl, Amendt, & Dorner, 1979). In addition, rather than a single morning peak in cortisol levels, most newborns exhibit two peaks approximately 12 hours apart and unrelated to time of day (Rivkees, 2003).

There is much debate about the timing of the establishment of adult-like diurnal pattern in cortisol production with levels highest in the morning and lowest in the evening. Various studies have observed the onset of the diurnal rhythm from as early as 2 weeks to as late as 9 months (Antonini, Jorge, & Moreira, 2000; de Weerth & van Geert, 2002; de Weerth, Zijl, & Buitelaar, 2003; Larson, White, Cochran, Donzella, & Gunnar, 1998; Kiess et al., 1995; Mantagos, Moustogiannis, & Vagenakis, 1998; Onishi, et al., 1983; Spangler, 1991). More recent work suggests that the diurnal rhythm continues to develop well into the preschool years, when afternoon naps become less common (Gunnar & Donzella, 2002; Watamura, Donzella, Kertes, & Gunnar, 2004). Specifically, the expected small decreases in cortisol from late morning to late afternoon are not observed in children until around 4 years of age (Gunnar & Donzella, 2002). Thus, researchers interested in studying adrenocortical activity in young children should not assume the emergence of an adult-like diurnal rhythm until at least 4 or 5 years of age.

In addition to the development of diurnal rhythm of HPA axis activity, there are also developmental shifts in adrenocortical responsiveness to stressors early in human development. Newborns display highly reactive adrenocortical responses to stressors, such as physical exam and heel-stick blood draw, in the first weeks of life (Gunnar, 1989; 1992). Interestingly, repeated exposure to stressors will alter the physiological response (increased or decreased reactivity depends on the stressor type), but behavioral reactivity

will remain at the same level (Gunnar, 1989; 1992; for review see Gunnar & Donzella, 2002). There is mounting evidence that human infants gradually develop a dampened cortisol response to stressors during the first year of life (Gunnar, Broderson, Krueger, & Rigatuso, 1996; Ramsay & Lewis, 1994; for review see Gunnar & Donzella, 2002). Beginning at 12 weeks of age, infants no longer display adrenocortical responses to physical exam even though they continue to show behavioral distress (Larson et al., 1998). While Lewis and Ramsey (1995) observed cortisol reactivity to well-baby exam and inoculation between 2 and 6 months of age the magnitude of both behavioral and adrenocortical reactivity decreased with age. However, there was more stability in both behavioral and physiological reactivity between 4 and 6 months than between 2 and 4 months. Gunnar and colleagues (1996) confirmed this observation, but also observed further decreases in cortisol reactivity between 6 and 15 months. At 15-months, cortisol reactivity to exam and inoculation were no longer observed at the group level. In other domains, social stressors such as separation from a parent result in cortisol reactivity in 9-month old-infants, but not in 13 month-old infants (Gunnar et al., 1992; Gunnar & Nelson, 1994). Stressors designed to elicit distress to wariness or inhibition similarly do not elicit cortisol responses in most toddlers (Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996; Spangler & Schieche, 1998). Interestingly, one study of 12 to 18 month-olds did observe adrenocortical reactivity to maternal separation, but not to exposure to novel events (remote controlled spider, boisterous clown) in the same children (Goldberg et al., 2003). This suggests the timing of hypo-responsiveness to different types of stressors is not absolute. However, in general, the majority of children do appear to enter an adrenocortical hyporesponsive period by the end of the first year. It is unknown how

long the stress hyporesponsive period persists, but is currently hypothesized to last well into the preschool years (Gunnar & Donzella, 2002).

In summary, both the SNS and HPA axis continue to experience developmental change related to maturation until middle childhood or early adolescence. Resting levels of sympathetic activity appear to gradually increase across early childhood, but SNS reactivity does not appear to be age related. The diurnal rhythm of the HPA axis begins to emerge during the first year although there are wide-varying individual differences in its inception. In addition, while cortisol reactivity is strong and dependable during the first 6 months, this reactivity becomes attenuated by the end of the first year. Currently, this stress hyporesponsive period is believed to persist well into the preschool years (Gunnar & Donzella, 2002). Especially during the first year of life, the two major stress responsive physiological systems are undergoing rapid developmental change. As such, there is great potential for the reorganization of their function in response to social and environmental perturbation during this period.

Assessment of Stress Physiological Systems in Saliva

Stress research has rapidly proceeded as methodological advances have allowed the non-invasive assessment of biomarkers of stress reactivity in saliva. Such advances allow the assessment of physiological responses to stress outside of the laboratory environment. Despite the promise of these methods, stress researchers, especially those working with young children, need to be aware of potential limitations to the accurate assessment of these physiological systems of interest.

Assessment of HPA Axis Activity in Saliva

Cortisol is a steroid hormone derived from cholesterol and is not soluble in an aqueous solution, such as blood. In order to travel in the bloodstream, cortisol must be bound to a carrier protein, such as cortisol binding globulin (CBG). Thus, at any given time, the majority of circulating cortisol is inaccessible to receptors on target tissues (Griffin & Ojeda, 2000). As a result, assessment of cortisol in serum reflects the total concentration of cortisol in blood, rather than the portion that is free to act on tissues (Kirschbaum, Read, & Hellhammer, 1992). In addition, venipuncture requires trained medical personnel and can be a painful, stress-inducing procedure with the potential to confound the interpretation of cortisol levels (Kirschbaum &Hellhammer, 1994).

Over the last few decades, assessment of salivary cortisol has proven to be a simple and non-invasive measure of the activity of the HPA axis. Salivary sampling is inexpensive, can be conducted is naturalistic settings, is relatively innocuous compared to blood sampling, and has proven especially valuable for use with special populations, including children (Granger, Harmon, Hibel, Rumyantseva, 2006; Kirschbaum & Hellhammer, 1994). Additionally, cortisol is believed to passively diffuse into saliva through the oral mucosa due to its small size and hydrophobic nature. Acinar cells are believed to prevent larger, hydrophilic molecules, such as carrier proteins, from entering saliva. Therefore, cortisol levels in saliva are believed to reflect only the portion of cortisol in the bloodstream that is free to act on target tissues (Kirschbaum et al., 1992; Kirschbaum & Hellhammer, 1994), making them a more meaningful measure of this steroid hormone than serum cortisol levels.

Saliva Collection Procedures

Compared to blood sampling, saliva collection is an easy and innocuous process. A relatively small volume of saliva is required by most commercial tests available today (e.g., 25µl in singlet; Salimetrics, State College, PA). Collection can be conducted in a variety of ways including passive drool, absorption into cotton-based collection material, or use of a hydrocellulose microsponge. Collection of unstimulated, whole saliva may be the best method for ensuring that collection materials will not interfere with the assay (Shirtcliff, Granger, Schwartz, & Curran, 2001). However, this type of collection may not be feasible in some populations, including infants, young children, and the elderly (Granger, Harmon, et al., 2006; Kirscbaum & Hellhammer, 1994).

Alternatively, cotton-based collection methods have been found to be a suitable collection method when cortisol is the only steroid hormone the researcher is interested in collecting (Shirtcliff et al., 2001). Subjects can chew on pieces of cotton dental rope or small pieces of cotton swabs. Once the cotton has absorbed the sample, the saliva can be expressed either using a 5cc needleless, plastic syringe or the cotton can be centrifuged to remove the saliva from the collection material. Cotton-based collection devices are often used successfully with infants (Gunnar, Connors, & Isensee, 1989; Kirschbaum & Hellhammer, 1994). However, obtaining sufficient sample using a cotton dental roll can be difficult if infants are asleep or fussy. Obtaining a small sample volume may be especially problematic if a cotton-based collection device is used. A recent study found that the percentage of cortisol recovered from samples absorbed in cotton was strongly related to the original sample volume. Thus, use of cotton-based collection devices may

be especially problematic with young children or other populations that typically produce small sample volumes (Granger, Harmon, et al., 2006).

Moreover, it can be difficult to use cotton-based collection methods with older infants and toddlers. Beginning around the onset of locomotion, many infants begin to develop stranger anxiety and an aversion to novelty (Lamb, Bornstein, & Teti, 2002). At this age, it can become difficult for researchers to coax young subjects to place large cotton-based collection devices in their mouths. Some researchers have attempted to resolve this problem by dipping cotton dental rolls in flavored drink mix crystals in order to make the collection device more pleasant. However, oral stimulants have been found to interfere with the assay and inflate cortisol measurements (Schwartz, Granger, Susman, Gunnar, & Laird, 1998). Conversely, a more recent study suggests that these problems may not be as severe as originally thought if the researcher limits the amount of oral stimulant used (Talge, Donzella, Dryzer, Gierens, & Gunnar, 2005).

Finally, researchers have recently begun to use a hydrocellulose-based microsponge for collection of saliva from special populations (Granger, Harmon, et al., 2006). These microsponges are small and seem to be less aversive and threatening to young children. In addition, they can be easily inserted into the mouth of a sleeping infant. However, their size also limits the amount of saliva that can be collected to approximately 200µl. Given the inability to express saliva from the device at the time of collection (it must be centrifuged), it can also be difficult to know in the field if sufficient sample has been collected. In summary, collection of saliva for assay of cortisol and other salivary biomarkers is easy and relatively non-aversive to the subjects. However, researchers need to be aware of the potential limitations associated with the various

collection techniques, and take care to select a technique appropriate for the population of interest, especially when working with young infants as in the current study.

Limitations to the Use of Salivary Cortisol

Many common behaviors, medications, and saliva collection procedures can add unforeseen additional variance to the measurement of salivary cortisol. Thus, it is important for researchers interested in HPA axis activity to be aware of the potential limitations in the measurement of the salivary biomarker. As mentioned previously, saliva collection procedures have both advantages and disadvantages and should be tailored to the population of interest (Schwartz et al., 1998; Shirtcliff et al., 2001; Talge et al., 2005). Saliva samples also need to be frozen relatively quickly in order to avoid degradation of cortisol in the sample and complications associated with bacterial growth (Kirschbaum & Hellhammer, 1994; Whembolua, Granger, Singer, Kivlighan & Marguin, 2006). Blood leakage into the oral cavity due to poor dental care or teething is also problematic for the measurement of many steroid hormones, but this does not appear to be problematic for cortisol (Kivlighan et al., 2004). Health behaviors may also affect cortisol levels and should be taken into account. Smoking has been observed to immediately elevate salivary cortisol levels, but also reduces adrenocortical activity in habitual smokers (Rohleder & Kirschbaum, 2006). Common medications, including the use of glucocorticoids, oral contraceptives, aspirin, opiods, and acetaminophen in children, have also been observed to alter cortisol levels and reactivity (Hibel, Granger, Kivlighan, Blair, & the FLP Investigators, 2006; Kirschbaum, Pirke, & Hellhammer, 1995). Infection or illness that results in increased immune system activity can elevate cortisol levels, especially if a fever is present (Hibel et al., 2006). Caution may be

especially important for those interested in studying early childhood populations. For researchers interested in very young infants, colic has been associated with a blunted diurnal rhythm (White, Gunnar, Larson, Donzella, & Barr, 2000). Later in infancy, napping and riding in cars has been associated with a decrease in salivary cortisol levels (Larson, Gunnar, & Hertsgaard, 1991). Thus, researchers interested in incorporating salivary cortisol, especially those interested in working with young children, should be cautious and carefully consider both the advantages and disadvantages of this technology.

Assessment of Sympathetic Nervous System Activity in Saliva

Psychophysiological methods have long been available to assess individual differences in SNS activity and typically involve electrodes, computerized recording apparatus, and sophisticated data reduction algorithms (Lovallo & Thomas, 2000). Measurement of skin conductance exploits increased perspiration in the hands or feet (Hernes et al., 2002; Lader, 1983). The degree of pupil dilation in response to stressful tasks can also be monitored (Steinhauer, Siegle, Condray, & Pless, 2004). Finally, some researchers attempt to extract the sympathetic contribution to the regulation of heart rate or pre-ejection period (PEP). Specifically, PEP is the interim between the onset of ventricular depolarization and the beginning of ventricular contraction. It is believed that greater sympathetic regulation of cardiac activity is associated with a shorter PEP (Berntson, Lozano, Chen & Cacioppo, 2004). Such psychophysiological measures are often difficult to employ outside of the laboratory environment and are particularly difficult to use with children. Much of the apparatus is cumbersome, requiring the attachment of several electrodes (especially PEP; Alkon et al., 2003; 2006). In addition, movement can be problematic for some of these methods and restriction of movement

may be especially difficult to accomplish with young children. Shortening of protocols may be required in order to achieve successful measurement in these populations (Alkon et al, 2003).

Alternatively, serum measures of catecholamine activity are available. However, catecholamines are peptide hormones and as such, are metabolized very quickly, resulting in a short half-life. As a result, blood sampling is either done with an indwelling catheter or venipuncture is done almost immediately after the completion of a stressor (Kirschbaum & Hellhammer, 1994). Venipuncture can be painful and constitute a stressor itself, possibly confounding the effects of the stressor of interest. In addition, most parents are unwilling to subject young children to repeated blood sampling. Unfortunately, while salivary measures of cortisol have long been available, direct measurement of catecholamine activity in saliva does not appear to reflect the activity of the SNS (Schwab, Heubel & Bartels, 1992). However, there is mounting evidence that salivary α-amylase might serve as a non-invasive, surrogate marker of SNS activity.

Salivary α-Amylase as a Surrogate Marker of SNS Activity

Salivary α-amylase (sAA) is an enzyme primarily responsible for the digestion of complex carbohydrates. It is released into saliva from the parotid gland in response to direct sympathetic stimulation of β-adrenergic receptors. While individual acinar cells in the parotid are innervated by both the sympathetic and parasympathetic nervous systems, sAA secretion is regulated via release of norepinephrine from the sympathetic terminals and stimulation of β-adrenergic receptors. In contrast, fluid and electrolyte secretion are regulated by parasympathetic stimulation and to a much lesser extent, by stimulation of α-adrenergic receptors by norepinephrine (for review, see Baum, 1987).

Based on the work in animal models demonstrating sympathetic regulation of sAA secretion, Chatterton and colleagues (1996) conducted a series of studies with human subjects to support sAA as a surrogate marker of sympathetic activity in saliva. These studies demonstrated that sAA paralleled sympathetic responses to both physical (exercise, heat, and cold stress) and psychological (exam stress) stressors in this sample. During both the exercise and exam stressors, sAA concentrations were associated with catecholamine reactivity and recovery. In general, links were stronger with norepinephrine than with epinephrine (Chatterton et al, 1996; see also Rohleder, Nater, Wolf, Ehlert & Kirschbaum, 2004). This strong association led Chatterton and colleagues to suggest that sAA may be a surrogate marker of norepinephrine activity specifically, and later reports from this group interpreted sAA activity in this way (Chatterton, Vogelsong, Lu, & Hudgens, 1997; Skosnik, Chatterton, Swisher, & Park, 2000). While more recent studies have confirmed that sAA is both stress responsive and associated with sympathetic tone during stress, they have not observed support for a specific association with either norepinephrine or catecolaminergic activity in general (Nater et al., 2005; 2006). As such, these studies question sAA as a surrogate marker for stress-related changes in catecholamines specifically.

Despite this setback, there is strong evidence supporting an association between sAA and SNS activity. A recent placebo-controlled study demonstrated that stress-related increases in sAA can be inhibited by administration of the β-adrenergic blocker propranolol (van Stegeren, Rohleder, Everaerd, & Wolf, 2006). In addition, β-adrenergic agonists are capable of stimulating sAA release without increasing salivary flow (Gallacher & Petersen, 1983). This pattern of evidence confirms that secretion of sAA is

physiologically regulated, at least in part, by the activity of the SNS in humans. Thus, while sAA levels may not be tightly linked to catecholamine activity in the blood (Nater et al., 2006), there is mounting support for sAA as being reflective of sympathetic activation in general.

Diurnal rhythm and stress reactivity of salivary α-amylase. Similar to salivary cortisol, sAA has also been demonstrated to show a diurnal rhythm. However, the pattern of this rhythm is the opposite of that observed for cortisol. Salivary α-amylase begins low, and upon waking shows a sharp decrease, followed by a gradual increase to the highest levels in the late afternoon and evening (Jenzano, Brown, & Mauriello, 1987; Rohleder et al., 2004). Examination of the circadian rhythm of sAA release from the parotid gland in rats suggests that this rhythm is endogenous and regulated by the ANS (Bellavia, Sanz, Sereno, & Vermouth, 1992).

Salivary α-amylase reactivity to stress is distinct from the response profile measured by salivary cortisol (Nater et al., 2005; 2006; Rohleder et al., 2004; Takai et al., 2004; see Dickerson & Kemeny, 2004 for review on cortisol). Salivary α-amylase reaches its peak response by approximately 5 to 10-minutes post-stressor (Granger, Kivlighan et al., 2006). Recovery to pre-task levels has also been observed to be faster for sAA than for cortisol (Takai et al., 2004). These differences may be due to the faster time course of sympathetic reactivity and the more sensitive threshold of reactivity in the SNS as compared to the HPA axis. Theoretically, SNS reactivity would occur to challenges that are more mild than those required to activate the HPA axis (Lovallo & Thomas, 2000).

Sample Collection and Assay of Salivary α-Amylase

Researchers interested in examining sAA levels can use the same saliva collection techniques used for salivary cortisol. Passive drool, absorption in cotton-based collection materials, or the hydrocellulose-based microsponge are all viable options for examination of sAA. In addition, neither multiple freeze-thaw cycles or delayed freezing (up to 96 hours) had any effect on sAA activity levels (Granger, Kivlighan, et al., 2006, in press). While the concentration of sAA protein in saliva has been linked to flow-rate, the enzymatic activity of sAA is not (Rohleder, Wolf, Maldonado Montero, & Kirschbaum, 2006). As such, the enzymatic activity of salivary α-amylase can be measured effectively using a kinetic reaction assay employing a chromagenic substrate. The action of sAA on the substrate 2-cloro-p-nitrophenol linked to maltotriose releases 2-cloro-p-nitrophenol which can be measured spectrophotometrically using a standard plate reader. The amount of sAA activity is proportional to the increase in absorbance over a 2-minute period (Granger, Kivlighan, et al., 2006; in press).

Limitations to the Use of Salivary α-Amylase.

Less work has been done to examine potential influences on sAA activity that might confound our interpretation of its meaning, than with cortisol. However, there are a few issues that researchers should be aware of and take measures to control for in their studies. Levels of sAA increase following stimulation with food products such starch, sugar, salt, and citric acid (Froehlich, Pangborn, & Whitaker, 1987). Ideally, researchers should restrict food intake, or at least monitor and record time since last meal. In addition, avoiding the use of oral stimulants for saliva collection purposes seems prudent. Common drugs may also influence sAA levels and reactivity. The use of SNS stimulant

drugs, such as caffeine, increase sAA reactivity to stress (Klein, Whetzel, Bennett, Ritter, & Granger, 2006). In contrast, cigarette smoking has been observed to reduce sAA activity and the aldehydes in cigarette smoke are believed to functionally alter the structure of this enzyme (Zappacosta et al., 2002). Finally, researchers interested in measuring sAA in infants should be aware that it can also found in human breast milk (Hamosh, Henderson, Ellis, Mao, & Hamosh, 1997; Lonnerdal, 2003). Therefore, infant intake of breast milk should also be carefully controlled.

Development and Salivary α-Amylase

Salivary α-amylase activity levels also show developmental changes during the first year of life. Levels of sAA are nearly undetectable at birth, but increase steadily during the first 3 months, when they are detectable at approximately two-thirds the activity observed in adults (Sevenhuysen, Holodinsky, & Dawes, 1984). Salivary α-amylase activity then continues to show a sharp rise during early childhood, particularly between 11 months and 2 years, reaching maximum levels by 5 to 6 years of age (O'Donnell & Miller, 1980). Levels of sAA have also been observed to be responsive to stress in childhood populations ranging from infancy through adolescence (Granger, Kivlighan, et al., 2006; in press). However, while sAA reactivity has been observed on the group level in older children and adolescents, these studies did not observe sAA reactivity to stress on average in infants or preschool aged children. In each of these studies, there were wide varying individual differences in sAA response patterns to stress. Approximately 44% of the infants and 40% of the preschoolers in these studies showed a sAA increase in response to stressor tasks (Granger, Kivlighan, et al., 2006). More recently, Shea and colleagues (2006) also observed a sAA in response to noise burst and

arm restraint in 39% of her infants. Thus, while sAA may be responsive to stress in some individuals during early childhood, it is not yet clear if changes in sAA have the same meaning in young children as they do in older populations.

In summary, sAA is responsive to stress and appears to be a promising salivary biomarker of sympathetic activity. However, despite this promising outlook, researchers interested in incorporating sAA into studies of health and human development should proceed with caution and be aware of potential methodological limitations to this usefulness. Much work remains in order to firmly establish sAA as a surrogate marker of sympathetic activity or alternatively as a unique biological marker of stress.

Summary: Stress Physiology, Development, and Methodological Issues

The conceptualization of stress has evolved since the field of stress research began with the work of Cannon and continues to do so today. Likewise, our understanding of the complexity of stress responsive physiological systems continues to grow as methodological advances and new technology become available. In particular, the assessment of cortisol in saliva has proven to be a major catalyst for research into the function of the HPA axis and downstream consequences of the activity of this physiological system, especially in childhood populations. Given the possibility that sAA may be a surrogate marker for the activity of the SNS, we may soon be able to fill a major gap in our knowledge regarding the role of the sympathetic activity in child development. As a consequence of these advances, our understanding of stress responsive physiology has progressed. We have come to understand that these systems do not mechanically respond to all stressors, regardless of type, with a stereotypical set of

responses designed to maintain homeostasis and prevent change. Instead, we now believe that the functioning of these systems is specific to the challenge at hand and that these systems are meant to change and develop in order to ensure survival in the face of an ever-changing environment. Neither the SNS, nor the HPA axis are fully developed at birth and due to this early plasticity, environmental influences may have a greater impact on the functioning of these systems during early childhood that at any other time in the lifespan. Thus, given the propensity of stress responsive physiological systems to change and develop in response to the demands of the environmental context, it becomes imperative that we begin to understand how some of the most common and potent contextual influences can alter the functioning of these systems, especially early in life.

Chapter 3

THE MOTHER-INFANT RELATIONSHIP AND THE CO-DEVELOPMENT OF STRESS-RESPONSIVE PHYSIOLOGICAL SYSTEMS

Given the plasticity of stress responsive physiological systems early in life and the life long consequences of early stress, it is imperative that we understand the contextual influences most salient to the development of stress physiology. In particular, early economic disadvantage is believed to be a context of heightened risk for the development of allostatic load and stress-related disorders (Evans, 2003; McEwen, 2001). However, during the first year of life, the relationship with the primary caregiver is believed to be the most significant context for child development (Hofer, 1994). Children living under conditions of poverty are at heightened risk for unresponsive parenting and separation from their biological parents (Evans, 2004) and this may be one means through which poverty mediates its effects on the SNS and the HPA axis. The literature clearly demonstrates the importance of the mother-infant relationship for the development of stress responsive physiological systems during infancy (for review, see Gunnar & Donzella, 2002). However, human development does not abruptly end when adulthood is reached, but continues throughout life. Through the process of allostasis (Sterling & Eyer, 1988), maternal physiology continues to evolve in response to environmental demands and the mother-child relationship also represents a significant social context for the mother. Surprisingly, much less work has examined the influence of the infant on physiological responses to stress in the mother (Walker et al., 2004). The mother-infant dyad represents a dynamic or ever-changing system with bi-directional influences on the development of the dyadic relationship (Belsky, Taylor, & Rovine, 1984; Field, 1992).

The shared experiences of daily challenges faced by the mother-infant dyad should shape the development of stress responsive physiological systems in mother and infant simultaneously (Field, 1992; Rosenblum & Andrews, 1994). Both behavioral and physiological attunement may be the result. While the importance of behavioral attunement for the mother-infant relationship has been well documented (Harrist & Waugh, 2002), it has been suggested that physiological attunement may also be an integral component of the mother-infant relationship (Field, 1992; Sethre-Hofstad et al., 2002). The current review provides support for the centrality of the mother-infant relationship for the development of the stress physiological systems and seeks to demonstrate the salience of this relationship for the functioning of maternal stress physiology and the potential importance of physiological attunement for the mother-infant dyad.

The Influence of the Infant on Stress Physiology in Mother-Infant Dyads

The influence of infant temperament and gender on the development stress responsive physiology during infancy has received substantial attention (e.g., Davis & Emory, 1995; Gunnar, Larson, Hertsgaard, Harris, & Broderson, 1992; Kagan et al., 1987; Lewis & Ramsay, 2005). Far fewer studies have addressed the impact of infant behaviors and characteristics on maternal psychological and physiological responses to stress (Walker et al., 2004). A more complete understanding of how individual differences in child behavior can influence maternal functioning may help us to understand why some mothers have more difficulty with the transition in motherhood and what consequences of maternal stress are for child development.

Temperament and Stress Physiology During Infancy

Individual differences, such as temperament and previous developmental experiences, will influence whether or not events in the environment are deemed as stressful and thus, necessitate a physiological response (McEwen & Seeman, 1999). During infancy, temperament may be a key influence on infant perceptions of stress. According to Rothbart and Derryberry (1981), temperament can be conceptualized as constitutionally based individual differences in reactivity and self-regulation. Reactivity refers to the excitability or arousability of behavioral, endocrine, autonomic, or central nervous system response to stimuli and can have either a positive and negative valence. This level of arousal can be modified by self-regulatory behaviors such as behavioral approach or withdrawal, attentional orientation, or other behaviors that serve to enhance or reduce reactivity. As will be discussed, research on stress physiology during infancy and early childhood has often focused on linking physiological reactivity with behavioral reactivity and regulation (e.g., Gunnar, et al., 1992; Kagan et al., 1987; Lewis & Ramsay, 2005). Due to the ease and non-invasive nature of salivary measurement of cortisol, much of this work has centered on adrenocortical activation (Granger, Kivlighan, et al., 2006; in press). However, a small, but growing literature has begun to address sympathetic activation and its link with temperament during infancy.

Fear, Sympathetic, and Adrenocortical Reactivity in the Infant

Negative reactivity includes both expressed and felt distress and can be further differentiated by the type of stimuli inducing the reactivity. Fear is defined as distress towards novel stimuli, while frustration or anger is due to distress to limitations or goal blockage (Lemerise & Dodge, 2000; Rothbart, 1986; Rothbart, Derryberry & Posner,

1994). Several studies have examined the relationship between fear and adrenocortical activity in young children. Behavioral inhibition is a distinct behavioral style marked by characteristically shy or withdrawn behaviors in novel social situations and signs of increased anxiety when children are presented with novel challenges (Garcia-Coll, Kagan, & Resnick, 1984). In an early study of behavioral inhibition, Kagan and colleagues (1987) observed that cortisol levels both at home and in the laboratory were higher for inhibited children as compared to their uninhibited counterparts. Other findings have supported this initial observation. Across infancy and the toddler period, studies have confirmed that temperamentally fearful behavior in response to novel people or situations is associated with both higher basal cortisol levels and reactivity (van Bakel & Riksen-Walraven, 2004; Wilson, Megel, Fredrichs, & McLaughlin, 2003; Zimmerman & Stansbury, 2004). Asymmetrical activation of the right frontal hemisphere has also been associated with withdrawal behavior and a fearful temperament in both adults and children (Davidson, 1995; Fox et al., 1995), and Buss and colleagues (2003) demonstrated that 6 month-old infants with greater right frontal activation of the prefrontal cortex had both higher levels of basal cortisol and greater cortisol reactivity to a stranger approach. It is also important to note that direct associations between fear / withdrawal behavior and cortisol levels and reactivity have not always been reliably replicated, especially in studies that do not use an extreme group methodology (Schmidt, et al., 1997; Buss et al., 2003).

Research has also focused on the relation between temperamental fearfulness and sympathetic arousal during childhood. In addition to elevations in HPA axis activity, Kagan and colleagues (1987) also found group differences in sympathetic nervous system

activity in his study of inhibited children. At 5 ½ years of age, these children had higher heart rates and larger pupillary diameters than their uninhibited counterparts, which indicate greater sympathetic activation. In addition, urinary measures of norepinephrine activity were associated with measures of inhibited behavior recorded at 4 and 5 ½ years (Kagan et al., 1987). Freezing behavior is the tendency to reduce activity in response to threat and it has recently been suggested that "out-of-context" freezing, that is, freezing in response to low-risk situations, is a sign of a dysregulated fear response (Buss, Davidson, Kalin, & Goldsmith, 2004). Buss and colleagues (2004) found that freezing behavior across three stranger approach situations in 24 month-olds, was associated with the fastest PEP at baseline, a measure of greater sympathetic regulation of heart rate. In particular, the association between freezing and a faster PEP was highest during the lowest-risk situation. Thus, there is evidence that a fearful temperament may also be associated with greater sympathetic activation and that this may be particularly true for individuals with dysregulated responses to fear.

Frustration, Sympathetic, and Adrenocortical Reactivity in the Infant

Anger or frustration may also be related to cortisol reactivity. At 9-months, Gunnar and colleagues (1992) found that infants rated by their parents as being highly distressed to limitations showed greater behavioral (i.e., more fussing and crying) and cortisol reactivity to maternal separation than fearful infants. Among 3 to 5 year-old preschool children participating in a competitive challenge, only 15% demonstrated a cortisol response to the challenge. When examining this sub-group, it was found that these children were significantly more surgent and showed more tense or angry behavioral responses when they believed they were losing the challenge (Donzella et al.,

2000). Children with a surgent behavioral style have been repeatedly found to respond to goal blockage with greater distress, frustration, or anger (Donzella et al., 2000). Thus, there is evidence, that anger may also be related to adrenocortical reactivity.

Lewis and Ramsey (2005) have recently suggested that the expression of anger in response to goal blockage should be considered positive because it represents an attempt made by an individual to control the situation. In addition, they also suggested that an association between cortisol reactivity and anger should not be expected, unless it becomes clear that the effort to overcome goal blockage is not effective. Evidence was provided for this theory by examination of the emotional and adrenocortical responses of 4 month-old infants to a contingency learning situation and 6 month-old infants to a still-face paradigm. In both situations, sadness but not anger, was associated with cortisol reactivity to the tasks. Thus, anger may only be associated with adrenocortical activity when individuals have not been successful at using this emotion to re-gain control of a situation. In most of the studies listed above, the infants and children were unable to use their anger to effect a change in their situation (e.g., maternal separation, a planned losing streak), and their adrenocortical responses may be the result of this loss of control.

Few studies have examined the association between anger or frustration and sympathetic activity in children. However, research on adults may suggest some alternative patterns. In a study of male undergraduates, males who reported using more outward expressions of anger showed a greater decrease in PEP from baseline in response to a mental arithmetic task (Burns, Friedman, & Katkin, 1992). Similarly, exposure to a psychological stressor that resulted in a significant increase in anger was also associated with cardiovascular activation and elevated plasma norepinephrine levels in adults

(Atanackovic, Brunner-Weinzierl, Kroger, Serke, & Deter, 2002). These studies suggest that anger may be related to increases in sympathetic activity. Alternatively, a study of normotensive adults found that anger expressed outwardly was actually associated with lower heart rate and norepinephrine reactivity to a mental arithmetic task (Mills, Schneider, & Dimsdale, 1989). This finding may be in line with the ideas of Lewis and Ramsay (2005) regarding anger as a potentially regulatory emotion. Thus, both distress to novelty and frustration have been linked to activation of both the SNS and the HPA axis during infancy.

Emotion Regulation and Physiological Stress Reactivity

Outward expressions of behavior do not always reflect temperamental tendencies to react to stimuli, especially later in development. This is a result of an increase in the ability to self-regulate with greater maturity. According to Stansbury and Gunnar (1994), if a stressor has the potential to activate the HPA axis, the child will move through a series of "phases" during which the HPA axis will be activated or the stress response will be extinguished. During a "pre-evaluation" phase, the child undertakes appraisal of the emotional meaning of a potentially threatening event and potential coping or emotion regulatory resources. As a result of this appraisal, the event may be considered non-threatening and the stress response extinguished or the event may be confirmed as threatening, leading to adrenocortical activation. Thus, it is very likely that several potentially threatening stimuli will never result in adrenocortical activity. If an event is perceived as threatening, there are several emotion regulatory processes that may reduce the likelihood of adrenocortical reactivity. Perceived control of threatening events, distraction or attentional regulatory strategies, self-soothing behavior (i.e., self-grooming,

sucking, rhythmic movements, stroking), and social support (e.g., sensitive caregivers) are all believed to reduce the need for a cortisol response. However, it seems likely that the greatest adrenocortical activation will result when behavioral coping or emotion regulation strategies are not available (Spangler & Scheubeck, 1993).

The relationship between regulatory behaviors and reduced adrenocortical activation is expected to begin shortly after birth and is evident during infancy. In newborns between 1 and 6 days of age, those with a high orientation to social and non-social stimuli had little or no increase in cortisol in response to a mild stressor (Brazelton Neonatal Behavioral Assessment Scale) while infants with a low orientation show marked cortisol increases to this procedure. The authors of this study hypothesized that the high orientation infants were able to use this ability as a behavioral coping strategy (Spangler & Scheubeck, 1993). Beyond infancy, emotion regulatory abilities have also been found to impact adrenocortical activation. Zimmermann and Stansbury (2004) found that 3 year-olds with better regulatory abilities were less likely to show a cortisol response to stranger approach. Among pre-school and school-aged children in center-based daycare, greater increases in cortisol across the day, a pattern opposite that expected by the diurnal rhythm, were related to poorer self-regulatory skills (Dettling, Gunnar, & Donzella, 1999; Dettling, Parker, Lane, Sebanc, & Gunnar, 2000). Thus, across early development, there is clear support for theories suggesting the use of effective coping and emotion regulation will alleviate the need for adrenocortical activation in response to emotion-eliciting stimuli.

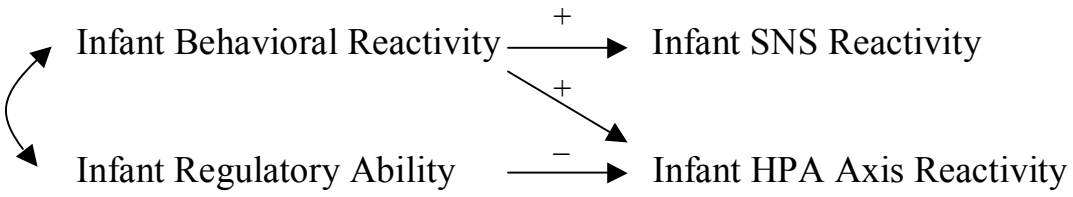
Again, there are few studies that examine the effect of emotion regulation or coping on sympathetic activation in childhood. According to Eriksen, Olff, Murison, and

Ursin (1999), the purpose of coping is to abolish general activation of the stress responsive systems. However, even with a coping response to stress, there will be a brief period of initial arousal characterized by activation of the sympathetic nervous system and an initial release of catecholamines into the bloodstream. The magnitude of this initial response will probably be the same as that observed during longer-term responses to stress. There is support for this hypothesis in the literature. Previous exposure to a particular stressor may better prepare an individual to cope with that stressor on subsequent occasions. In two studies of adult men and women exposed to the same laboratory stress paradigm on multiple occasions, the catecholamine response to the stressor was maintained at the same magnitude. However, for the group, ACTH and cortisol both showed significantly decreased responsiveness to the stressor on the subsequent exposures (Gerra et al., 2001; Schommer, Hellammer, & Kirschbaum, 2003). Thus, the HPA axis and the sympathetic nervous system may be influenced differently by an infant's ability to cope with a stressor as manifested by observable regulatory strategies.

In summary, infant physiological responsiveness to challenge is likely to be influenced by both temperamental reactivity and regulatory ability. Due to individual differences in infant distress to novelty and frustration, infants have been demonstrated to show unique levels of behavioral reactivity in response to the same challenge. In addition, temperament, maturity, and developmental experiences are believed to influence each infant's ability to regulate his or her emotional response to the challenge. The combination of reactivity and regulation may also result in the unique patterns of sympathetic and adrenocortical response to infant challenge. Both distress to novelty and

frustration have been linked to increases in sympathetic and adrenocortical activity during infancy. Moreover, an infant's regulatory ability is believed to be inversely associated with adrenocortical reactivity to stress, while the degree of sympathetic reactivity should be uniform regardless of regulatory ability. Based on previous research, Figure 1 schematically presents the theoretical associations between behavioral manifestations of reactivity and regulatory ability with SNS and HPA axis reactivity to stress.

Figure 1. Previous research suggests that infant behavioral reactivity may be positively associated with the degree of infant SNS and HPA axis response to challenge. In addition, behavioral reactivity may also interact with infant regulatory ability such that better regulatory ability will reduce the need for HPA axis reactivity.



Temperament and Maternal Physiological Response to Infant Distress

There is limited work on the effects of individual differences in infant temperament on either psychological or physiological response to stress in mothers. One study reported that maternal ratings of temperament at 6 weeks and 4 months were predictive of maternal adaptation (i.e., role satisfaction, irritability, stress, and coping) when infants were 10 months of age (Hagekull & Bohlin, 1990). In another, mothers of fussier babies, as measured by maternal report questionnaire at 6 weeks and 9 months, reported being less attached to their infants (Denham & Moser, 1994). Both of these studies relied on maternal report of infant temperament and maternal outcomes. Thus, links between these measures might be confounded. A more recent investigation

examined of the effects of persistent crying, as rated by clinic staff, on maternal psychological well-being in a sample of families referred to a clinic for colic and a group of control families (Papousek & von Hofacker, 1998). Mothers of extreme and moderate criers reported being significantly more depressed, exhausted, and anxious than mothers of control infants. They also reported lower feelings of self-efficacy and higher marital distress. These findings were recently confirmed in a report of increased incidence of moderate to severe depressive symptoms in mothers of infants being treated in an outpatient colic clinic (Maxted et al., 2005).

There was some early interest in the psychophysiology of maternal response to infant cues and infant temperament. An early study presented mothers of 3 month-old infants with images of a smiling infant followed by images of a crying infant of the same age. Researchers found that both heart rate and skin conductance responses to the stimuli decreased as the stimulus shifted from the smiling infant to the crying infant and that the degree of cardiac decrease was related to maternal ratings of their own child's temperament. Specifically, mothers who rated their own infant's temperament as difficult, showed less of a cardiac decrease in response to the change in infant emotional cues (Donovan, Leavitt, & Balling, 1978). Given that attentional responses are linked to decreases in heart rate (Porges, 1995), this might suggest that mothers who are exposed to highly difficult infants become less attentive to infant distress cues. In contrast, Wiesenfeld and Klorman (1978) only observed cardiac decelerations when mothers observed crying infants that were not their own. In this study, when mothers watched videotapes of their own infants crying, they showed both large cardiac accelerations and increases in skin conductance. Thus, sympathetic activation may be characteristic of a

mother exposed to her own infant's distress. While there is one report on maternal adrenocortical reactivity to infant distress, to our knowledge, no research has addressed links between individual differences infant temperament and maternal cortisol reactivity to infant distress. This study of mothers observing their 3 month-old infants receive inoculations reported no adrenocortical response to infant distress in these mothers as a group (Braarud & Stormark, 2006).

In summary, maternal self-report of psychological stress has been linked to both maternal reports of infant temperament and objective ratings of the degree of infant cry problems. Thus, individual differences in infant behavioral reactivity do appear to have an impact on maternal psychological responses to stress. Maternal SNS response the distress of an unfamiliar infant has also been associated with a mother's perceptions of her own infant's temperament. However, while mothers' observations of their own children's distress have been shown to induce sympathetic activation, this activation has not been linked to the degree of infant behavioral reactivity. In addition, there does not appear to be evidence of maternal adrenocortical reactivity to infant distress. However, it seems likely that maternal physiological response to infant behavioral reactivity and regulation will be dependent on a woman's perception of her infant's distress. Therefore, infant reactivity and regulation should interact to produce an overall level of negative reactivity observable by the mother. Maternal sensitivity may play a pivotal role in determining maternal physiological response to this observable distress will be discussed further.

The Effect of Infant Gender on Stress Physiology in Mother-Infant Dyads

Gender Differences in Infant Physiological Reactivity to Challenge

The adult literature suggests the existence of sex differences in biobehavioral responses to stress (Hinojosa-Laborde, Chapa, Lange, & Haywood, 1999; Klein & Corwin, 2002; Taylor et al., 2000; Taylor, Dickerson, & Klein, 2002). Hinojosa-Laborde and colleagues (1999) propose that there is strong evidence that women may have attenuated sympathetic activity compared to men due to greater inhibitory control at every level of neural regulation. In support of this, men are generally observed to respond to stressors with greater blood pressure, heart rate, and catecholamine levels than do women (Frankenhaeuser, Dunne, & Lundberg, 1976; for review see Hinojosa-Laborde et al., 1999). These sex differences also extend to the functioning of the HPA axis with men also showing greater adrenocortical reactivity to stress than women in general (for review see, Kajantie & Phillips, 2006). To better understand these differences, Taylor and colleagues (2000) proposed an evolutionary-based theory to explain sex differences in stress response behaviors, such that the classic conceptualization of the fight-or-flight response may not be totally applicable to women. However, while this theory provides a solid explanation for the adaptiveness of such sex differences in adulthood, it is unknown if the proposed mechanisms (i.e., the attachment-giving system) will hold up in infancy.

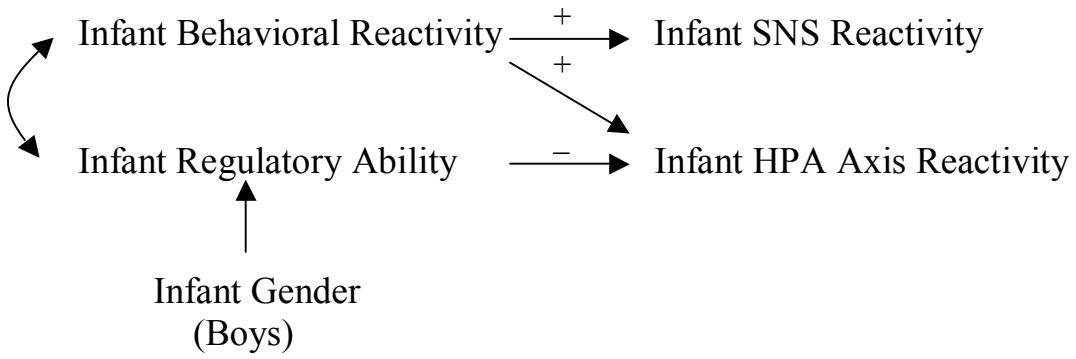
The literature on sex differences during the first year of life is sparse and inconsistent. There are few studies to suggest sex differences in stress physiology from birth. In line with the adult literature, sex differences in the total urinary excretion of catecholamines have been observed in young infants (aged 1 day to 3.5 months) such that the total amount of urinary epinephrine and norepinephrine excreted was lower for

female than male infants (Dalmaz & Peyrin, 1982). Similarly, Nagy, Loveland, Orvos, and Molnar (2001) have also observed greater cardiac reactivity in male than female neonates during testing for the Moro reflex, a normal startle reflex of newborns. Davis & Emory (1995) reported sex differences in neonatal stress reactivity to a mild behavioral stressor (Neonatal Behavioral Assessment Scale), such that females displayed a greater change in heart rate immediately after assessment, but demonstrated no cortisol elevation, whereas an elevated cortisol response was observed in males 10-15 minutes after the stressor. Most other studies have reported no sex differences in cortisol levels or reactivity during infancy (Gunnar, Connors, Isensee, & Wall, 1988; Gunnar, Isensee, & Fust, 1987, for review see Kajantie & Phillips, 2006). Thus, the available evidence does not consistently suggest greater SNS or HPA reactivity to stress in either males or females during infancy.

Gender differences have been observed in emotion regulation ability during infancy. Weinberg, Tronick, Cohn, & Olson (1999) observed that boys displayed greater negative reactivity to the still face procedure and had greater difficulty maintaining emotion regulation than girls. In addition, males were observed to be more socially oriented, and the authors suggested that this was necessary in order to communicate their greater need for external emotion regulation to their mothers. In a separate but related study, 6 month-old boys were observed to be three times less likely than girls to use self-comforting behaviors to regulate emotional state (Tronick & Weinberg, 2000). Stifter and Spinrad (2002) found that infant boys who show high levels of negative reactivity also display less self-regulation than other boys, but the same was not true for girls. Thus, male infants may be more likely to display an adrenocortical response to a challenge not

accompanied by maternal support due to their greater difficulty maintaining emotion regulation on their own. In contrast, females might be more likely to regulate independently and thus, would primarily show a sympathetic response to challenge. Figure 2 demonstrates how infant gender might influence the relationship between infant temperament and physiological reactivity to stress.

Figure 2. Male infants may rely more on external maternal support for emotion regulation during challenge, and thus may be more likely to show an HPA axis response to a challenge when maternal support is unavailable than girls.



Infant Gender and Maternal Responses to Infant Distress

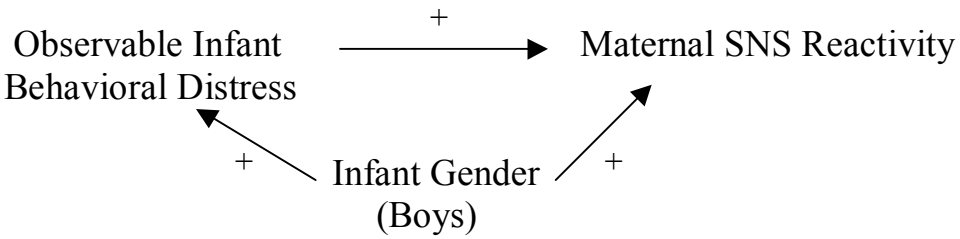
To the best of my knowledge, no studies have examined differences in maternal physiological response to infant challenge by infant gender. The literature has documented differences in maternal behaviors by infant gender during either interaction with infants or infant challenge. Soon after birth (days 2 and 4), mothers have been observed to show more interactive and skin-to-skin contact behavior with female than male infants, such as talking, smiling, patting, rubbing, kissing, and touching behaviors. In contrast, mothers of boys direct more touching behavior to clothed areas of the infant, such as patting or adjusting clothing (Hwang, 1978). During the first year, Tronick and Cohn (1989) observed differences in mother-infant interaction behaviors by infant

gender. The amount of time spent in matching activities (social attend, social play, object attend, and object play) and synchrony (temporal coordination of interaction behaviors) during mother-infant interaction was greater for dyads with male rather than female infants. Finally, observations of mother-toddler interaction revealed that mothers rated as being more sensitive allowed their boys to control the flow of interactions to a greater extent that they did with their girls (Biringen, Robinson, & Emde, 2000). Thus, both the affectionate behaviors of mothers with their infants and the content of mother-child interactions appear to differ based on infant gender.

Maternal responses to infant distress or challenge also seem to differ by infant gender. One early study examined maternal perceptions of their 4 month-old infants while crying. While there were no objective sex differences observed in the amount of infant crying, mothers perceived their daughters more negatively and as less powerful, and their sons as more powerful as the intensity of crying increased (Teichner, Ames, & Kerig, 1997). Another study manipulated the degree of infant distress to maternal separation by either providing toys to 10-month old infants or leaving them completely alone during separation from the mother (Corter & Bow, 1976). Mothers of the boys left without toys (more vocal distress) during separation watched a video screen displaying infant behavior significantly less and smiled less than mothers of boys with toys (less vocal distress). Researchers also observed that these mothers spent more time looking at the doorway as though debating whether to retrieve the infant. Mothers of boys also terminated the separation early and retrieved the infant more often than mothers of girls, even though there were no gender differences in overall levels of distress behavior in the infants. Thus, while crying in male infants is perceived more positively by mothers,

mothers might have a more difficult time observing infant distress in males. Therefore, given the greater amount of synchrony in mother-infant dyads with boys and observations of greater maternal concern over infant distress in boys, mothers of boys might display more physiological arousal, most likely SNS reactivity, to distress in male rather than female infants. Figure 3 demonstrates how infant gender might influence maternal physiological response to infant distress through effects on both infant behavior and maternal perceptions of infant distress.

Figure 3. Given that male infants may rely more on external maternal support for emotion regulation during challenge and that their mothers may have more difficulty with passively observing infant boys in distress, mothers of infant boys may show more SNS reactivity to infant challenge than mothers of girls.



The Influence of the Mother on Stress Physiology in Mother-Infant Dyads

The relationship with the primary caregiver is the most immediate and salient social environmental context for the development of stress responsive physiological systems (Hofer, 1994). Both the presence of the mother and the quality of maternal care have been investigated as potentially important influences for early development in both animal and human models. The absence of or long-term separation from a primary caregiver represents one of the most significant stressors that can be experienced in early childhood (Gunnar, Morison, Chisholm, & Schuder, 2001; Luecken & Appelhans, 2006; Levine, 2005). When the mother is present, maternal behavior can act a filter through

which all of the influences of the larger environmental context must pass. Under disadvantaged conditions, mothers can either buffer their offspring from these conditions, or they can magnify the effects of disadvantaged social or physical environment through reduced quality of maternal caregiving behavior (Belsky, 1984).

Research in both animal and human models has informed mid-level models regarding the link between early maternal care and the long-term development of stress responsive physiological systems. Both rodent and primate models allow flexibility in the manipulation of early environments that is not possible in human research. In addition, rodents mature at a rate that permits longitudinal work that takes years or decades in primates or humans. Early environmental experiences in both the rodent and the primate have been observed to permanently alter the functioning of stress responsive physiological systems (for reviews, see Anisman, Zaharia, Meaney, & Merali, 1998; Levine, 2001; 2005; Sanchez, Ladd, & Plotsky, 2001). In rodents, the presence and behavior of the rat dam have the potential to impact the HPA axis functioning of pups during adulthood. While extended maternal separations in the rat appear to produce stress vulnerable animals (Ladd, Owens, & Nemeroff, 1996; Plotsky & Meaney, 1993), brief separations are associated with stress resistant animals, due to an increase in the frequency of certain maternal caregiving behaviors, such as increased licking and grooming (Meaney, Mitchell, et al., 1991; Meaney, Viau, et al. 1991; Meaney, Aitken, Sharma, Viau, & Sarriau, 1989; Meaney, Aitken, van Berkel, Bhatnagar, & Sapolsky, 1988). In addition, rat dams that naturally show high levels these caregiving behaviors also have offspring that that tend to be more stress resistant (Francis, Champagne, Liu, & Meaney, 1999; Liu et al., 1997). Thus, the rodent work has provided a clear example

where manipulations in the early social environment, specifically, maternal caregiving behavior, have the capacity to permanently alter the functioning of the HPA axis.

In primates, while such behavioral alterations have been observed, the evidence for long-term physiological alterations is less consistent. Similar to the effects of short separations and handling in the rat, brief maternal separations in primates may inoculate primate infants against future stress (Levine & Mody, 2003; Parker, Buckmaster, Sundlass, Schatzberg & Lyons, 2006). Individual differences in maternal care have also been experimentally induced in primates through the manipulation of foraging conditions (Champoux, Hwang, Lang, & Levine, 2001; Lyons, Kim, Schatzburg, & Levine, 1998; Parker et al., 2006). Attenuated HPA axis reactivity has been observed in adult animals raised under high foraging demand conditions that reduced the quality of maternal care (Parker et al., 2006). Such findings support the observations in separation paradigms that exposure to mild stress during infancy may actually produce more a more stress-resistant adult primate. No consistent pattern of long-term effects in stress reactivity has been observed for more extreme forms of disruption of early caregiving in primates (Levine, 2005; Mason, 2000). However, these alternative rearing conditions have been observed to produce devastating permanent alterations in the socio-emotional behavior of primates. Thus, even though long-term effects of extreme deprivation on the functioning of the HPA axis have not been consistently observed, it seems clear that extremely stressful early environments can have serious consequences for adult primates (Levine, 2005; Mason, 2000).

The use of animal models allows researchers to manipulate both maternal presence and behavior and these studies have revealed that the length of maternal

separation and the quality of maternal care can result in distinct stress responsive phenotypes. Such studies cannot be conducted in humans and thus, these findings inform the observations of a myriad of studies that attempt to address similar questions about early stressful environments in human models using naturalistic observation. In the human literature, there also is evidence that early experiences related to maternal caregiving behavior can have long-term consequences for the development of stress-responsive physiological systems. Disruption in caregiving due to parental loss has been retrospectively associated with elevated blood pressure and cortisol reactivity during a speech task (Luecken, 1998). In addition, a blunted cortisol awakening response and elevated cortisol levels across the day has been observed in individuals that lost a parent during childhood (Meinlschmidt & Heim, 2005; Nicolson, 2004). Severe neglect has long-term effects on HPA axis function in children. Conditions in Romanian orphanages during the early 1990's were extremely poor, and infants living in this environment received little social or physical stimulation and had no opportunities to form attachment relationships with caregivers (Gunnar & Donzella, 2002). In a study of 2 year-old children reared in a Romanian institution, Carlson and colleagues observed a flat, low pattern of daytime cortisol production across the day. This pattern is in striking contrast to the normal diurnal rhythms of the home-raised Romanian children that constituted the comparison group for this study (Carlson et al., 1995; Carlson & Earls, 1997). In addition, long-term effects of these orphanage-rearing conditions have also been observed. In a sample of Romanian children that had been adopted by families in British Columbia prior to 3 years of age, overall cortisol levels differed between late-adopted children, early-adopted children, and a Canadian-born comparison group (Gunnar et al.,

2001). Late-adopted children had higher cortisol levels as compared to either the early adopted or Canadian born children. The period of time spent in the orphanage before adoption was also associated with the cortisol levels for these children. Thus, both early disruptions in care, such as the loss of a parent, and severe early neglect have the capacity to leave a long-term imprint on the functioning of the HPA axis in humans.

In summary, early caregiving experiences have been observed to have long-term effects on the functioning of stress physiological systems in both animal and human models. Long-term maternal separation in rodents, as well as disruptions in early care due to parental loss, and severe institutional neglect in human children have all been observed to result in long-term increases in stress vulnerability for offspring. In addition, animal models suggest that more mild forms of early stress related to maternal care may promote resilience in the long-term. Given the complexities involved in the link between the mother-child relationship and stress vulnerability, it becomes imperative that we understand the specific aspects of the mother-child relationship that influence the development of stress-responsive physiological systems.

Maternal Sensitivity and Stress Physiology in Mother-Infant Dyads

Perhaps the most salient development experience shaping an infant's regulatory ability is the history of interactions with the primary caregiver. For decades, attachment researchers have demonstrated the importance of sensitive maternal behavior for child development in a variety of domains (Ainsworth, Blehar, Waters, & Wall, 1978; for reviews, see Beckwith et al., 2002; Belsky & Cassidy, 1995). Maternal sensitivity is defined as a mother's ability to accurately perceive and interpret her infant's signals and to respond to these signals promptly and appropriately (Ainsworth et al., 1978).

However, a sensitive mother not only perceives her infant's affective signals, but also shares in them by synchronizing her affective behaviors with those of her infant (Field, 1994; Stern, 1985). Thus, in order for a mother to be sensitive, she must also be emotionally available to her infant. For example, if the mother is focused on her own emotions, she cannot sensitively respond to the signals of her infant. This increases the likelihood that the interactions between mother and child will become either intrusive or detached (Biringen & Robinson, 1991). Intrusive interactions are defined as adult-centered rather than child-centered and the maternal agenda becomes increasingly imposed on the child. Intrusive maternal behavior involves more rough physical contact (e.g., tickling, poking, tugging) and a louder, faster vocal style. In contrast, detached interactions occur when a caregiver is underinvolved and unresponsive to the infant's behavior and bids for attention. This withdrawn interaction style involves flat maternal affect and reduced touching or vocal interaction (Beckwith et al., 2002; Field et al., 2001; NICHD Early Child Care Research Network, 1997, 1999).

Maternal Sensitivity, Attachment, and Infant Stress Physiology

Maternal sensitivity is believed to be an important external source of emotion regulation for the developing infant (Ainsworth et al., 1978; Spangler, Schieche, Ilg, Maier, & Ackerman, 1994). In support of this idea, Braungart-Rieker, Garwood, Powers, and Wang (2001) found maternal sensitivity predicted infant affective regulation at 4 months. Over the last several years, the importance of maternal sensitivity and the attachment relationship for the development of physiological regulation has also been considered. Spangler and colleagues (1994) observed cortisol increases during a mother-infant free play session for 3 and 6 month-old infants of highly insensitive mothers only.

They hypothesized that sensitive maternal behavior acts as buffer against even mild stress at this age, while intrusive maternal behavior might actually cause stress via over-stimulation of the infant and result in activation of the HPA axis. In a related study, Bugental and colleagues (2003) found that infants of mothers that show frequent emotional withdrawal displayed elevated baseline levels of cortisol. Thus, either intrusive or detached maternal behaviors can have hormonal costs for infants.

Repeated exposure to insensitive maternal behavior might also serve to permanently alter the activity of the HPA axis. In support of this, Essex, Klein, Cho, & Kalin (2002) found that preschool children exposed to high levels of maternal stress (and potentially greater maternal insensitivity) both concurrently and during infancy had elevated cortisol levels compared children who were not exposed to high levels of maternal stress. Children exposed to maternal stress during a single period of development (either concurrently or during infancy) did not show elevated cortisol levels relative to children never exposed to maternal stress. Thus, long-term exposure to maternal stress (or insensitive maternal behavior) may be necessary for permanent alteration of adrenocortical functioning.

The mother-child attachment relationship has also been studied in connection with child stress physiology. The security of this relationship is believed to reflect a history of interactions between mother and child. Secure children are believed to develop internal working models that hold their caregivers as trustworthy and themselves as worthy of love and attention. In contrast, insecurely attached children are believed to have been exposed to either inconsistent or intrusive maternal caregiving behavior (for review, see Belsky & Cassidy, 1995). In the studies examining links between attachment and

adrenocortical activity, temperament is also found to play a key role. Nachmias and colleagues (1996) observed cortisol elevations to the Strange Situation only for behaviorally inhibited toddlers in insecure attachment relationships. The authors hypothesized that mothers of these inhibited children interfere with their children's coping strategy for dealing with novelty or over-stimulation. Rather than allowing these children to avoid novel stimuli or to approach it cautiously, these intrusive mothers attempt to shape their child's behavior by encouraging them to approach novelty and still remain calm. Spangler and colleagues have also independently confirmed this pattern of adrenocortical elevation during the Strange Situation only for behaviorally inhibited children in insecure attachment relationships (Spangler & Scheiche, 1998; Schieche & Spangler, 2005). Thus, while sensitive maternal care can provide a buffer for temperamentally vulnerable children, insensitive maternal care does not and may even produce elevations in HPA axis activity (for review, see Gunnar & Donzella, 2002).

In addition to temperament, van Bakel and Riksen-Walraven (2004) observed an interaction between cognitive functioning and attachment security in the prediction of adrenocortical reactivity to novel stimuli. Among 15-month old infants exposed to a stranger and a novel robot, the highest cortisol reactivity was observed for insecurely attached infants that scored the highest on the Bayley Scales of Infant Development, a measure of infant cognitive ability. This finding confirmed the hypothesis that insecurely attached and cognitively developed infants would be most aware of the threat posed by the novel encounter, but would not have the benefit of a sensitive caregiver, and would thus show an adrenocortical response to the situation. Thus, links between the attachment relationship and adrenocortical reactivity seem to confirm associations

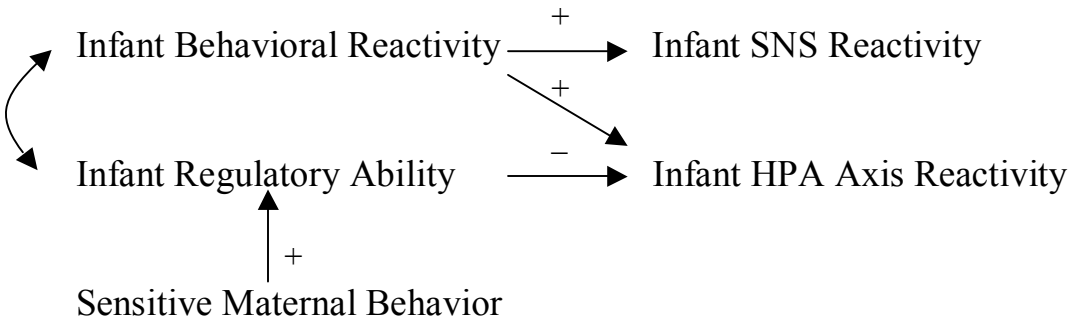
between maternal behavior and infant stress physiology, such that sensitive maternal behavior acts as an external organizer of infant regulation and reduces adrenocortical reactivity to stress. In addition, they underline the importance of individual differences in infant characteristics in determining whether an adrenocortical response to a challenge is more likely to be observed for a particular child.

Until very recently, little work had been done to examine links between maternal behavior or attachment and SNS responses to challenge during infancy. The dearth of work in this area is likely due to the methodological difficulties associated with the measurement of sympathetic activity in children (Granger, Kivlighan, et al., 2006; in press). In the last few months, Hill and colleagues (personal communication, February 2006) observed elevated sAA levels for infants classified as having an avoidant attachment relationship. This finding supports the theoretical position that avoidantly attached infants are externally regulating their behavior in order to avoid attracting the attention of their caregiver (Belsky & Cassidy, 1995), but that the Strange Situation does exact an internal (physiological) toll on these infants.

There is a great deal of evidence that maternal behavior (sensitive vs. insensitive) and the quality of the attachment relationship are associated with the functioning of the HPA axis in infancy. For the most part, adrenocortical elevations to mild challenge have been observed in the infants and young children of highly insensitive mothers. Alternatively, behaviorally inhibited or cognitively advanced children in insecure attachment relationships, believed to be the result of insensitive parenting, also show adrenocortical elevations to mild stressors. Such findings support the role of the mother as an external organizer of infant physiological regulation (Spangler et al., 1994). In

addition, the majority of children enter an adrenocortical hyporesponsive period during the first year (for review, see Gunnar & Donzella, 2002), and it has been suggested that the dampened cortisol response to stress during this period is supported by sensitive maternal behavior, which acts as a buffer against even mild stress at this age (Spangler et al., 1994). Thus, infants of insensitive mothers (i.e., intrusive or withdrawn) are more likely to show adrenocortical elevations to even mild challenge due to the absence of external regulation by their mothers. Figure 4 demonstrates how maternal behavior may enhance or impede an infant's behavioral and physiological regulatory abilities.

Figure 4. Sensitive maternal behavior supports infant affective and physiological regulation ability, thus reducing the need for activation of the HPA axis. However, insensitive maternal behavior (i.e, intrusive or withdrawn) may reduce infant regulatory resources and result in adrenocortical elevations to challenge.



Maternal Sensitivity and Maternal Stress Physiology

Quality of maternal caregiving behavior has been studied extensively as a key factor in the developing mother-child relationship (for reviews see Beckwith et al., 2002; Belsky & Cassidy, 1995). However, there are individual differences in women's ability to perceive infant cues and this might be linked to maternal arousal and vulnerability to stress. A sensitive mother should be able to perceive both the presence and magnitude of infant distress in response to a challenge and respond to it appropriately (Ainsworth et al.,

1978). Thus, if a mother is sensitive to her infant's signals, the presence or level of her infant's distress, may determine the nature of her physiological response to infant challenge. Therefore, if an infant does not display behavioral distress when presented with a mild challenge, a sensitive mother should not respond physiologically to the situation. However, if the child does respond negatively to a challenge, the maternal physiological response of a sensitive mother should reflect the magnitude of the infant's behavioral distress (Sethre-Hofstad et al., 2002). This is because a sensitive mother not only perceives her infant's affective signals, but also synchronizes her affect with that of her infant (Field, 1994; Stern, 1985).

Few studies have examined links between maternal behavior and maternal physiological arousal to stress. There is some evidence to suggest that mothers show SNS reactivity to infant distress and this may be linked to maternal interest in the infant. Maternal physiological responsiveness to infant distress stimulus appears to be greater when a women hears her own infant as compared to the cries of an unfamiliar infant (Weisenfeld & Klorman, 1978). Cardiac decelerations, consistent with attentional focus (Porges, 1995), are observed when mothers listen to the cries of unfamiliar infants (Donovan et al., 1978; Wiesenfeld & Klorman, 1978). In contrast, cardiac accelerations and elevations in skin conductance are observed when a woman is presented with videotapes of her own child crying (Wiesenfeld & Klorman, 1978). Thus, hearing one's own child cry appears to be a potent stimulus for SNS activity in mothers.

Very few studies have observed links between sensitive maternal behavior and adrenocortical activity. There is some evidence that basal levels of cortisol during the postpartum period are associated with maternal behavior. One group found higher basal

cortisol levels associated with more positive maternal attitudes towards infants and higher levels of non-instrumental or affectionate contact with infants (Fleming, Steiner, & Anderson, 1987). In a later study, maternal experience effects were observed for this relationship such that higher cortisol levels were associated with more affectionate contact in primiparous mothers and more instrumental behavior in multiparous mothers (Fleming, Steiner, & Corter, 1997). Mothers with higher postpartum cortisol are also better able to identify a recording of their own infant's cry (Stallings, Alison, Corter, Worthman, & Steiner, 2001). Thus, higher cortisol levels in the postpartum period may prime mothers to show more caregiving behavior, but the nature of this care depends on previous maternal experience. In line with these experience-related effects, another study found age differences in the relationship between cortisol levels and maternal caregiving behavior. Among young mothers (19-25 years), higher cortisol levels while holding infants were associated with more affectionate maternal behavior. There was no association between cortisol levels and affectionate maternal behavior in teen mothers (15-18 years) or mature mothers (26-40 years). However, teenage mothers with higher cortisol levels showed lower levels of instrumental care while holding their child.

While cortisol levels have been found to be associated with maternal behavior, no studies have observed cortisol reactivity to the stimulus of infant crying or challenge. In a study of 3 year-old children and their mothers, Sethre-Hofstad and colleagues (2002) observed no cortisol reactivity in mothers as a group to observing their children walk a balance beam for the first time. In another study, mothers observing their 3 month-old infants receive inoculations similarly displayed no adrenocortical response as a group (Braarud & Stormark, 2006). Thus, while the degree of SNS reactivity to infant cry and

overall cortisol levels appear to be linked to maternal caregiving behavior, maternal cortisol reactivity to infant distress does not appear to be related to sensitive maternal behavior.

Only one study, to my knowledge, has examined hormonal correlates of insensitive maternal behavior. Schechter and colleagues (2004) observed that among mothers with histories of violent trauma lower baseline cortisol levels were marginally related to their high levels of "atypical" parenting behavior during a 30-minute play paradigm involving separation and reunion. "Atypical" or "frightening or frightened" maternal behavior appears as though the mother perceives the child as a threat and is commonly observed in traumatized mothers (Lyons-Ruth & Jacobvitz, 1999). Thus, such behavior would constitute an extreme form of insensitive maternal behavior.

Abuse may also represent a form of extremely insensitive maternal behavior. There is some work examining SNS responses to crying infants in abusive mothers or those at risk for committing abuse. Crying is often the precipitating stimulus for abuse of children and may be more aversive and arousing to abusive parents than non-abusive parents (Frodi & Lamb, 1980). In one study, abusive and non-abusive mothers were shown two videotapes of the same infant either crying or smiling. Both abusive and non-abuse parents showed heart rate and skin conductance responses to the crying stimulus, but the abusive mothers had significantly greater physiological responses than non-abusive mothers. The abusers also reported finding the baby less pleasant and themselves as more annoyed and less sympathetic to the baby than their counterparts. Non-abusive parents responded to the smile stimulus with either no change or decreases in SNS activation, but the abusive parents showed the same physiological reactivity to both the

smiling and crying infant stimulus. They also reported being more indifferent to, annoyed by, and less willing to interact with the smiling baby (Frodi & Lamb, 1980). Thus, abusive parents appear to be less interested in infants in general and find crying infants especially arousing and aversive.

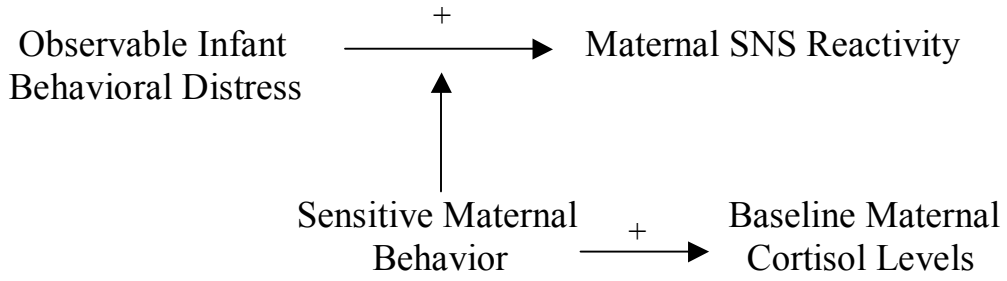
Adults abused as children are at elevated risk for becoming abusive parents themselves (Narang & Contreras, 2005). In a related study of mothers with a history of child abuse, previously abused women displayed increases in skin conductance while viewing a videotape of a smiling infant, but did not respond to a crying infant. In contrast, non-abused women showed increased physiological responses (skin conductance) to the crying infant, but not to the smiling infant as in the previous study (Casanova, Domanic, McCanne, & Milner, 1994). Thus, the mothers with a history of abuse in this study partially show the same physiological pattern evidenced by the abusive mothers in the previous study. Both showed physiological arousal to the smiling infant. However, while the abusive mothers showed exaggerated SNS arousal to the crying infant as compared to non-abusive parents, the previously abused women showed significantly less arousal to infant behavior than other mothers. Therefore, both over- and under-arousal to infant distress may be considered a risk factor for sub-optimal maternal behavior.

Such findings are reminiscent of the nature of intrusive and detached maternal behavior as being either over- or under-stimulating for the infant. While no studies, per se, have examined physiological correlates of intrusive or detached maternal behavior, it is possible that the sympathetic and adrenocortical responses of intrusive and detached mothers will reflect their behavioral interactions with their children. If intrusive

interactions are based on a mother's focus on her own emotions and agenda (Beckwith et al., 2002; NICHD, 1999), mothers displaying these behaviors may be more focused on their own distress during infant challenge than on the distress of the infant. Such behaviors are in line with the emotional and physiological reactions of abusive parents to infant distress (Frodi & Lamb, 1980). In contrast, detached mothers are generally unresponsive to their infant's signals (Beckwith et al., 2002; NICHD, 1999). Thus, similar to their lack of behavioral response, these mothers may be likely to display a lack of physiological stress response to their infants as well. While there was no measure of detached behavior per se, the lack of physiological response by the previously abused mothers could have represented an emotional withdrawal in response to infant distress.

In summary, it has been suggested that a sensitive maternal physiological response to infant challenge should reflect the magnitude of infant distress (Sethre-Hofstad et al., 2002). There is evidence that mothers do show SNS reactivity to observing their own infant's cry. More affectionate maternal care is also related to higher cortisol levels in the early postpartum period, but maternal adrenocortical reactivity in response to infant distress has not been observed. Finally, over- or under-activation of the SNS to infant distress and lower basal cortisol levels have been observed in mothers displaying extreme forms of insensitive maternal behavior including atypical maternal behavior and child abuse. Thus, there is some evidence that the quality of maternal care is linked to maternal SNS reactivity and basal levels, but not reactivity, of the HPA axis. Figure 5 presents the potential role of maternal sensitivity as a moderator of the link between observable infant distress and the degree of maternal SNS reactivity.

Figure 5. Maternal sensitive (or insensitive) behavior may moderate the relationship between observed infant distress and maternal SNS reactivity, such that SNS reactivity in more sensitive moms will be more closely linked to observable infant distress. In addition, there is also evidence that maternal sensitivity may be positively associated with basal maternal cortisol levels.



Maternal Psychopathology and Stress Physiology in Mother-Infant Dyads

Maternal Psychopathology and Infant Stress Physiology

The negative impact of maternal depression on parenting behaviors and infant development has been well documented (for reviews, see Beckwith et al., 2002; Field, 1998). Behaviorally, infants of mothers reporting depressive symptoms differ from infants of non-depressed mothers (for reviews, see Beckwith et al., 2002; Goodman & Gotlib, 1999). During the postpartum period, Abrams, Field, Scafidi, and Prodromidis (1995) examined infant performance on the Neonatal Behavioral Assessment Scale (NBAS) in a sample of low-income, young mothers (age 14-21) and their infants. They found that infants of depressed mothers displayed poorer orienting skills, depressed motor tone, and lower activity levels as compared to the infants of non-depressed mothers in this sample. Among 12 month-old infants and their depressed mothers, infants of intrusive-depressed mothers engage in less object exploration and display more negative affect, while infants of withdrawn-depressed mothers showed more dampened affective responses (Hart, Jones, Field, Lundy, 1999). A longitudinal study aimed to examine the

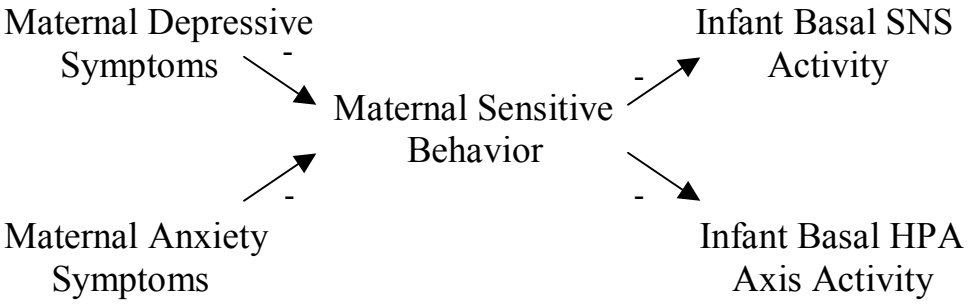
long-term effects of postpartum depression at 3 months in mother-infant dyads (Righetti-Veltema, Bousquet, & Manzano, 2003). Eighteen month-old infants of depressed mothers scored less well on tests of object concept and were more often found to have insecure attachments to their mothers than infants of non-depressed mothers. Finally, maternal depressive symptoms during the first 30-months of life were found to predict both problem behaviors and lower competency scores for boys, but not girls (Carter, Garrity-Rokous, Chazan-Cohen, Little, & Briggs-Gowan, 2001). Thus, it is well-established that maternal depression can have deleterious consequences for infant developmental outcomes.

Physiologically, infants of mothers reporting postpartum depressive symptoms also differ from infants of non-depressed mothers. Within two-weeks of delivery, infants of depressed mothers have significantly elevated urinary norepinephrine and cortisol levels as compared to infants of non-depressed mothers (Diego, Field, Hernandez-Reif, Cullen, Schanberg, & Kuhn, 2004). In addition, older infants of depressed mothers showing detached or withdrawn behaviors also have elevated basal cortisol levels (Bugental et al., 2003). Thus, the infants of depressed mothers show both heightened SNS and HPA axis activity and these effects may be the result of altered maternal behavior. Even among non-clinical samples, mothers with more severe symptoms of depression are less responsive, more disengaged, and show more hostility in interactions with their infants (Cohn & Campbell, 1992).

Maternal anxiety can also impact a mother's ability to respond sensitively to her child and correspondingly, child development. Children of parents with panic disorder are more likely to be behaviorally inhibited and to form insecure attachments (Manassis,

Bradley, Goldberg, Hood, & Swinson, 1994; Rosenbaum et al., 1988; 2000). Physiologically, at both 4 months and 14 months, infants of anxious mothers (i.e., panic disorder) also show elevated basal cortisol levels during infancy (Warren et al., 2003). Mothers with anxiety or panic disorder are less warm, more intrusive, more likely to express anger in disciplinary situations, and show reduced contingent responsiveness during exploratory play (Nover, Shore, Timberlake, and Greenspan, 1984; Weinberg & Tronick, 1998; Whaley, Pinto, & Sigman, 1999). Biringen (1990) also found that mothers scoring high in covert anxiety (i.e., concern about other's perceptions) were less sensitive and interacted less harmoniously with their infants. Thus, symptoms of both anxiety and depression may reduce maternal sensitivity, resulting in elevated SNS and HPA axis activity in their infants. The role of the quality of maternal behavior as a potential mediator of the effects of maternal depression or anxiety on infant stress physiology is presented in Figure 6.

Figure 6. Symptoms of maternal depression and anxiety may reduce sensitive maternal behavior, leading to elevated basal levels of both SNS and HPA axis activity in infants.

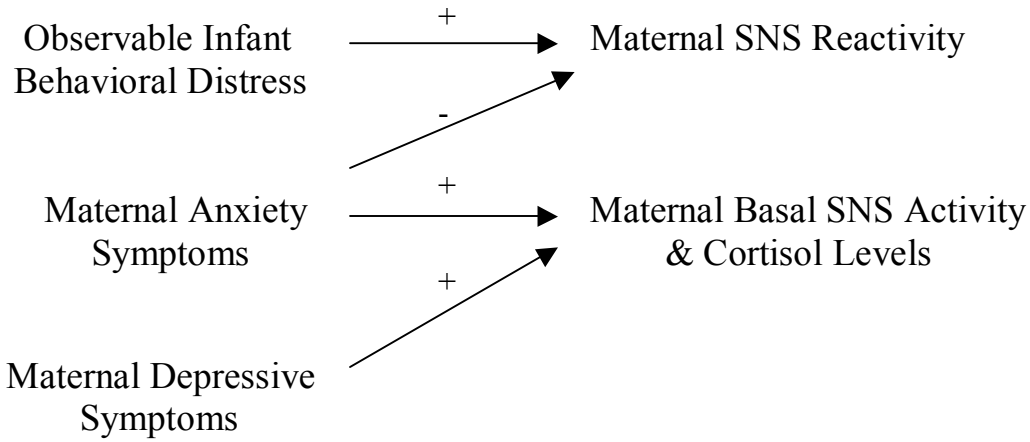


Maternal Psychopathology and Maternal Stress Physiology

Depression and anxiety in mothers may also directly impact the functioning of the two major stress responsive systems: the SNS and the HPA axis. In general, both depression and anxiety are associated with elevated basal cortisol levels, a flattening of the diurnal rhythm, and non-suppression in response to dexamethasone administration (Stokes, 1995; Gold, Goodwin, & Chrousos, 1988). Individuals with anxiety disorders also show dysregulation of the SNS (Brawman-Mintzer & Lydiard, 1997), such that individuals with anxiety disorders show less flexible autonomic responses to stress characterized by weaker responses to novel situations, slower habituation, and return to baseline (Hoehn-Saric & McLeod, 1988). In support of this, Hoehn-Saric, McLeod, and Zimmerli (1989) observed sympathetic *inhibition* in response to performance stress among female patients with generalized anxiety disorder. Thus, symptoms of depression and anxiety in may directly alter SNS and HPA axis responsiveness to infant challenge. In general, mothers depressed both pre- and postpartum show elevated cortisol and norepinephrine levels at 2 weeks post-partum compared to non-depressed mothers (Diego et al., 2004). In contrast, Shea and colleagues (2006) recently reported seeing lower salivary α-amylase levels in depressed mothers compared to controls. However, no studies, to our knowledge, have examined the physiological responses of depressed or anxious mothers to infant distress. It seems likely that mothers showing symptoms of depression or anxiety will show reactivity patterns in line with physiological profiles often associated these psychopathologies. Based on the literature, while mothers with either anxiety or depressive symptoms may show elevated SNS activity and cortisol levels, anxious mothers may show reduced SNS reactivity to infant distress. Figure 7

demonstrates the potential associations between maternal depression and anxiety with both basal and reactive levels of SNS and HPA axis activity.

Figure 7. Both anxious and depressed mothers may have elevated basal levels of SNS and HPA axis activity, but anxious mothers will show reduced SNS reactivity to infant distress.



Physiological Attunement in Mother-Infant Dyads

The confluence of infant and maternal behaviors and characteristics may promote or reduce the likelihood of both behavioral and physiological attunement in mother-infant dyads. During infancy, synchronous dyadic interactions characterized by mutual regulation and reciprocity are believed to be an important aspect of the mother-child relationship (Field, 1987; Tronick & Giannino, 1986; for review see Harrist & Waugh, 2002). Such interactions are key for the facilitation of perceptual processing, secure attachment, and both physiological and affective homeostatic regulation (Stern, 1977; 1994). The existence and meaning of physiological attunement is also beginning to be explored by researchers interested in early child development (Bornstein & Suess, 2000; Sethre-Hofstad et al., 2002; for review, see Field, 1992). Depending on the physiological

system of interest, the degree of this attunement has been alternatively measured either using correlational analysis to examine links between the basal or reactive levels of a particular physiological system between dyad members (Bornstein & Suess, 2000; Sethre-Hofstad et al., 2002) or by examining second-by-second coherence in physiology using methods such as time series analysis (DiPietro, Irizarry, Costigan, & Gurewitsch, 2004; Field, 1985). According to Field, (1992), both physiological and behavioral synchrony in mother-infant dyads develops as each partner continually modulates the other's level of arousal. Thus, the multiple harmonious interactions and shared affective states that occur during daily activities form the basis for mother-infant dyadic relationship (Field, 1987; Tronick & Giannino, 1986). Interestingly, some theorists have suggested that mothers might actually be biologically pre-adapted to attunement with their infants (Brazelton, 1984; Emde, 1984, Stern, 1985; Papousek & Papousek, 1981, 1987).

While it is unknown how early the development of mother-infant dyadic synchrony begins, recent research suggests that this process may have its roots during the prenatal period. DiPietro and colleagues (2004; 2006) have observed biobehavioral synchrony in maternal-fetal dyads. This synchrony consists of temporal associations between fetal movement and maternal heart rate and skin conductance, such that maternal physiological measures increased in response to fetal movement. In addition, the degree of linkage between fetal movement and maternal response is consistent across gestation and appears to represent stable individual differences between maternal-fetal pairs (DiPietro et al., 2004; 2006). While little is known about the function or purpose of maternal-fetal synchrony, the authors suggest that fetal stimulation of maternal

physiological response might serve to entrain maternal arousal patterns to meet the needs of the newborn or alternatively, to prepare new mothers to be receptive to infant cues and subsequently provide more sensitive care for their infants (DiPietro et al., 2004). At birth, the biological rhythms of the infant also appear to be connected to the development of behavioral synchrony in mother-infant dyads. Recently, Feldman (2006) found that sleep-wake cycles, vagal tone, newborn orientation and arousal modulation as measured by the NBAS at term were each predictive of the degree of mother-infant behavioral synchrony when infants reached 3 months of age. Thus, either prior to or at birth, both mothers and infants appear to be uniquely equipped for the development of behavioral and physiological synchrony.

Later in infancy, it has been observed that physiological synchrony may also extend to sleep. In a study of 2 to 4 month-old infants, McKenna and colleagues (1994) observed and monitored mother-infant dyads with polysomnography while sleeping in adjacent rooms or co-sleeping in the same bed. The found that while co-sleeping, mothers and infants spent more time in the same state of sleep or wakefulness than when they slept in adjacent rooms. During co-sleeping the mother is believed to act as an external regulator of infant sleep, reducing the amount of time the infant spends in deep sleep, and possibly lowering the likelihood of sudden infant death syndrome (SIDS; McKenna et al., 1994).

During wakeful periods, physiological attunement has been observed in other domains. Borntein and Suess (2000) were interested in examining stability in infant and maternal vagal tone response, a measure of parasympathetic activity, to attention tasks between early infancy (2 months) and at 5 years. Baseline vagal tone was measured in

both the children and their mothers, age-appropriate attention tasks were administered to both dyad members, and vagal tone suppression to these tasks was assessed. While there was no correlation or concordance between child and maternal baseline vagal tone at either age, there was a consistent positive association between the degree of vagal tone suppression to these tasks between mothers and infants at both 2 months and 5 years. This concordance in vagal tone responsiveness, but not in resting vagal tone, suggests an attunement between mothers and children's response to environmental challenge due to co-experiential factors, such as repeated interactions and modeled affective styles, rather than genetic ones (Bornstein & Suess, 2000). The same processes might also hold true for the co-development of adrenocortical responses to challenge. Sethre-Hofstad and colleagues (2002) observed concordant adrenocortical reactivity to child challenge for dyads with highly sensitive mothers. Mothers observed their 3 year-old children walk a balance beam for the first time on a monitor in a separate room. For dyads with sensitive mothers only, there was a positive association between child and maternal cortisol change across the tasks. Thus, the reactivity of both the parasympthatic nervous system and the HPA axis appear to be linked within some mother-child dyads.

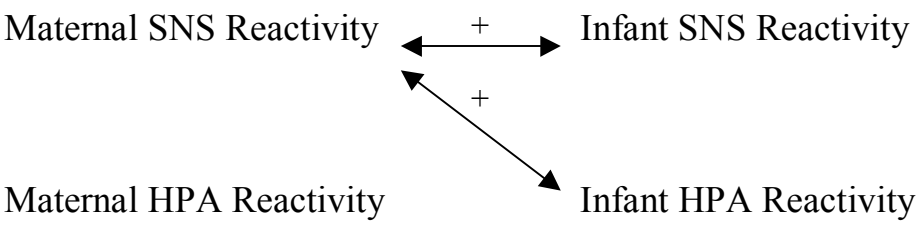
The type of shared experiences, whether positive or negative, may also influence the nature of the affective, behavioral, and physiological attunement. Field, Healy & LeBlanc (1989) examined heart rate and behavior states during mother-infant interaction with depressed and non-depressed mothers and their 3 month-old infants. Temporal synchrony was observed for both behavior states and heart rate and there was higher coherence between infant behavior and maternal heart rate for the non-depressed dyads. More specifically, dyads with depressed mothers spent a greater percentage of their time

in shared negative affective states (i.e., angry, protesting states) as compared to non-depressed dyads and these states were accompanied by a high coherence in heart rate. While high levels of affective and physiological attunement were also observed in the non-depressed dyads, the nature of the shared affective state was more positive than in the depressed dyads. This suggests that if a mother-infant dyad is regularly confronted with environmental stressors that serve to increase negative affect, such as persistent conflict, the resulting attunement between mother and child may be characterized by greater distress and negative affect (Field et al., 1989; Field, 1992). Such negative attunement is such cases might be linked to poorer developmental outcomes as compared to a more positive attunement.

In summary, there is growing evidence for the existence of physiological attunement, in addition to behavioral attunement, in mother-infant dyads. While behavioral attunement has been found to be extremely important for child development, it is currently unknown if physiological attunement will serve a similar function in these dyads. However, there is evidence that attunement in sleep stages during co-sleeping may actually promote infant survival. Physiological attunement may begin as early as the prenatal period and appears to be linked to the normal biological rhythms of newborns. In addition, one study suggests that attunement can either center on positive interactions and emotions or negative interactions and emotions. Whether or not behavioral and physiological attunement will have a positive or negative effect on child development may depend on the emotional valence (i.e., positive vs. negative) of the attunement. Finally, concordance in the reactivity of physiological systems (e.g. PNS, HPA axis) rather than basal or baseline levels in mother-child dyads suggests that attunement may

be due to experiential factors rather than to genetic ones. Thus, during a challenging situation, it may be more likely that physiological reactivity, rather than baseline activity levels of physiological systems, will be concordant in mother-infant dyads. Figure 8 presents to the potential for attunement between maternal SNS reactivity and both infant SNS and HPA axis reactivity.

Figure 8. Given that mothers respond to their infant's cries more often with SNS reactivity, maternal SNS reactivity may be concordant with either infant SNS or HPA axis reactivity during infant distress.



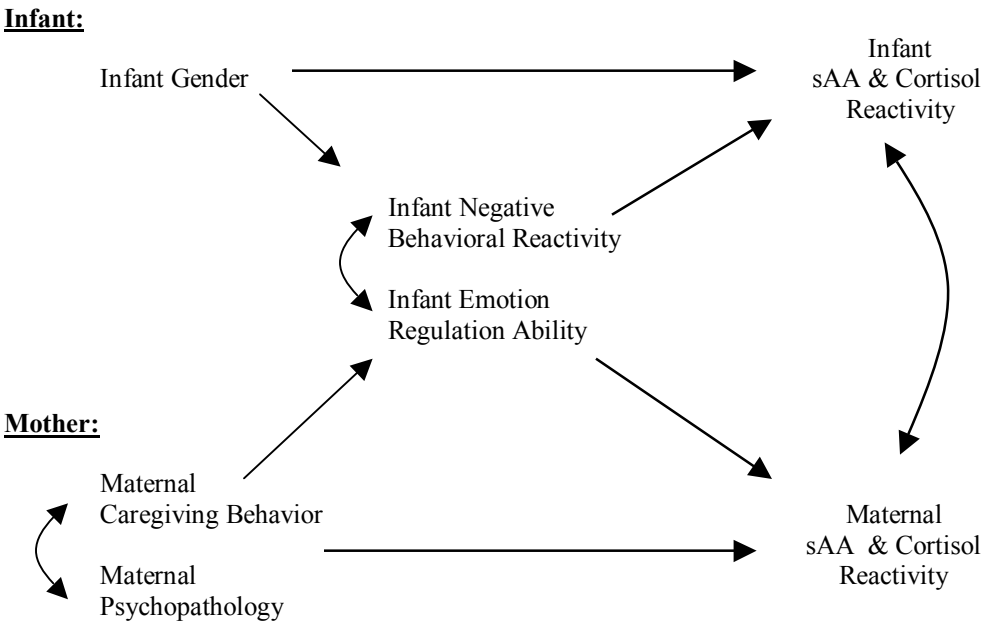
Summary: The Co-Development of Stress Physiological Systems in Mother-Infant Dyads

Work in both animal and human models clearly support that early maternal caregiving can influence the development of stress responsive physiological systems with long-term effects. While a great deal of work has focused on the impact of the mother-infant relationship on infant stress physiology, much less work has examined the influence of the infant on physiological responses to stress in the mother. Therefore, understanding what specific aspects of this bi-directional relationship will influence the co-development of the stress responsive physiology in both mother and child represents a significant knowledge gap. The impact of infant influences, such as temperament and infant gender, on the development and reactivity of the HPA axis have been examined, but no studies to our knowledge have addressed the impact of these characteristics on

maternal stress physiology. This is surprising given the links between the frequency of infant crying in extreme groups, such as colicky infants, and maternal psychological well-being. In addition, much work has examined the effects of maternal behavior (i.e., sensitive vs. insensitive behavior) and psychopathology (i.e., depression and anxiety) on infant HPA axis activity, but we know little about how these individual differences in maternal influences might influence maternal physiological response to infant distress. In particular, it has been observed that mothers frequently display SNS responses to hearing their own infant cry, but we know very little about how this physiological response is linked to a mother's ability to read her infant's cues and respond to them appropriately. Given that abusive mothers show elevated levels of SNS reactivity to infant distress and highly anxious mothers may have blunted SNS reactivity in general, it may be important to know how these physiological responses to infant distress related to parenting behavior in these extreme groups.

Finally, the aspects of the mother-infant relationship that influence both infant and maternal stress physiology will contribute to the development of these systems over time. It also seems likely that these experiences will result in concordant physiological responses to stress in mother and child, as the daily challenges faced by the mother-infant dyad are believed to form the basis for physiological attunement. Thus, the early caregiving environment clearly influences the co-development of stress physiological systems in both mother and child with implications for their health and welfare. Figure 9 presents a schematic representation of the proposed relationships between infant and maternal characteristics with physiological responses to infant challenge in the mother-infant dyad.

Figure 9. Conceptual model of dyadic stress vulnerability.



Chapter 4

THEORETICAL AND METHODOLOGICAL ISSUES IN STRESS RESEARCH

Moving Stress Research Forward: Examination of Individual Differences and Multiple Stress Physiological Systems

A review of the literature on stress research reveals two major issues that need to be addressed in order for the field to advance. First, researchers interested in stress physiology should move beyond focusing on physiological responses observed at the level of the aggregate towards investigating the meaning of physiological responses at the level of the individual. Second, stress responsive physiological systems provide a biological context for each other and concurrent investigation of the activity of multiple stress responsive systems may uncover previously hidden hormone-behavior relationships. The following review seeks to elaborate on these issues and to suggest a potential resolution.

Understanding Individual Differences in Physiological Responses to Stress

Early theorists assumed that physiological responses to stress were unidimensional and non-specific in nature. For example, Selye proposed that physiological responses to stress proceeded through a specific series of stages regardless of the type of stimulus (Selye, 1950). Since Selye's time, a variety of factors, such as stressor type, perception, and coping style have been shown to contribute to the physiological stress response profile of each individual in a given situation (for review, see Biondi & Picardi, 1999). In addition, it has been observed that neuroendocrine and adrenocortical responses to stress may be tied to specific perceptions of a particular

challenge. Lundberg and Frankenhaeuser (1980) examined links between self-reported subjective responses to different types of laboratory stressors (e.g., reaction time task, vigilance task, color-word conflict task) and the neuroendocrine responses to these tasks in adult men and women. They found that norepinephrine and epinephrine were more closely tied to self-reports of effort, tenseness, and concentration while cortisol was linked with impatience, boredom, tiredness and irritation. Such observations led stress researchers to conclude that SNS responses to stress are linked to feelings of alertness and effort (Frankenhaeuser, Lundberg, & Forsman, 1980; Lundberg & Frankenhaeuser, 1980), while HPA axis activation occurs when an individual perceives a challenge as unpredictable and/or feels either threatened or distressed (Mason, 1968). Henry (1992) has modified these ideas slightly by stating that an individual's perceived control over a situation may be the most important factor for determining the specific pattern of neuroendocrine responses to stress. Specifically, he suggests that challenges perceived as easy to cope with result in elevated norepinephrine levels. As challenges become more difficult, epinephrine secretion will increase coupled with rising feelings of anxiety. Finally, as perceived controllability declines, feelings of distress rise along with secretion of cortisol from the adrenal cortex. Thus, the specific activation of individual stress responsive physiological systems appears to be linked to an individual's perception of the stressor and their ability to cope with it.

Despite these observations, there does not always appear to be a tight link between psychological states and physiological responses to stress. According to Biondi and Picardi (1999), "the complex, multilevel interplay between psychological and biological factors is still poorly understood." This may be partially due to our

methodological approaches to examining hormone-behavior relationships. The majority of the statistical modeling techniques rely on linking behavioral or psychological factors with the mean physiological responses of the aggregate or deviations from this mean pattern. However, an examination of individual responses will reveal enormous reactivity in one individual and a lack of response in another that cannot be captured by examining the mean or deviations from the mean response. Moving beyond examining physiological responses to stress at the group level towards understanding the meaning of individual response patterns would likely advance our understanding of the links between psychological and physiological responses to stress. Moreover, an attempt to understand individual differences in physiological stress response profiles would also be responsive to calls by developmental scientists to focus our models on the individual rather than the group (Cairns, 1998).

Examination of the Simultaneous Activity of Multiple Stress Responsive Physiological Systems

The activity of one physiological system may also act as context for the activity of another. As such, considering the physiological context of the HPA axis is responsive to arguments that developmental models of biosocial phenomena need to include multiple measurements of biological processes (Donzella et al., 2000; Granger & Kivlighan, 2003; Repetti et al., 2002). Based on the early work of Frankenhaeuser and colleagues (1980), Bauer, Quas, and Boyce (2002) have suggested that coordination or dissociation of the activity of the SNS and HPA axis responses to stress might result in differential risk for the development of behavior problems. Specifically, if the activity of each system performs the same protective function for the body during stress, then the activation of

both systems could be considered additively in relation to risk. Thus, similar to the inverted "U-shaped" curve between arousal and performance proposed by Yerkes and Dodson (1908), this additive model suggests that both low and high arousal in both systems would be linked to lower functioning and greater risk, while a combination of low and high arousal in both systems would yield an optimal medium level of arousal and low risk. In contrast, if the activity of the SNS and HPA axis perform complementary functions in relation to protecting an individual from stress, then the activity of the two systems could interact to predict risk for developing behavioral problems. Thus, the interactive model predicts the lowest risk of developing behavior problems for children with balanced SNS and HPA axis activity, whether low or high, and the greatest risk for children with unbalanced activity between these two systems (Bauer et al., 2002). This model provides a first attempt at a theoretical framework for understanding how the SNS and the HPA axis provide a context for each other in relation to internalizing and externalizing behavior problems.

A few recent studies have sought to confirm the models proposed by Bauer and colleagues (2002). In support of the interactive model, which predicts the lowest risk for children with balanced SNS and HPA activity, El-Sheikh, Mize, and Granger (2005) found that lower levels of both salivary cortisol and sAA reactivity were associated with fewer child behavior problems. In contrast, Gordis, Granger, Susman, and Trickett (2006) found lower sAA and cortisol reactivity was associated with higher levels of parent-reported aggression, providing support for the additive model which predicts higher risk for children either low or high levels of activity in both the SNS and the HPA axis (Bauer et al., 2002). In addition, while this particular model is specific for

explaining children's behavior problems, it may be worthwhile to pursue a similar multiple system approach to stress research that seeks to identify individual differences in stress response patterns and links them to a variety of risk factors (e.g., quality of early environment) and outcomes (e.g., mental and physical health). New methods and models may be necessary in order to address these issues and move the field of stress research forward.

Applying Growth Mixture Models to Stress Physiological Research

The developmental literature has a long history of theorizing about the existence of qualitatively different developmental pathways within the population not readily identifiable by observable characteristics such as gender (Bauer & Curran, 2003; Nagin, 1999; Nagin, 2005). The purpose of such theories is to explain individual differences in development over time by using the theorized subgroups as approximations of a more complex reality (Cudeck & Henly, 2003; Nagin & Tremblay, 2001). Growth mixture modeling is an analytical strategy derived to answer the question posed by these models: "Do certain types of people tend to have distinctive developmental trajectories" (Nagin, 1999)? The myriad of individual differences in physiological responses to stress represent a similarly complex reality and the field of stress research may benefit from the use of similar methods.

Growth mixture modeling blends conventional growth models with finite mixture models. Conventional growth models are commonly estimated using hierarchical linear modeling (HLM; Raudenbush & Bryk, 2002), multilevel modeling (MLM; Mehta & West, 2000; Willett & Sayer, 1994), or latent curve analysis in structural equation

modeling (SEM) programs (McArdle & Epstein, 1987). Unconditional growth models use a continuous distribution function to estimate the means and the covariance structure of a set of repeated measures. Conditional growth models link variability about the mean trajectory to one or more predictors of interest. Thus, such models provide the analyst with the intercept and the shape of the trajectory (e.g., linear, quadratic, cubic) at the group level as well as the deviation from that trajectory for each individual. Such models assume that the shape of the developmental trajectory is similar for all members of the population (i.e., all linear paths)[1] and the parameters of the model follow the multivariate normal distribution in the population (Bauer & Curran, 2003; Nagin, 2005). Thus, use of these models is most suitable when the population is believed to be homogenous and individuals are believed to follow a similar trajectory. However, if the population is believed to be composed of distinct subgroups and the grouping variable is unobserved, traditional growth models may not be appropriate (Muthen & Shedden, 1999; Nagin, 1999).

Finite mixture models were developed as a means to identify qualitatively distinct subgroups within a population (Pearson, 1894; Rindskopf, 1990; for review see Bauer & Curran, 2003). These models use maximum likelihood estimation to estimate model parameters, thus extracted groups are not the product of a cluster analysis (Nagin, 2005). These models assume that a non-normal distribution is a mixture of a number of normal distributions. In fact, non-normality is a necessary condition for the application of finite

[1] While the shape of the developmental trajectory is assumed to be similar, the intercept and the slope of these pathways is allowed to vary across individuals. Thus, each case in the model will have an entirely unique developmental pathway, even if all pathways are linear.

mixture models. If the distribution of a variable of interest is normal, then only a single "group" is necessary to adequately model the distribution (Bauer & Curran, 2003).

Growth mixture models take finite mixture modeling into a longitudinal sphere and assume that there are distinct subgroups of longitudinal trajectories to be found within the population (Jones, Nagin & Roeder, 2001; Nagin, 1999; 2005; Nagin & Tremblay, 2001). Similar to conventional growth models, growth mixture models begin by trying to link the change in the dependent variable with time or age. Often this link is specified by a polynomial equation of time where the coefficients determine both the intercept and the shape of the trajectory of the dependent variable (i.e., linear, quadratic, cubic). One polynomial function is calculated for each subgroup specified by the model and the shape of these trajectories is not assumed to be the same across the various groups that are extracted. Thus, one group may show an entirely different pattern of change over time from another. In addition to the shape of the trajectory in each group, the growth mixture model also offers means for determining the optimal number of groups to include in the model, the proportion of the population that belongs to each group, and the probability that a particular individual belongs to each group (Nagin, 1999; 2005). It is because the grouping variable is unobserved (and the "true" number of groups is unknown), that the proportion of cases in each group as well as an individual's probability of belonging to any one group must also be estimated. In fact, each case or individual contributes to the parameter estimates for each latent class with its probability of membership in that class (Muthen & Muthen, 1998; for review see Bauer & Curran, 2003).

One of the major criticisms raised about growth mixture modeling is that it can be difficult to determine if the "correct" number of groups has been extracted. Generally, investigators will fit a series of models with different numbers of classes and then compare these models with fit statistics, such as the Bayesian Information Criteria (BIC), to determine the optimal number of groups (Bauer & Curran, 2003; Nagin, 2005). Bauer and Curran (2003) warn of the potential of growth mixture modeling methods to "over-extract" the optimal number of groups within a population. Specifically, using simulation procedures, they demonstrated that fit statistics can falsely indicate a two-group solution for a homogenous population with a non-normal distribution. In rebuttal, Cudeck and Henly (2003) suggest that due to the subjective nature of modeling, any attempt to simulate the "true" model is a farcical prospect from the very beginning and results will always be abstract and artificial. Psychological (and biobehavioral) problems are inherently complex and thus, they suggest that definitive mathematical models of such problems are impossible to discover. Therefore, they posit, "speculation about the 'true' number of subpopulations is pointless because there is no true number to find." Instead, they suggest that all statistical models of development serve three purposes: 1) to summarize the data and describe the process of change algebraically, 2) to formalize an opinion about the observed development with empirical support, and 3) to make useful predictions using the available model. In other words, the purpose of these models is not find the "true" number of groups within the aggregate, but instead to use these groups in order to better understand the complex reality that we observe in our data. While Nagin (2005) advocates the use of fit criteria to select the optimal number of groups, he also suggests that theoretical knowledge about the domain of interest is invaluable in the

model selection process. Thus, both the fit criteria and the theoretical meaning of the observed change in the groups should always be used to select the number of groups in mixture growth models.

As mentioned previously, there is a long history of theoretical models proposing the existence of distinct developmental trajectories within the population. Previous attempts to test these theories and gain access the heterogeneity of the larger group have involved the use of *a priori* assignment rules. For example, stress researchers have often assigned individuals to categories based on observed physiological responses to stress. One common strategy in the literature includes dividing our samples into two groups based on their pre- to post-stressor change in cortisol: "reactors" are defined as showing an increase over their baseline level, and "decliners" are defined by a decrease from baseline. While these rules have been useful in the past, they provide a good example of some of the potential pitfalls associated with using such assignment rules. First, the existence of certain groups must be assumed a priori and thus, the discovery of other potentially interesting groups is not possible (Nagin, 2005). For example, using these assignment rules, the "reactors" are all grouped together even though some may only show a minor cortisol increase and others may show an enormous increase over baseline. However, it is possible that there might be distinct differences between the weak and the strong reactors and that a growth mixture model might capture these differences. Among those who show a very small change from baseline, it is difficult to know if this change is meaningful and there might be some error in classifying these individuals as either "reactors" or "decliners". Growth mixture modeling quantifies uncertainty about group membership through the calculation of group membership probabilities for each

individual (Nagin 1999; 2005). Finally, using the change from baseline does not take into account the starting point or "intercept" of each individual. This is surprising given that individual differences in cortisol reactivity might be heavily influenced by the basal levels of HPA axis activity. For example, a difference score of 0.10 μg/dl may be a large change for an individual with a baseline level of 0.05 μg/dl of cortisol, but it would be a minor change for an individual with a baseline of 0.50 μg/dl. Growth mixture models take into account both the intercept and the shape of the trajectory over time (Nagin, 1999; 2005). Thus, while the use of assignment rules has been usefully applied in stress research in the past, growth mixture modeling may provide information about heterogeneous groups that might not be accessible through the use of assignment rules.

Applying Joint Trajectory Models to Examine Relationships Between Stress Physiological Systems

Growth mixture modeling may also be able to provide guidance for dealing with the second issues raised by this section as well. Namely, how do we concurrently investigate the activity of multiple physiological systems within a person? A related question might also ask, if we assume that our data contains a mixture of groups representing individual differences in physiological responses to stress, how do we test for the presence of "attunement" in physiological systems across persons?

Typically, examination of a relationship between two continuous variables involves the use of a summary statistic, such as a correlation, to examine the covariance between the two variables. However, examination of correlations makes poor use of a repeated measures design (Nagin & Tremblay, 2001). For example, in stress research, we might be able examine correlations between sAA and cortisol at each time point, but

this tell us little about how the change in sAA relates to the change in cortisol over time. Alternatively, we might calculate change or percent change scores and calculate correlations between these new variables. However, we can only examine the relationship between the changes in each salivary biomarker over a small part of the total stress response curve at time. Specifically, we could only look at "reactivity" or the initial response to the stressor or we could look at "recovery" or the return of physiological reactivity to baseline levels. However, we are never able to understand how the complete pattern of physiological response in the SNS relates to the complete pattern of physiological response in the HPA axis.

Additionally, there is another problem with using correlations to assess constructs like physiological attunement between mother and child. Correlation coefficients are assumed to apply evenly to the total population (Magnusson & Bergman, 1990; Pulkkinen & Tremblay, 1992). Thus, if we observe a correlation of 0.50 between maternal and infant cortisol levels at baseline, we would assume that that this correlation implies the magnitude of the relationship between maternal and infant cortisol levels for the whole population. However, if the population is heterogeneous and composed of distinct subpopulations, then for some of these populations the correlations might actually be higher and for others there might be no association at all (Magnusson & Bergman, 1990; Pulkkinen & Tremblay, 1992).

Nagin and Tremblay (2001) have tried to address these issues by describing a model that jointly estimates growth mixture models for two distinct, but related measurement series. The major output of these models, in addition to those described above for the univariate models, is the joint probability of membership in trajectory

groups across behaviors. The joint probabilities allow investigators to characterize the relationships between two dependent variables as they unfold over a specified period of time. As such, it allows taking full advantage of repeated measures designs, while at the same time, realizing that the nature of relationships between these dependent variables might differ between different subgroups in the population.

One caveat should be pointed out. The purpose of the joint trajectory model is to characterize the relationship between two dependent variables (i.e., behaviors, hormones, etc.). In effect, this model is improving upon the correlation coefficient as a measure of the relationship between these dependent variables (Nagin & Tremblay, 2001). In addition, the incorporation of predictor variables into this model should be interpreted as examining a link between group membership in one of the dependent variables, controlling for membership in the other variable (Nagin, 2005). Thus, to use this model, one should *a priori* expect a relationship between the two dependent variables of interest. In the case of examining the responses of sAA and cortisol to stress within the same person, use of the dual trajectory model is attractive, but does not seem tenable. Several recent studies have observed that there appears to be absolutely no relationship between the reactivity of these two biomarkers to stress (Granger, Kivlighan, et al., 2006; Nater et al, 2005; 2006; Rohleder et al., 2004). However, examination of physiological attunement between mother and child seems to be a perfect application of the dual trajectory model. Several studies have observed evidence for such physiological attunement in mother-infant dyads (Bornstein & Suess, 2000; Field et al., 1989; Sethre-Hofstad et al., 2002). Thus, application of the dual trajectory model to this question seems entirely appropriate.

Summary: Addressing Theoretical and Methodological Issues in Stress Research

In summary, physiological responses to stress are complex and the relationships between the physiological and psychological aspects of the stress response are not yet well understood. This section attempted to call to attention two major issues raised by the literature. First, stress research needs to move beyond focusing on patterns observed at the group levels towards investigating the meaning of physiological response patterns at the level of the individual. Second, stress responsive physiological systems provide a biological context for each other and concurrent investigation the activity of multiple stress responsive systems may uncover previously hidden hormone-behavior relationships. The use of growth mixture modeling was proposed as a possible solution for these two problems. Application of univariate growth mixture models would aid our understanding of the very complex reality we observe when studying physiological responses to stress. In addition, these models may also address many of the problems inherent in using group assignment rules in stress research to access the underlying heterogeneity in physiological responses to stress in the population. The extension of these models to examine the relationship between distinct, but related dependent variables also appears as though it might be responsive to the second problem on the surface. However, the application of joint trajectory models to the question of physiological attunement within mother-infant dyads might be more appropriate than to the problem of understanding the interaction of SNS and HPA axis responses to stress.

Chapter 5

RESEARCH QUESTIONS AND HYPOTHESES

The purpose of the current project is to examine the impact of the mother-infant relationship on both infant and maternal stress physiology through concurrent examination of sAA and cortisol responses to infant challenge in a sample of low-income mothers and their 6 month-old infants. Separately for infants and mothers, a semi-parametric, growth mixture modeling method (Jones, Nagin, & Roeder, 2001; Nagin, 1999; 2005) will be used to identify unique response profiles of sAA and cortisol, link them with infant and maternal behaviors and characteristics, and examine associations between infant and maternal profiles in order to address the following research questions and hypotheses:

1) Are infant behavioral reactivity, regulatory behavior, and infant gender associated with sympathetic and adrenocortical responses to challenge in mother-infant dyads?

Hypothesis 1: *Infant behavioral reactivity and regulatory behavior will interact to predict physiological stress response profiles in mother-infant dyads.*

Infant sAA response to challenge will be positively associated with behavioral reactivity, but will not be influenced by regulatory behavior. However, infant behavioral reactivity and regulatory behavior will interact to predict cortisol responses to infant challenge, such that more regulatory behavior will reduce cortisol reactivity. Specifically, infants that show high behavioral reactivity to the challenge tasks and a high number of regulatory behaviors will have a physiological reactivity profile characterized by high sAA reactivity and low to moderate cortisol reactivity with recovery. In contrast, infants that show high behavioral reactivity to the challenge tasks, but few regulatory behaviors

will have a physiological reactivity profile characterized by high sAA reactivity, and high cortisol reactivity with no recovery.

Infant behavioral reactivity and regulatory behavior will produce an overall level of negative reactivity observable by the mother. Maternal sAA, but not cortisol responses to infant distress will be associated with this observable negative reactivity. Mothers of highly behaviorally reactive infants who show many regulatory behaviors will observe less overall distress, and thus will show low to moderate sAA reactivity, but no cortisol reactivity to infant challenge. Mothers of highly behaviorally reactive infants who show few regulatory behaviors will observe more overall distress and thus, will have higher sAA reactivity than mothers of infants using more regulatory behaviors, but no cortisol reactivity to their infants' distress.

Hypothesis 2: *Infant and maternal physiological stress response profiles will differ by infant gender.*

Infant boys may rely more on external maternal support for emotion regulation, and thus will be more likely to show higher cortisol reactivity to the challenge than girls when maternal support is unavailable. Mothers may have more difficulty passively observing infant boys in distress as opposed to infant girls. Therefore, mothers of infant boys will show higher sAA reactivity to infant challenge than mothers of girls, but no cortisol reactivity.

2) Do maternal sensitivity and psychopathology influence the relationship between infant behavioral reactivity and sympathetic and adrenocortical responses to challenge?

Hypothesis 3: *Maternal sensitivity will be associated with both infant and maternal physiological stress response profiles.*

Sensitive maternal behavior supports infant affective and physiological regulation, reducing the need for activation of the HPA axis. However, insensitive maternal behavior (i.e, intrusive or withdrawn) will reduce infant regulatory resources and result in adrenocortical elevations to challenge. Therefore, infants of highly sensitive mothers will show lower cortisol reactivity to infant challenge as compared to infants of highly insensitive (i.e., intrusive or detached) mothers.

Maternal sympathetic, but not adrenocortical reactivity has been observed in response to infant distress. Maternal sensitivity has been associated with higher baseline cortisol levels, but over- or under-activation of the SNS to infant distress has been linked to extreme forms of insensitive maternal behaviors. Therefore, highly sensitive mothers will show sAA reactivity but not cortisol reactivity to infant distress. They will also have higher baseline cortisol levels than highly insensitive mothers (i.e. intrusive or detached). Highly intrusive mothers will be physiologically over-reactive and will show greater sAA and cortisol reactivity than low intrusive mothers regardless of the level of their infants' distress. Highly detached / disengaged mothers will be physiologically under-reactive and will show no sAA or cortisol reactivity in the context of infant distress.

Hypothesis 4: *Maternal psychopathology will be associated with both infant and maternal physiological stress response profiles.*

Symptoms of maternal depression and anxiety have been associated with elevated basal levels of both SNS and HPA axis activity in infants. Therefore, infants of mothers with either a high number of depression or anxiety symptoms will have elevated baseline sAA and cortisol levels as compared to infants of mothers with a low number of depression or anxiety symptoms. Similarly, both anxiety and depression are associated with elevated SNS activity and cortisol levels. Anxiety disorders are also linked to weak SNS reactivity to stress. Thus, mothers with a high number of depression or anxiety symptoms will have elevated baseline sAA and cortisol levels as compared to infants of mothers with a low number of depression or anxiety symptoms. In addition, mothers with a high number of anxiety symptoms will also show less sAA reactivity to observed infant distress than mothers with a low number of anxiety symptoms.

3) Is there attunement or linkage between mothers' and their infants' physiological (i.e., sympathetic or adrenocortical) responses to challenge early in infancy.

Hypothesis 5: *There will be attunement between infant and maternal physiological stress response profiles.*

Baseline maternal sAA levels will be associated with infant sAA levels. Baseline maternal salivary cortisol levels will be associated with infant salivary cortisol levels. In addition, previous studies have observed concordance in child and maternal PNS and adrenocortical response to challenge. Given that maternal SNS, but not adrenocortical reactivity has been observed to infant distress, maternal sAA reactivity profiles to

observed infant distress will be associated with infant sAA and/or cortisol reactivity profiles to infant challenge. There will be no associations between maternal and infant salivary cortisol reactivity to infant challenge.

Chapter 6

RESEARCH METHODS

Overview of the Family Life Project

The Family Life Project, an NICHD program project (1P01HD396667-01A1), was designed to examine the early developmental competence of children living in rural America, especially in areas where the rural poverty levels are highest. The project was designed to study families living in two of the four major geographical areas of high child rural poverty (Dill, 1999). Eastern North Carolina (Sampson, Wayne and Wilson counties) and Central Pennsylvania (Blair, Cambria and Huntington counties) were selected to be indicative of the Black South and Appalachia, respectively. Complex sampling procedures were used to recruit families in hospitals at the birth of a child, over-sampling for low-income families in both states, and African American families in North Carolina. Families were designated as low income if they reported household income less than 200% of the poverty rate, use of social services requiring a similar income requirement (e.g., food stamps, WIC, Medicaid), or had less than a high school education.

In Pennsylvania, families were recruited in person from three hospitals. These three hospitals represented a weighted probability sample (hospitals were sampled proportional to size within county) of seven total hospitals that delivered babies in the three target PA counties and provided 89% coverage. In North Carolina, families were recruited in person in three of the hospitals that delivered babies in the target counties and by phone for families who resided in target counties, but delivered in non-target county hospitals. In both states, recruitment occurred seven days per week over the 12-month

recruitment period spanning September 15, 2003 through September 14, 2004 using a standardized script and screening protocol (See Appendix A). In total, recruiters identified 5471 (57% NC, 43% PA) women who gave birth to a child during the recruitment period, 72% of which were eligible for the study. Eligibility criteria included residency in target counties, English as the primary language spoken in the home, and no intent to move from the area in the next three years. Of those eligible, 68% were willing to be considered for the study. Of those willing to be considered, 58% were invited to participate. Mothers, fathers, and other adults residing with and assisting the mother in the care of her child were asked to take part in the study. Of those selected to participate, 82% of families completed their first home visit, at which point they were considered enrolled in the study. A total of 1292 families were successfully enrolled. The final ethnic makeup of the sample is 59% European-American families and 41% African American. Sixty-five percent of the total sample is below 200% of federal poverty guidelines. Home visits for the purpose of data collection are made to the participants' homes when the target child reached 2, 6, 15, 24, and 36 months of age.

Overview of the Present Study

The data for the present study was taken from the 6-month assessment of the Family Life Project. Home visits were made to the participants' home on two separate occasions. All of the measures used in the current project were collected during the second visit, which focused on the primary caregiver and infant exclusively. During the approximately three hour visit, mothers and children were asked to participate in a free-play session and a series of three infant challenge tasks taken from the Laboratory

Temperament Assessment Battery (LAB-TAB; Goldsmith & Rothbart, 1996). Saliva samples were collected before, 20-, and 40-minutes after the challenge tasks. Primary caregivers were also asked to fill out a series of questionnaires independently. Observational coding of maternal caregiving behaviors was conducted from digital recordings of a free-play session during this assessment. Similarly, infant temperament behaviors were coded from digital recordings of the challenge tasks. Measures of salivary sAA and cortisol were obtained from saliva samples collected around these tasks.

Participants

For this project, a sub-sample of mothers and their infants were selected in order to control procedurally for influences that might affect salivary biomarker levels, reactivity, or physiological attunement. Mother-infant dyads were excluded if either the mother or infant had a fever greater than 101°F, were taking steroid-based medication, β-blockers or agonists, or if mothers were pregnant or taking prescription anti-depressants, anti-psychotics, narcotics or any other medication or substance that would significantly alter perception. Dyads were selected for inclusion in this analysis if the primary caregiver was the biological mother of the child, if the child was less than 10-months of age in order to control for developmental differences in infant response to the challenge tasks, if the child had completed at least one of the three challenge tasks, and if mother and infant had complete sAA and cortisol data at both a baseline and 20-minute post-challenge collection point. Given that the primary analysis used in this study is capable of handling missing data in the salivary biomarker variables, a semi-complete cases

strategy was used to deal with missing data. Dyads were further excluded if they were missing data in any covariates or predictor variables used in this study.

The final sub-sample for the analysis consisted of 284 mother-infant dyads. Sixty-four percent of the sub-sample was recruited in North Carolina and the remaining 36% resided in Pennsylvania. The economic breakdown reflected that observed in the larger sample (36% in poverty, 27% low income, 37% middle or upper income; M = 2.01, SD = 1.95). Infants (138 girls; 49%) ranged in age from 5.26 to 9.86 months (M = 7.42 months, SD = 1.06) and 47% were of African-American descent. Biological mothers ranged in age from 14.70 to 44.42 years (M = 26.62 years, SD = 5.98) and 45% were single and had never been married. Maternal years of education ranged from 8 to 21 years (M = 14.52, SD = 3.01).

Procedures and Measures

Behavioral Assessments

Challenge Tasks

During the 6-month home visit, the infants participated in a set of developmentally appropriate "challenge" tasks. The task procedures have been previously validated (e.g., Buss & Goldsmith, 1998; Kochanska, Coy, Tjebkes, & Husarek, 1998; Stifter & Fox, 1990; Stifter & Braungart 1995; Stifter & Spinrad, 2002). Tasks took place consecutively and were digitally recorded for later coding. The first task was a toy reach task designed to examine attention and latency to reach. The three distress eliciting tasks selected for the 6-month home visit were a mask task designed to elicit distress to novelty, a barrier task, and an arm restraint, both designed to elicit

distress to frustration. The toy reach involved alternating 1-minute presentations of stimulating and non-stimulating toy sets (two presentations each) and was designed by Robthbart (1988) to assess approach/withdrawal tendencies. The mask, barrier, and arm restraint tasks were all derived from the Laboratory Temperament Assessment Battery (LAB-TAB; Goldsmith & Rothbart, 1996). For the mask task, the experimenter sat beside the child for consecutive 10-second presentations of four scary masks. The experimenter spoke the child's name while moving his/her head slowly from side to side and then leaned toward the child. The toy removal task involved familiarizing each child with a toy for 30 seconds and then placing it behind a plexi-glass barrier where it could be seen, but not touched for an additional 30 seconds. This was repeated three times. Lastly, for the arm restraint task, an experimenter crouched behind the infant and gently restrained the child's arms for 2 minutes or until 20-seconds of hard crying ensued. During the first two tasks, the mother remained seated beside her child, but was asked not to interact with the child during the task. For the last two tasks, mothers were out of the child's sight, but within hearing range. Mothers were asked not to intervene, but were told they could stop the tasks at any time. Following the challenge tasks, infants were allowed to self-soothe for 1 minute, and then mothers were told they could comfort their child as they normally would.

Teams of undergraduate coders were trained to reliability to assess behaviors related to negative reactivity and regulation during the "challenge" tasks (Braungart & Stifter, 1991; Stifter & Braungart, 1995, Stifter & Fox, 1990; Stifter & Spinrad, 2002) Interrater reliabilities were established using Cohen's Kappa (.84 to .93) and reliability

was assessed on 20% of the tapes. In addition, interrater reliabilities were regularly checked in order to prevent coder drift.

Negative Behavioral Reactivity. Negative vocalizations were assessed on 4-point scale (0-3) where, 0 = "no reactivity", 1 = "mild reactivity" (whimper or fuss), 2 = "moderate reactivity" (continuous crying), 3 = "high reactivity" (hysterical crying). Based on this scale, for each task, proportions of time spent at each level of intensity of negative vocalization for each task were calculated by dividing the total amount of time in seconds at each level of intensity by the total time spent on the task.

Summary variables were also computed to reflect negative behavioral reactivity the entire task series (Stifter, personal communication, October, 2005). The mean intensity of negative reactivity during each of the three challenge tasks (masks, barrier, and arm restraint) was computed by multiplying the total number of seconds at each intensity level by the code used to reflect that intensity (0 for "no reactivity", 1 for "mild reactivity", 2 for "moderate reactivity", and 3 for "high reactivity"). This score is then divided by the total number of seconds in the task. For example, the mean intensity score for a child that displayed 3 seconds at each intensity level during the arm restraint would be (3*0) + (3*1) + (3*2) + (3*3) = 18/12 = 1.5 for that task. Once the mean intensity was computed for each task, these scores were summed across the three tasks to reflect total negative behavioral reactivity across the task series for each infant. See Table 1.

Table 1. Means and standard deviations for the proportion of time spent at each intensity level of negative behavioral reactivity during the challenge tasks series.

Negative Reactivity:	**N**	**Mean**	**SD**
Mask Task (Proportion of Total):			
No Negative	283	.88	.19
Low Negative	283	.05	.10
Moderate Negative	283	.04	.12
High Negative	283	.00	.02
Mean Intensity	283	.13	.32
Barrier Task (Proportion of Total):			
No Negative	259	.87	.23
Low Negative	259	.07	.14
Moderate Negative	259	.05	.15
High Negative	259	.00	.02
Mean Intensity	259	.18	.38
Arm Restraint Task (Proportion of Total):			
No Negative	234	.53	.37
Low Negative	234	.19	.20
Moderate Negative	234	.27	.31
High Negative	234	.02	.08
Mean Intensity	234	.77	.70
Task Series:			
Sum of Mean Intensity Across Tasks	284	.93	.87

Given that home visitors were instructed to terminate the task series if a child cried hard for 20 seconds or if requested by the mother, a small number of the infants in this study did not complete the entire task series. However, in order to be included in the current sample, the child had to complete at least one task, usually the mask task given the order of tasks in the series. Requiring completion of the task series did not seem plausible for the current study because it was those infants that did not complete the series that were most reactive to the tasks. Thus, summary variables for negative behavioral reactivity and regulatory behavior scores should be an accurate reflection of the total amount of negative reactivity and regulatory behavior actually expressed by each infant during the task series and sometimes includes behavior during only one (8%) or two (11%) of the tasks.

Regulatory Behavior. Regulation behaviors were coded continuously (every second) in order to compute the duration of each behavior. Three categories of regulatory behaviors were assessed: orienting, self-comforting, and avoidance behavior. For all three categories of behavior, the total amount of time during which no regulation was used was recorded. For *orienting* behavior, coders recorded the total amount of time spent orienting away from the toy (infant's eyes were focused on the environment for 1 second or longer), and the total amount of time spent looking at the mother or the experimenter (experimenter looks are not coded during the mask task). For *self-comforting* behavior, coders recorded the amount of time the infant spent making small, fine motor movements (e.g., sucking own or others hands or fingers, rubbing eyes, hands or face, twisting hair or clothes). Finally, duration of *avoidance* behavior was coded if the infant displayed an arched back, struggled against the restraint, physically turned

his/her head away, or pushed against the high chair tray. The proportion of time spent using each regulatory strategy was calculated for each task by dividing the total amount of time using each strategy by the total time spent on the task. To reflect the total proportion of time using any regulatory strategy within each task, the proportions for each regulatory strategy were then summed within each task. Finally, these variables were then summed across all three tasks to represent total attempts at regulatory behavior during the task series. See Table 2.

Parent-Child Interaction

As part of the larger study, mothers and infants participated in a free-play episode (Vandell, 1979; NICHD Early Child Care Research Network, 1997; 1999; 2003) and were presented with three standard, age-appropriate toys. Mothers were instructed to play with their infants, as they normally would while seated on a baby blanket on the floor. Free-play episodes lasted about 10 minutes and were digitally recorded for later coding of infant and maternal social interaction behaviors.

Coders were trained to reliability to assess maternal, infant, and dyadic interaction behaviors during the parent-child free-play episode (NICHD Early Child Care Research Network, 1997, 1999. 2003). Interrater reliabilities were established using Cronbach's alpha (α = .81 to .95) on a set of practice tapes, as well as on the total sample in order to prevent coder drift. All interaction behaviors were globally coded on a 5-point scale (1-5) where 1 = "not at all characteristic", 2 = "minimally characteristic", 3 = "somewhat characteristic", 4 = "moderately characteristic", and 5 = "highly characteristic".

Table 2. Means and standard deviations for the proportion of time using each regulatory behavior during the challenge tasks series.

Regulatory Behavior:	**N**	**Mean**	**SD**
Mask Task (Proportion of Total):			
Orienting	283	.04	.09
Self-Comforting	283	.04	.14
Avoidance	282	.02	.07
Total Proportion of Time Spent Regulating	283	.11	.18
Barrier Task (Proportion of Total):			
Orienting	266	.07	.09
Self-Comforting	269	.03	.07
Avoidance	267	.04	.08
Persistence	267	.05	.08
Total Proportion of Time Spent Regulating	270	.18	.17
Arm Restraint Task (Proportion of Total):			
Orienting	239	.20	.23
Self-Comforting	238	.04	.07
Avoidance	240	.18	.19
Total Proportion of Time Spent Regulating	241	.41	.32
Task Series:			
Total Proportion of Time Spent Regulating	284	.63	.47

The following maternal interaction behaviors were assessed at the 6-month assessment: The *maternal sensitivity* scale focused on how appropriately the parent observed and responded to the child's social gestures, expressions, and signals as well as to cries, frets, or other expressions of negative affect. The *maternal intrusiveness* scale focused on identifying intrusive, insensitive parent interaction behaviors that were adult-centered instead of child-centered. The *detachment / disengagement* scale identified parents that were emotionally uninvolved, disengaged, or unaware of the child's needs for appropriate interaction in order to facilitate involvement with objects or people. See Table 3 for means and standard deviations.

Table 3. Means and standard deviations for maternal sensitivity, intrusiveness, and detachment.

Maternal Caregiving Behavior:	**N**	**Mean**	**SD**
Maternal Sensitivity	284	2.61	.84
Maternal Intrusiveness	284	2.96	.86
Maternal Detachment / Disengagement	284	2.83	1.02

Brief Symptom Index (BSI-18)

The BSI-18 is a short, highly sensitive, self-report screening index for psychological distress, and the most recent addition to a family of tools that includes the Symptom Checklist 90-Revised (SCL-90-R, Derogatis, 1994) and the Brief Symptom Inventory (BSI, Derogatis, 1993). The BSI-18 contains eighteen items that are divided evenly across three dimensions: somatization, depression, and anxiety. A total score, referred to as the Global Severity Index (GSI), can also be computed by summing scores for three individual scales. Raw scores were also converted to standard scores using the BSI-18 manual for somatization, depression, anxiety, and GSI scales. Reliability and

validity have been previously validated. Internal consistency for somatization (.74), depression (.84), anxiety (.79), and Total or GSI (.89) is based a community sample of 1,134 (male N = 605; age range = 20-69). Correlations for subscale and GSI (total) scores from the BSI-18 and the SCL-90-R ranged from .91 to .96 within a community sample (Derogatis, 2000).

Participants in the current study were presented with a list of physical and emotional symptoms and asked to respond to the question, "How much has each problem distressed or bothered you in the last 7 days including today?" on a 5-point ordinal scale (0= "Not at all", 1 = "A little bit", 2 = "Moderately", 3 = "Quite a bit", 4 = "Extremely").

Depression. The depression scale consisted of the sum of six items: Item 2 (Feeling no interest in things), Item 5 (Feeling lonely), Item 8 (Feeling blue), Item 11 (Feelings of worthlessness), Item 14 (Feeling hopeless about the future), and Item 17 (Thoughts of ending your life). Descriptive statistics for this sample were calculated for the 6-month assessment (M = 1.99, SD = 2.93, N = 284).

Anxiety. The anxiety scale consisted of the sum of six items: Item 3 (Nervousness or shakiness inside), Item 6 (Feeling tense or keyed up), Item 9 (Suddenly scared for no reason), Item 12 (Spells of terror or panic), Item 15 (Feeling so restless you couldn't sit still), and Item 18 (Feeling fearful). Descriptive statistics for this sample were calculated for the 6-month assessment (M = 1.85, SD = 2.68, N = 284).

Biological Assessments

Saliva Collection and Health Screen

Saliva samples were collected around the challenge tasks in order to assess HPA axis and sympathetic reactivity to these tasks. A baseline saliva sample was taken prior

to the challenge tasks and subsequent samples were collected 20-minutes after the tasks to assess reactivity and 40-minutes after the tasks to assess recovery. These saliva collections were designed for optimal measurement of salivary cortisol and thus it is possible that we might have missed the peak of sympathetic arousal for some individuals. For saliva collection, mothers were asked to donate 1-2 mLs of unstimulated saliva into a cryogenic vial. Infants mouthed either a 6-inch sterile cotton rope for 2 to 3 minutes or an absorbant hydrocellulose micro-sponge for 1 minute (Granger, Harmon, et al., 2006). If the rope was used, once saturated, it was placed in the barrel of a needleless 5cc plastic syringe and compressed to express the saliva from the rope. If the micro-sponge was used, the sponge was centrifuged at 3000 rpm for 10 minutes in order to retrieve the saliva sample. Mothers were asked to restrict eating prior to sample collection and to drink a glass of water at least 10 minutes prior to saliva collection. Child intake of breast milk, formula, or other dairy products was restricted for 20-minutes prior to each saliva collection and the child's mouth was rinsed with water if food restriction was not possible (Magnano, Diamond, Gardner, 1989). Samples were transported on ice to temporary storage in a -20°C freezer at local data collection sites until transfer to permanent storage in an -80°C freezer.

Prior to saliva collection, a medical history was taken in order to assess health issues for both the infant and the mother. Mothers answered questions regarding both her own and her child's health and health-related behaviors. Questions addressed issues related to the schedule of eating and sleeping behavior (i.e. When did you/the baby last eat/drink and last sleep? What was the total time spent sleeping?), smoking (i.e., How many cigarettes have you smoked in the previous 48 hours?), drinking (i.e., How many

alcoholic beverages did you drink in the previous 48 hours?), dental health (i.e., Do you/the baby have cuts or sores in your mouth, bleeding gums from brushing teeth, untreated cavities? Is the baby teething?), current health (i.e., Would you say your/the baby's health in the last 48 hours was poor, good, very good, or excellent?), childhood immunizations (i.e., Has your infant had an immunization shot within the past 72 hours?), breast feeding (Is the baby still drinking breast milk? Has the baby had breast milk in the last week?), and pregnancy (i.e., Are you currently pregnant?). In addition, both mothers' and infants' weight in kg was recorded. Finally, in an attempt to screen for current systemic infection, both mother's and infant's body temperatures (°F) were recorded in duplicate using an infrared forehead thermometer.

Mothers were also asked to report any over-the-counter or prescription medications they or their infant took in the previous 48 hours. Following Hibel and colleagues (2006), medication categories were coded separately for infants and mothers. Category assignment was based on active ingredients in consultation with the Physicians' Desk Reference (2001), and was confirmed by the Family Life Project core pediatrician. Medications taken by infants were coded into 9 categories; (A) acetaminophen (i.e., Tylenol ®), (B) teething gels, (C) non-steroidal anti-inflammatory drugs (NSAIDs), (D and E) OTC cough-cold stimulants or depressants, (F) antibiotics, (G) glucocorticoids, (H) beta-agonists, and others. Medications taken by mothers in the 48 hours prior to the interview were coded into the following categories; (A) acetaminophen (i.e., Tylenol®), (B) pure agonist opioids, (C) anti-depressants, (D) psychotropics, (E) blood pressure medications, (F) salicylic acid (i.e., Aspirin®), (G) NSAIDs (H) beta-agonists, (I) Hormones (not contraceptives), (J) glucocorticoids, (K) hormonal contraceptives (oral or

transdermal), and others. Medications were coded as "other" because they were very infrequently used or our literature search was unable to justify why they would have the potential to influence salivary cortisol.

Salivary Cortisol Assessment

All samples were assayed for salivary cortisol using an FDA 510K cleared, highly sensitive enzyme immunoassay (Salimterics, State College, PA). The test uses only 25 µl of saliva (for singlet determinations), has a lower limit of sensitivity of .007 µg/dl, range of sensitivity from .007 to 1.2 µg/dl, and average intra- and inter-assay coefficients of variation of less than 5% and 10% respectively. Values from matched serum and saliva samples showed the expected strong linear relationship, r (17) = .94, $p < .0001$. Performance is robust for samples with pHs ranging from 4.0 to 9.0.

Salivary α-Amylase Assessment

Samples were assayed for sAA using a kinetic reaction assay kit (Salimetrics, State College, PA). The assay employs a chromagenic substrate, 2-chloro-p-nitrophenol, linked to maltotriose. The enzymatic action of sAA on this substrate yields 2-chloro-p-nitrophenol, which can be spectrophotometrically measured at 405 nm using a standard laboratory plate reader. The amount of sAA activity present in the sample is directly proportional to the increase (over a 3 minute period) in absorbance at 405 nm. Saliva samples (10 µl) were diluted 1:200 in assay diluent and well mixed. Diluted sample or control (8 µl) were then pipetted into individual wells of a 96-well microtiter plate. Preheated (37°C) α-amylase substrate solution (320 µl; 2-chloro-p-nitrophenol, linked to maltotriose) was added to each well and the plate was rotated at 500-600 RPM at 37°C for 3 minutes. Optical density (read at 405 nm) was determined at exactly the one-minute

mark and again at the 3-minute mark. Results were computed in U/mL of amylase using the formula: [Absorbance difference per minute x total assay volume (328 ml) x dilution factor (200)]/ [millimolar absorptivity of 2-chloro-p-nitrophenol (12.9) x sample volume (.008 ml) x light path (.97)].

The theoretical detection limit (Chard, 1990), defined as the minimal concentration of sAA that could be distinguished from zero, was obtained by interpolating the mean minus 2 STDEV for replicates of a 0 U/mL sAA control. By this method, the limit of this assay's sensitivity is 3.1 U/mL. Intrassay variation (CV) computed for the mean of 10 replicate tests of low (17.7 U/mL), medium (108.8 U/mL), and high (474.6 U/mL) concentration samples were 7.2%, 6.7%, and 2.5%, respectively. Inter-assay variation computed for the mean of average duplicates for 8 separate runs for lower (10.6 U/mL) and higher (166.0 U/mL) concentration samples were 5.8% and 3.6%. Method accuracy was determined from known amounts of sAA (range 3.14 to 77.09 U/ml) added to five saliva samples containing various endogenous concentrations (range 29.99 to 123.28 U/mL). Recoveries ranged from 87.4% to 111.6% (M = 101.04%). Parallelism was evaluated by measuring sAA in lower (40.96 U/mL) and higher concentration samples (1943.16 U/mL), which were then serially diluted (1:2 thru 1:16; and 1:3 to 1:27) to the low end of the assay's range. Observed values were as expected across the entire range of measurement. The average recovery was 96.43% (range 83.2 to 108.5%).

Data Management and Transformation

For all preliminary analyses, variables were examined for skewness and transformed as necessary. For salivary biomarkers, a log transformation for salivary cortisol and a square root transformation for sAA were used to correct positively skewed distributions. In addition, outliers more than 3 SD from the mean were discarded. A semiparametric, growth mixture modeling method described by Nagin (1999; 2005) and Jones and colleagues (2001) was used to conduct the primary analyses for the present study. As mentioned previously, this and other growth mixture modeling procedures capitalize on non-normal distributions in order to identify subgroups within the sample population (Bauer & Curran, 2003). Thus, salivary biomarker distributions were not transformed before use in these models. However, extreme outliers at a single time point in a measurement series may result in the extraction of small, unstable trajectories, thus salivary biomarker scores above the 99^{th} percentile for each distribution were discarded in order to avoid this problem (Nagin, personal communication; May 24, 2006). Likewise, these models do not require covariates and predictors to be normally distributed, but extreme skewness can be problematic (Nagin, personal communication; June 6, 2006). Therefore, covariates and predictors with a skewness statistic greater than 2 were log transformed before use with these models. All continuous covariates and predictors were centered at the mean in order to increase interpretability.

The selected growth mixture modeling method is capable of handling missing data within the variables (i.e., sAA and cortisol) that make up the salivary biomarker response trajectories (Nagin, 2005). However, it was decided *a priori* that only dyads with complete sAA and cortisol data at the baseline and 20-minute post-challenge sample

collection periods would be included in this analysis. At the 40-minute post-challenge sample collection, 14% and 12% of the infant sAA and cortisol data were missing respectively. However, for mothers, only 1% of the data was missing for both maternal sAA and cortisol data. Given that missing data in the covariates and predictors cannot be accounted for in these models, a semi-complete cases missing data strategy was employed in this project. Only those cases with complete data for the covariates and predictors utilized in this study were included.

Analytical Strategy

In order to address the hypotheses for the current study, the analyses proceeded in three stages: 1) identification of subgroups of infants and mothers who displayed unique sAA and cortisol responses to infant challenge, 2) examination of associations between infant or maternal characteristics that might influence stress physiological systems and membership in these subgroups, and 3) determination of whether or not there is an association between subgroups of infants and mothers based on infant and maternal physiological responses to infant challenge. The semiparametric, growth mixture modeling method (Jones, Nagin, & Roeder, 2001; Nagin, 1999; 2005) was first used to identify unique response profiles of sAA and cortisol separately in mothers and their infants (4 models: infant-SAA, infant-cortisol, maternal-SAA, maternal cortisol), as well as to estimate the proportion of the sample that displayed each profile. Specifically, the Censored Normal model was used to fit this continuous data (Nagin, 2005). Polynomial equations were used to link the repeated measures of salivary biomarkers to the time course of the saliva collections. The models included parameters to describe the

intercept, linear, and quadratic elements of the response patterns in order to observe both reactivity and recovery in response to the challenge tasks. For each dependent variable (infant sAA and cortisol, maternal sAA and cortisol), a series of models were estimated with the number of groups ranging from 1 to 6. Following D'Unger, Land, McCall, and Nagin (1998) and Nagin (2005), the Bayesian Information Criteria (BIC) and theoretical knowledge about the meaningfulness of the observed physiological response profiles were used in order to select the optimal number of groups for each of the four models. For practical purposes, a model was not selected if any one of its groups contained less than 4% of the sample in the basic model (N=~11 individuals).

Once the optimal number of groups was selected, covariates and predictors could be added to the model in order to test hypotheses about links between infant and maternal characteristics and physiological response patterns to stress. Both covariates and predictor variables are linked to the model via the probabilities of group membership for each individual rather than being linked to the response profiles themselves. The significance tests to determine the contribution of a particular covariate or predictor variable are similar to those used in a discriminant analysis (Nagin, 2005). One group is designated as the reference group and the association of each predictor or covariate with membership in each group is compared to the association between with membership in the reference group. In addition, Wald tests were performed to determine if covariates or predictor variables also distinguished membership between non-reference groups (Nagin, 2005). For each of the models described above, the covariates were examined individually and then concurrently to assess their contribution to the models. If a

covariate significantly distinguished between at least 2 groups, it was retained in the model.

Given that covariates influence group membership and that the individuals in each subgroup contribute information about the trajectory shape for each group, adding covariates has the potential to alter trajectory shape. Nagin (2005) suggests that adding covariates to the models does not often alter the shape of the trajectories. However, to my knowledge, this is the first time that these models have been applied to examine trajectories of sAA and cortisol reactivity to challenge. As such, special attention will be paid to examination of the effects of adding covariates to the basic models for this study. Once covariates identified in preliminary analyses were added the models, predictor variables of interest in this study were tested to determine if they could distinguish between group memberships in each of the four models. Predictor variables used in this study were summary variables for infant negative behavioral reactivity, infant regulatory behaviors, an interaction term between infant reactivity and regulation, infant gender, maternal sensitivity, intrusiveness, and detachment, and maternal depression and anxiety. In a first step, we sought to determine if infant temperament predicted physiological response patterns in infants and their mothers. Since we believed that the infant behavior was the most proximal stimulus for physiological reactivity, if any of the temperament variables distinguished between group membership, those variables remained in all subsequent models as control variables. Thus, all other predictor variables had to make a unique contribution to predicting group membership above and beyond infant behavioral reactivity to the challenge tasks.

The final analysis sought to determine if there is any linkage between physiological response patterns observed for mothers and infants. To answer this question, a dual-trajectory analysis described by Nagin and Tremblay (2001) was used. This method will concurrently apply the group-based modeling techniques described above to two dependent variables. For the current analysis, four dual trajectory models were examined (infant-sAA with maternal-sAA; infant-cortisol with maternal-cortisol; infant-sAA with maternal-cortisol; and infant-cortisol with maternal-sAA). These models provide three metrics of the joint probability of group membership. This first is the probability of membership in the maternal groups given membership in the child groups. The second metric describes the probability of membership in the child groups given membership in the maternal groups. Finally, the third metric is the dyad's joint probability of membership in both the infant and maternal groups. These probabilities will suggest, for example, whether certain sAA or cortisol response patterns displayed by mothers are more likely to observed when infants show certain types of sAA or cortisol response patterns. Covariates will also be added to these models to determine if they alter the response patterns observed and the joint probabilities for each model.

Chapter 7

RESULTS

Preliminary analyses were first conducted to identify covariates and to examine infant and maternal sAA and cortisol reactivity to the challenge tasks at the group level. The semiparametric, growth mixture models were next employed to identify subgroups within the sample based upon the shape of their sAA and cortisol responses to infant challenge. For each of the four dependent variables (infant sAA and cortisol; maternal sAA and cortisol), model selection, addition of covariates, and examination of predictors are described below in their entirety for each model. Models will be presented in the following order: infant sAA, infant cortisol, maternal sAA, and maternal cortisol. Finally, the dual trajectory models employed to determine if there was linkage between infant and maternal sAA and cortisol response profiles are described.

Preliminary Analyses:

Covariates and Physiological Response to Challenge for the Entire Sample

Preliminary analyses were conducted to identify situational (e.g., sample collection time, medication use, sleep) and demographic factors (e.g., race, gender, maternal marital status) associated with both levels and reactivity of sAA and cortisol. Bivariate correlations and t-tests were conducted between potential covariates and baseline levels of sAA or cortisol or the change from pre-task to 20-minutes post-task. Analyses were repeated controlling those with sAA for hours since eating and analyses with salivary cortisol for time of day. Only those covariates that explained variance in the salivary biomarkers above and beyond these major control variables were included in

subsequent analyses. For a complete list of identified covariates for each salivary biomarker, see Table 4.

Table 4. Covariates identified as influencing sAA and cortisol levels and reactivity.

Infant		**Maternal**	
sAA	Cortisol	sAA	Cortisol
Hours since eating	Time of day	Hours since eating	Time of day
Cough-cold OTC stimulants	Hours since eating	Time of day	Hours since eating
Infant Race	Total hours of sleep	Hours since sleeping	Hormonal contraceptives
	Infant age	Body temperature	Maternal age
	Infant race	Cigarettes per day	Maternal race
	Infant Weight	Hormonal contraceptives	Marital status
		NSAIDs	
		Maternal age	
		Years of education	
		Marital status	

Next, in order to examine infant and maternal sAA and cortisol response to the infant challenge tasks in the entire sample, repeated measures ANCOVAs with 3 levels for time (baseline, 20-minutes post, 40-minutes post) were conducted separately for mothers and infants with either sAA or cortisol as the dependent variable. On average, infants showed no sAA change in response to the challenge tasks. See Table 5 for means and standard deviations. Despite the lack of response at the group level, approximately

43% of infants showed sAA increases of 10% over baseline or greater by 20-minutes post-challenge. In contrast, for maternal sAA, there was a main effect of time, F (2, 536) = 3.34, $p < .05$, such that maternal sAA levels increased between baseline and 20-minutes, F (1, 272) = 6.59, $p < .05$, but remained unchanged between 20- and 40-minutes post-challenge. Similar to the pattern observed for infant sAA, there was no salivary cortisol response to the challenge tasks on average for the infants. However, approximately 47% of the infants displayed salivary cortisol increases of 10% or more by 20-minutes post-challenge. For maternal cortisol, there was a significant main effect of time, F (2, 540) = 13.63, $p < .001$, such that maternal cortisol levels declined between baseline and 20-minutes, F (2, 276) = 15.19, $p < .001$, and again between 20- and 40-minutes post-challenge, F (1, 270) = 4.62, $p < .05$.

Table 5. Means (SD) of infant and maternal sAA and cortisol in before and after infant challenge tasks.

Salivary α-Amylase (U/ml)						
	N	Baseline	N	20-Minutes	N	40-Minutes
Infants:	284	36.12 (29.34)	284	35.45 (27.47)	242	38.83 (32.01)
Mothers:	284	69.78 (54.20)	284	75.11 (58.06)	279	76.59 (55.96)
Salivary Cortisol (µg/dl)						
	N	Baseline	N	20-Minutes	N	40-Minutes
Infants:	284	.21 (.18)	284	.23 (.16)	250	.21 (.14)
Mothers:	284	.20 (.13)	284	.15 (.08)	279	.13 (.07)

Growth Mixture Models: Linking Infant and Maternal Behaviors and Characteristics to Salivary α-Amylase and Cortisol Stress Response Profiles

Infant Salivary α-Amylase

The Basic Infant sAA Model

The four-group model was selected for infant sAA. Model selection criteria are presented in Appendix B. Figure 10 depicts the trajectories of the four-group model for infant sAA. The majority of infants (60.6%) belong to a group characterized by low sAA levels at baseline (intercept = 19.63 U/ml) and no change in response to the challenge tasks. Therefore, this group will be referred to as the "low flat" group. The next largest group (24.5%), the "mid flat" group, also displays no response to the challenge tasks, but the mean levels for this group are about twice as high as the previous group (intercept = 50.51 U/ml). The third group identified includes 8.6% of the sample will be referred to as the "mid increase" group. The baseline levels for this group are similar to those in the "mid flat" group. However, this group displays a 19% increase in sAA levels between baseline and 20-minutes, and then a further 44% increase by 40-minutes post-challenge. While the linear parameter for this group is non-significant suggesting the change between baseline and 20-minutes to be ignorable, the near significant quadratic term suggests the increase between 20- and 40-minutes may be more meaningful. Finally, the fourth group identified encompasses 6.4% of the sample and is readily identified by baseline levels twice as high as the last two groups (intercept = 107.83 U/ml). This "high decline-flat" group shows a near-significant 13% decrease in sAA levels by 20-minutes and then remains unchanged between 20- and 40-minutes post-challenge. The group membership probabilities and model parameters for each group are presented in Table 6.

Figure 10. Trajectories for the four-group model of infant sAA response to challenge tasks.

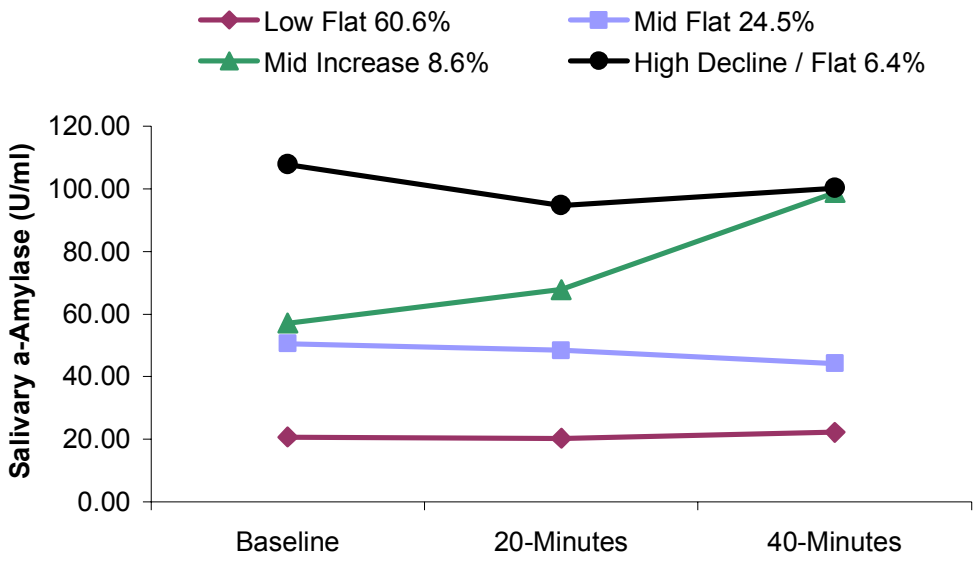


Table 6. Group membership probabilities, model parameters, and AvePP for the four-group model of infant sAA.

Basic Model – Infant Salivary *α*-Amylase

Group:	GM%	AvePP	Parameter	Estimate	SE	Sig
Low Flat:	60.57%	.94	Intercept	19.63	1.47	0.00
			Linear	-16.70	35.06	0.63
			Quadratic	126.41	170.24	0.46
Mid Flat:	24.49%	.85	Intercept	50.51	3.07	0.00
			Linear	-10.27	62.75	0.87
			Quadratic	-108.07	296.45	0.72
Mid Increase:	8.56%	.87	Intercept	57.11	5.82	0.00
			Linear	4.90	103.18	0.96
			Quadratic	1018.53	535.11	0.06
High Decline / Flat	6.37%	.92	Intercept	107.83	5.91	0.00
			Linear	-223.78	120.43	0.06
			Quadratic	928.31	551.86	0.09

In addition, the fit of this model to the data was examined using the average posterior probabilities (AvePP). The posterior probabilities represent that probability that each individual belongs in each of the four trajectory groups. Each infant was assigned to the trajectory group for which they had the largest posterior probability. Once assigned, the mean of the posterior probabilities was calculated for each of the four groups. Ideally, the AvePP for each group would be 1 if each infant could be clearly assigned to each group with no error. According to Nagin (2005), an AvePP of .70 or higher is indicative of good correspondence of the model to the data. For the four group model of infant sAA, the AvePPs for all groups are .85 or higher.

Adding Covariates to the Infant sAA Model

For the four-group infant sAA model, both infant race (white vs. non-white) and the use of over-the-counter cough-cold medications containing stimulants distinguished membership between groups. Model parameters are presented in Table 7. Infant race discriminated between membership in the "low flat" and the "mid increase" infant sAA groups, such that being non-white (coded as 0) increased an infant's likelihood of being in the "mid increase" group as compared to the "low flat" group, Estimate = -1.87, $p <$ .01. Use of OTC cough-cold medications discriminated between the "low flat" group and the "high decline / flat" infant sAA groups. Infants using these OTC stimulants (coded as 1) were significantly more likely to be in the "high decline / flat" group as compared to the "low flat" infant sAA group, Estimate = 2.02, $p < .01$.

Upon the addition of covariates or predictors to these models the interpretation of the shape of the model trajectories and the probabilities of group membership change slightly. Both should be interpreted as the shape of the trajectories and probability of

group membership when all covariates are equal to zero. In the current model, the trajectories and probabilities presented in Figure 11 reflect those observed when infants are non-white and are not taking OTC cough-cold medications.

Figure 11. While the trajectories of infant sAA response to the challenge tasks remain similar after adding covariates to the model, non-white infants are more likely to be in the "mid increase" group (8.6% in basic model) and those receiving OTC cough-cold medication containing stimulants are more likely to be in the "high decline / flat" infant sAA group (6.4% in basic model).

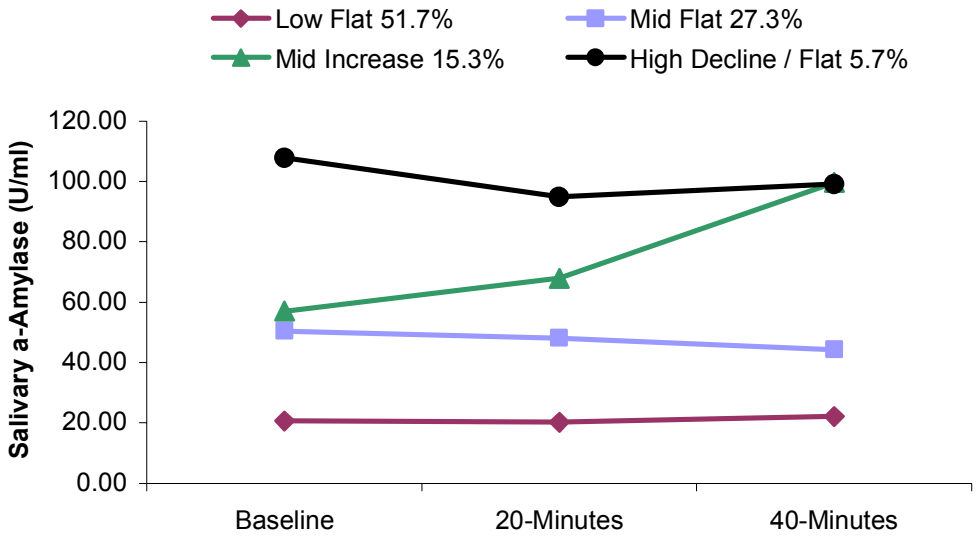


Table 7. Model parameters for four-group infant sAA model controlling for infant race and use of OTC cough-cold medications containing stimulants.

Covariate Model – Infant Salivary *α*-Amylase			
Group	**Estimate**	**SE**	**Sig**
Low Flat:			
Intercept	19.63	1.48	0.00
Linear	-17.02	35.07	0.63
Quadratic	128.08	170.31	0.45
Reference Group	-	-	-
Mid Flat:			
Intercept	50.48	2.97	0.00
Linear	-17.32	62.62	0.78
Quadratic	-67.53	297.32	0.82
Constant	-0.64	0.29	0.03
Race (0 = non-white)	-0.60	0.36	0.09
OTC Cough-Cold Stimulants (0 = no medication)	0.77	0.68	0.26
Mid Increase:			
Intercept	56.99	4.92	0.00
Linear	5.81	104.56	0.96
Quadratic	1037.71	545.13	0.06
Constant	-1.22	0.32	0.00
Race (0 = non-white)	-1.87	0.66	0.01
OTC Cough-Cold Stimulants (0 = no medication)	0.17	1.04	0.87
High Decline / Flat:			
Intercept	107.79	6.20	0.00
Linear	-215.73	126.29	0.09
Quadratic	862.88	576.71	0.14
Constant	-2.21	0.44	0.00
Race (0 = non-white)	-0.59	0.56	0.29
OTC Cough-Cold Stimulants (0 = no medication)	2.02	0.71	0.01

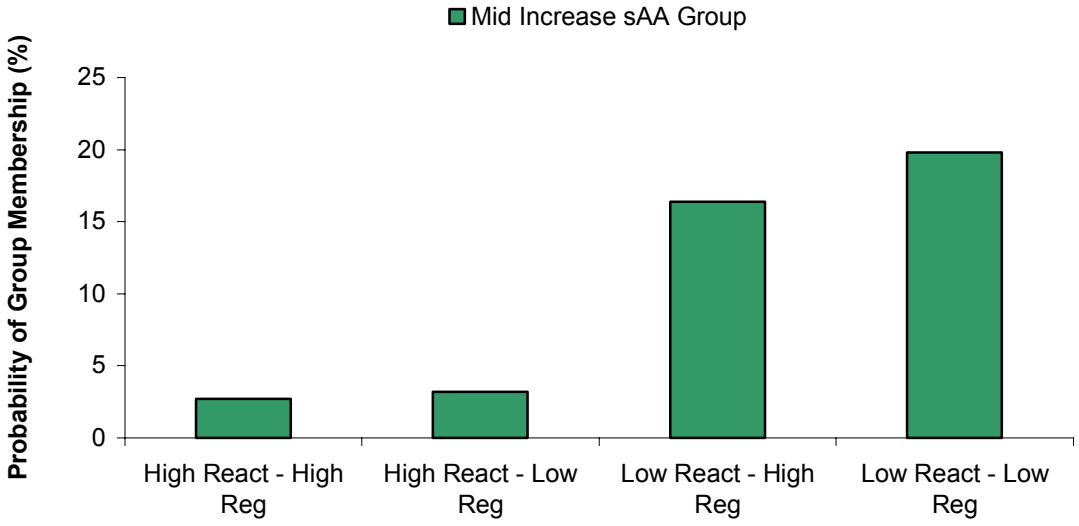
Adding Predictors: Are Infant and Maternal Characteristics Associated with Infant sAA Response Profiles?

Infant Reactivity and Regulation. In order to test if infant behavioral reactivity and infant regulatory behavior predict infant sAA response to the challenge tasks, the sum of the mean intensity of negative infant vocalizations across the challenge tasks (i.e., negative reactivity), the total proportion of time spent regulating across the challenge tasks (i.e., regulatory behavior), and the interaction of negative reactivity and regulatory behavior were added as predictors of group membership to the four group model of infant sAA controlling for infant race and use of OTC cough-cold stimulant medication. Adding infant behavioral reactivity and regulation to the model slightly altered the probabilities of group membership among the four identified infant sAA responses to infant challenge. At the sample mean for infant negative reactivity and regulatory behavior, 57.0% were estimated as being the "low flat" group, 28.0% in the "mid flat" group, 7.7% in the "mid increase" group, and 7.3% in the "high decline / flat" group.

Lower negative infant reactivity to the challenge tasks marginally increased the probability that an infant showed the "mid increase" sAA response as compared to the "low flat" response of sAA response to the challenge tasks, estimate = -1.20, SE = 0.64, p = .06. For infants displaying negative behavioral reactivity 1 SD above the mean, the probability of membership in the "mid increase" group dropped from 7.7% at the mean to 3.0%. However, for infants showing negative reactivity 1 SD below the mean, the probability of membership in the "mid increase" group rose to 18.1%. Thus, the less reactive infants were more likely to display a sAA increase across the assessment period.

In addition, negative behavioral reactivity and regulatory behavior interacted to discriminate between the "mid increase" and the "low flat" sAA responses to the challenge tasks, estimate = 1.19, SE = 0.32, $p < .001$. Infants that displayed high negative behavioral reactivity and made more attempts at regulatory behavior were the least likely to show the "mid increase" response (2.7%). In contrast, those showing low behavioral reactivity and making few attempts at behavioral regulation were most likely to show the "mid-increase" response (19.8%). Thus, high behavioral reactivity and regulation corresponded to low sAA levels and no change in response to the challenge tasks, while low behavioral reactivity and regulation were associated with moderate baseline levels and an increase in sAA across the assessment period. See Figure 12.

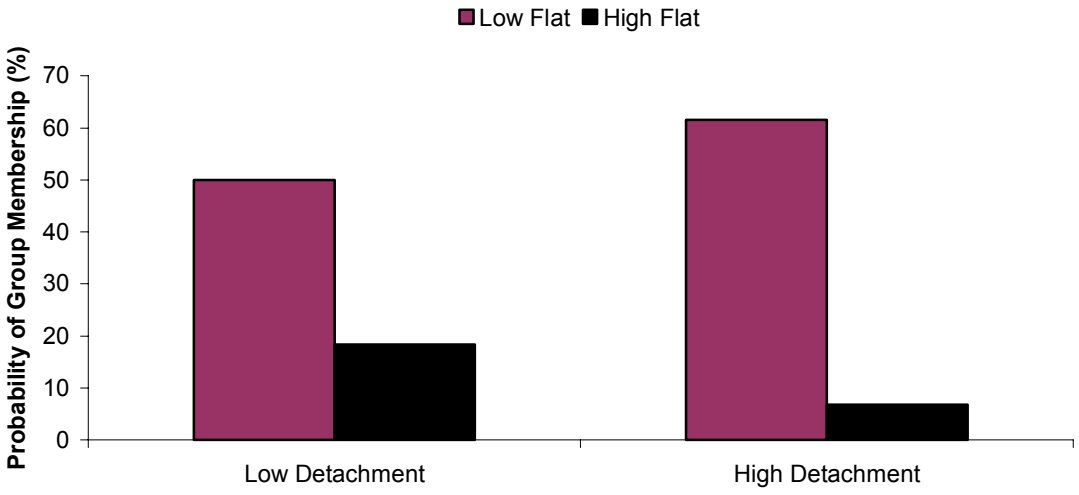
Figure 12. Infant negative reactivity and regulation behavior interact to discriminate the probability of membership between the "low flat" and "mid increase" infant sAA responses to challenge. Infants displaying low behavioral reactivity and low regulatory behavior were most likely to display the "mid increase" sAA pattern in response to challenge.



Maternal Caregiving Behavior. Maternal sensitivity, intrusiveness, and detachment were added individually as predictors to the four group model of infant sAA response to challenge controlling for infant race, OTC cough-cold stimulant medication

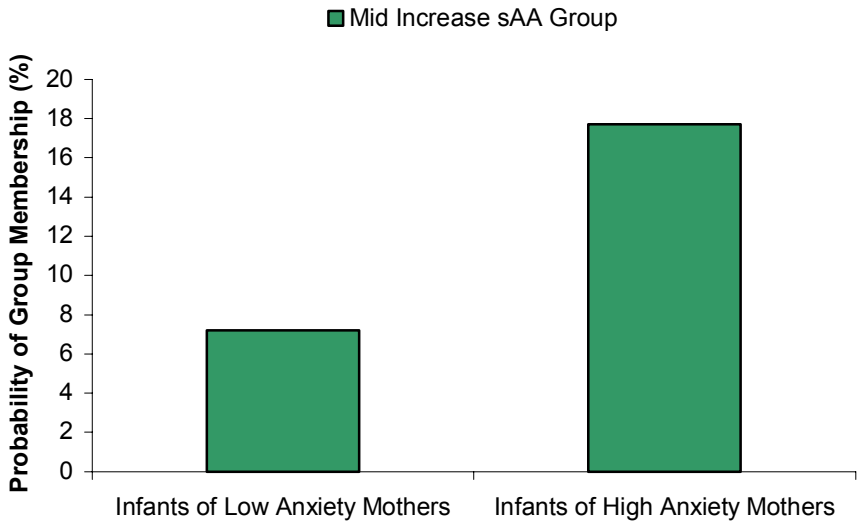
use, behavioral reactivity, and regulatory behavior. Maternal sensitivity and intrusiveness did not significantly distinguish between infant sAA response profiles. However, high maternal detachment increased the probability of being in the "low flat" infant sAA group as compared to the "high decline / flat" group, estimate = -0.59, SE = 0.25, $p < .05$. At the mean level of detachment, 56.4% of the infants were in the "low flat" group and 11.4% were in the "high decline / flat" group. However, at 1 SD above the mean for detachment, the probability of membership in the "low flat" infant sAA group increased to 61.6% and decreased to 6.8% for the "high decline / flat" group. In contrast, at 1 SD below the mean for maternal detachment, probability of membership in the "low flat" group decreased to 50.0%, while the likelihood of membership in the "high decline / flat" infant sAA group increased to 18.3%. Thus, infants of high detached mothers are more likely to show a low flat sAA response to infant challenge, while infants of low detached mothers are more likely to have the high sAA levels characteristic of the "high decline / flat" sAA group. See Figure 13.

Figure 13. Infants of detached mothers are more likely to show the "low flat" sAA response to the challenge tasks as opposed to the "high decline / flat" response.



Maternal symptoms of psychopathology. Maternal depression and anxiety scores were added individually as predictors to the four group model of infant sAA response to challenge controlling for infant race, OTC cough-cold stimulant medication use, behavioral reactivity, and regulatory behavior. While maternal depression was unrelated to infant sAA response to challenge, higher maternal anxiety marginally increased the probability of membership in the "mid increase" infant sAA group as compared to the "low flat" infant sAA response, estimate = 1.70, SE = 0.91, $p = .06$. At mean levels of maternal anxiety, 11.1% of the infants were expected to show the "mid increase" sAA pattern. However, at 1 SD above the mean on anxiety 16.7% were likely to show this sAA increase, while only 7.2% of the infants of mothers 1 SD below the mean for anxiety were likely to show this pattern. Thus, maternal anxiety amplified the likelihood that infants would show an increasing pattern of sAA during the challenge as opposed to a low flat pattern of sAA. See Figure 14.

Figure 14. Infants of high anxious mothers were more likely to show an increasing pattern of sAA during the challenge tasks as compared to infants of low anxiety mothers.



Infant Gender. Infant gender did not significantly discriminate between infant sAA response profiles in this sample.

Summary of Findings with Infant Salivary α-Amylase

In summary, infant negative behavioral reactivity and regulatory behavior during the tasks interacted to predict infant sAA responses to infant challenge. Highly reactive infants displaying high levels of regulatory behavior were most likely to display low flat sAA levels. In contrast, infants low in both reactivity and regulatory behavior were most likely to show a profile of moderate, increasing sAA levels across the challenge. Infants of highly detached mothers were more likely to show a profile of low flat sAA levels than infants of low detached mothers. Finally, infants of highly anxious mothers were mostly likely to show a profile of moderate, increasing sAA levels across the tasks than infants of low anxious mothers. Infant gender, maternal sensitivity, maternal intrusiveness, and maternal depression were unrelated to infant sAA response profiles.

Infant Salivary Cortisol

The Basic Infant Salivary Cortisol Model

The four-group model was selected for infant salivary cortisol (See Figure 15). Model selection criteria are reported in Appendix B. Again, the majority of the infants (71.0%) belong to a group characterized by low baseline levels (intercept = 0.16 μg/dl) and no change in response to the challenge tasks. These will be referred to as the "low flat" cortisol group. The next largest group included 17.9% of the infants and will be called the "low reactivity / non-recovery" group. These infants also begin with low baseline cortisol levels (intercept = 0.22 μg/dl), but then show a significant 64% increase, on average, in cortisol levels over baseline by 20-minutes post-challenge. However, the

cortisol levels remain elevated and show no change between 20- and 40-minutes post-challenge. The third group includes 5.0% of the sample and also begins with low baseline cortisol levels (intercept = .20 µg/dl). This group then proceeds to show a significant 230% increase in cortisol levels over baseline on average by 20-minutes, followed by a 36% decrease in cortisol by 40-minutes post-challenge. These infants will be referred to as the "high reactivity / recovery" group. Finally, the last group includes 6.1% of the infants in the sample and will be called the "high decline" cortisol group. These infants begin with very high baseline cortisol levels (intercept = 0.78 µg/dl) and then show significant decreases between baseline and 20-minutes, and then again between 20- and 40-minute post-tasks. For model parameters, see Table 8. The four-group infant cortisol model fits the model well and the AvePP for each group are all .85 or above.

Figure 15. Trajectories for the four-group model of infant salivary cortisol response to challenge tasks.

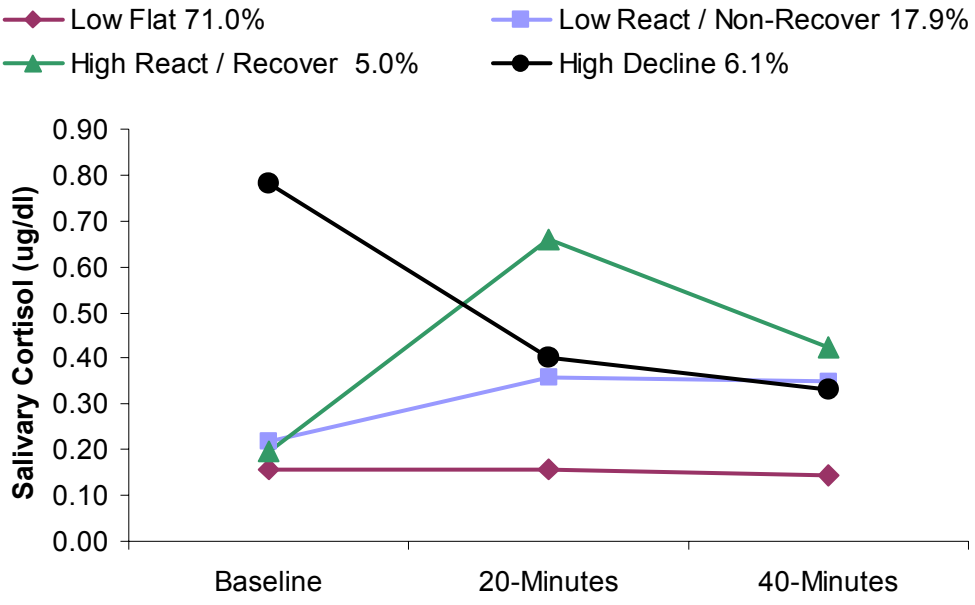


Table 8. Group membership probabilities, model parameters, and AvePP for the four-group model of infant salivary cortisol.

Basic Model – Infant Salivary Cortisol						
Group	**GM%**	**AvePP**	**Parameter**	**Estimate**	**SE**	**Sig**
Low Flat:	71.01%	.95	Intercept	0.16	0.01	0.00
			Linear	0.09	0.19	0.64
			Quadratic	-0.81	0.90	0.37
Low React / Non-Recover:	17.88%	.85	Intercept	0.22	0.02	0.00
			Linear	2.12	0.51	0.00
			Quadratic	-7.27	2.34	0.00
High React / Recover:	5.01%	.86	Intercept	0.20	0.03	0.00
			Linear	8.16	1.07	0.00
			Quadratic	-35.13	5.01	0.00
High Decline:	6.11%	1.00	Intercept	0.78	0.03	0.00
			Linear	-5.32	0.62	0.00
			Quadratic	15.39	2.94	0.00

Adding Covariates to the Infant Cortisol Model

Upon addition of covariates to the four-group infant salivary cortisol model, the two reactivity sub-groups were combined into one group and a second, small "low flat" group was extracted as the fourth group in the model. Given the redundancy between to the two "low flat" groups, it was decided that a three-group model for child cortisol would be a more parsimonious and interpretable representation of infant cortisol response to the challenge tasks. The basic three-group model extracted a "low flat" group (77.0%), a "reactivity / recovery" group (16.8%), and "high decline" group (6.2% of the sample). For the three-group model of infant cortisol, the hours since the child last ate was the only covariate that significantly distinguished between groups. More time since the last

meal significantly increased the likelihood of an infant belonging to the "reactivity / recovery" group as compared to the "low flat" group, estimate = 0.74, $p < .05$. Figure 16 presents the predicted trajectories and group membership probabilities for the three-group infant cortisol model at the mean of the variable representing the number of hours since the child had last eaten. Model parameters are presented in Table 9.

Figure 16. Following the addition of covariates to the four-group infant cortisol model, the reorganization of the groups suggested that three-group model for infant cortisol would be a more parsimonious and interpretable representation of infant cortisol response to the challenge tasks. For the three-group model of infant cortisol, the probability that a particular child belonged to the "reactivity / recovery" group increased the longer it had been since the child had last eaten a meal. The trajectories and probabilities are presented for the sample mean for hours since last eating.

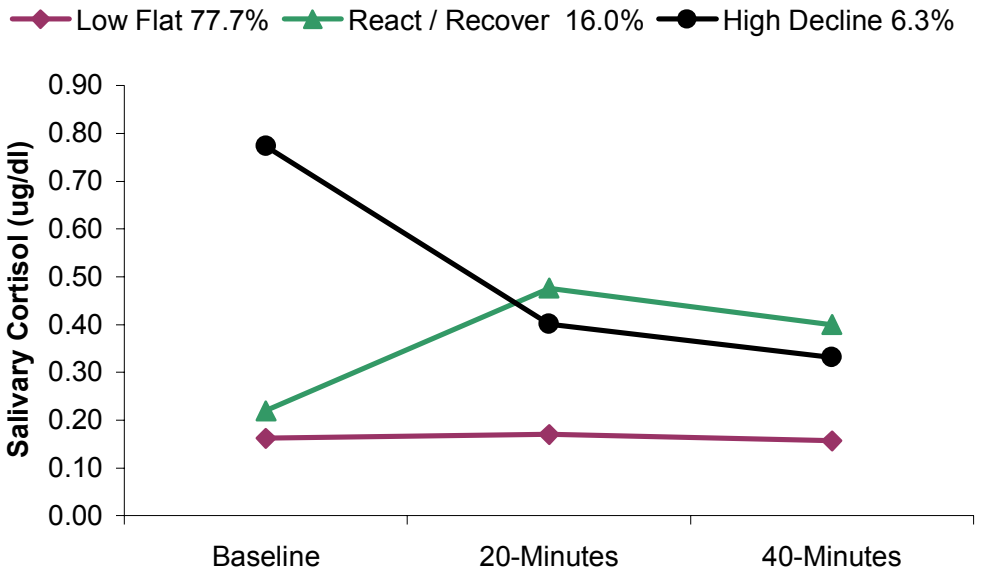


Table 9. Model parameters for three-group infant salivary cortisol model controlling for the number of hours since the child last ate.

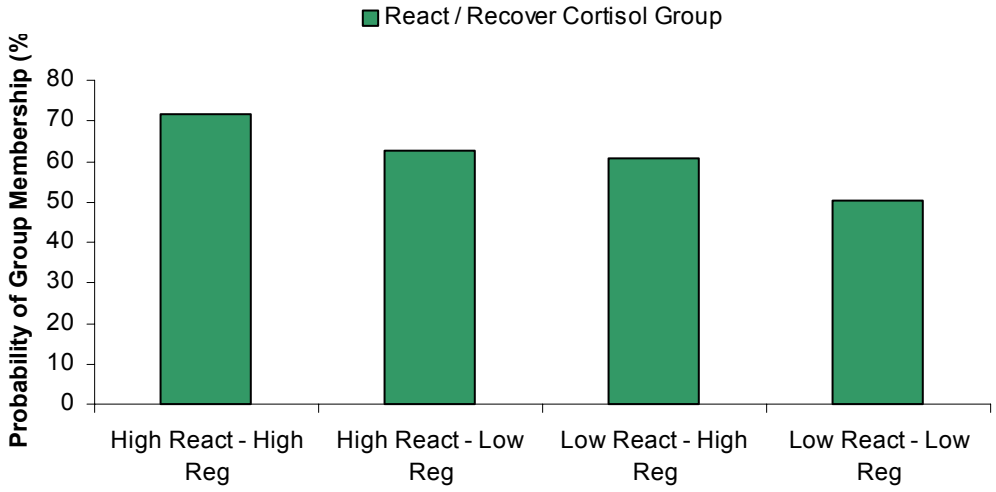
Covariate Model – Infant Salivary Cortisol			
Group	**Estimate**	**SE**	**Sig**
Low Flat:			
Intercept	0.16	0.01	0.00
Linear	0.20	0.18	0.27
Quadratic	-1.16	0.88	0.19
Reference Group	-	-	-
React / Recover:			
Intercept	0.22	0.02	0.00
Linear	4.25	0.45	0.00
Quadratic	-16.72	2.10	0.00
Constant	-1.59	0.20	0.00
Hours Since Eating	0.74	0.35	0.04
High Decline:			
Intercept	0.77	0.03	0.00
Linear	-5.24	0.67	0.00
Quadratic	15.13	3.14	0.00
Constant	-2.51	0.26	0.00
Hours Since Eating	0.30	0.52	0.57

Adding Predictors: Are Infant and Maternal Characteristics Associated with Infant Cortisol Response Profiles?

Infant Reactivity and Regulation. In order to test if infant behavioral reactivity and infant regulatory behavior predict infant cortisol response to the challenge tasks, infant negative reactivity, attempts at regulatory behavior, and the interaction of negative reactivity and behavior were added as predictors to the three-group infant cortisol model controlling for hours since the infant last ate. Interestingly, adding infant behavioral reactivity and regulation to the model dramatically altered the probabilities of group

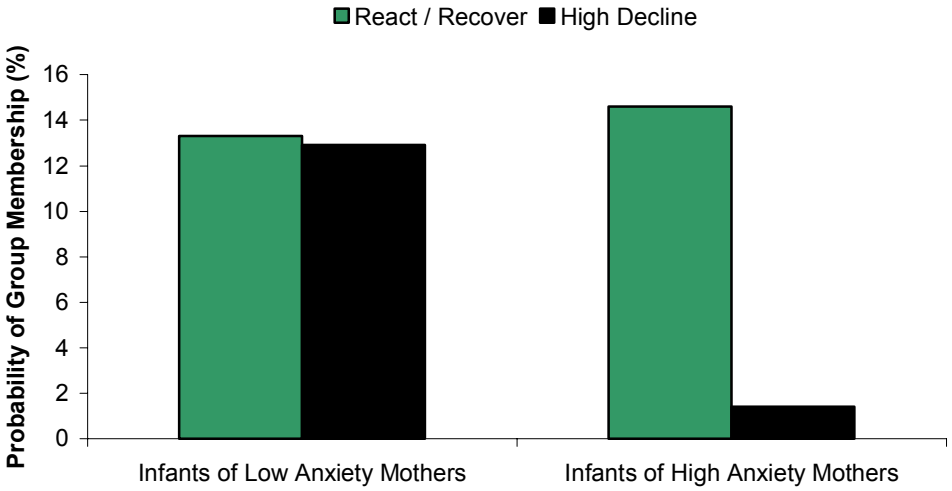
membership among the three identified infant cortisol responses to infant challenge. At the sample mean for infant negative reactivity and regulatory behavior, 11.0% were estimated as being the "low flat" group, 61.7% in the "reactivity / recovery" group, and 27.3% in the "high decline" group. Infant negative reactivity and regulatory behavior interacted to distinguish between the "low flat" and "reactivity / recovery" cortisol responses to infant challenge, estimate = -0.51, SE = 0.21, $p < .01$. Infants who responded to the challenge tasks with high negative behavioral reactivity and more attempts at regulatory behavior were the most likely to show the "reactivity / recovery" cortisol response (71.6%). In contrast, those showing low behavioral reactivity and few attempts at behavioral regulation were significantly less likely to show the "reactivity / recovery" response (50.6%). Thus, infants displaying both high behavioral reactivity and regulatory behaviors were more likely to show cortisol reactivity and recovery in response to the challenge than those displaying low reactivity and regulatory behaviors. See Figure 17.

Figure 17. Infant negative reactivity and regulation behavior interact to discriminate the probability of membership between the "low flat" and "reactivity / recovery" infant cortisol responses to challenge. Infants displaying both high negative reactivity and high regulatory behavior were most likely to show the "reactivity / recovery" cortisol response to challenge.



Symptoms of Maternal Psychopathology. Maternal depression and anxiety scores were added individually as predictors to the four group model of infant sAA response to challenge controlling for hours since the infant last ate, behavioral reactivity, and regulatory behavior. Similar to the model for infant sAA, symptoms of maternal depression did not influence infant cortisol response to the challenge tasks in this sample. However, high maternal anxiety was observed to decrease the likelihood that infants would show the "high decline" cortisol pattern as opposed to the "reactivity / recovery" infant cortisol response pattern, estimate = -4.27, SE = 1.99, $p < .05$. Adding maternal anxiety to the model again substantially redistributed membership among the three infant cortisol response groups, such that at the sample mean for maternal anxiety, the majority of infants (81.2%) displayed the "low flat" cortisol pattern, 14.4% displayed the "reactivity / recovery" pattern, and 4.4% showed the "high decline" pattern. The probability that infants of highly anxious mothers (1 SD above the mean) would show the "high decline / flat" pattern dropped to 1.4%. In contrast, infants of mothers low on anxiety (1 SD below the mean) were significantly more likely (12.9%) to show the "high decline" cortisol pattern. Thus, infants of highly anxious mothers were less likely to show a cortisol pattern characterized by high baseline cortisol levels with a steep decline than infants of low anxious mothers. See Figure 18.

Figure 18. Infants of highly anxious mothers were significantly less likely to show the "high decline" cortisol pattern as compared to infants of low anxiety mothers.



Infant Gender, and Maternal Caregiving Behavior. Neither infant gender nor maternal caregiving behaviors distinguished between infant cortisol response profiles in this sample.

Summary of Findings with Infant Salivary Cortisol

In summary, infant negative behavioral reactivity and regulatory behavior during the tasks interacted to predict infant cortisol responses to infant challenge. Highly reactive infants displaying high levels of regulatory behavior were most likely to display a profile of substantial cortisol reactivity and recovery in response to the tasks as compared to infants low in both reactivity and regulatory behavior during the tasks. Maternal symptoms of psychopathology also influenced infant cortisol responses to infant challenge. Infants of low anxious mothers more often displayed a profile of high baseline cortisol levels and steep decline across the assessment period than infants of high anxious mothers. However, infant gender, maternal caregiving behaviors, and symptoms of depression were unrelated to infant cortisol response profiles.

Maternal Salivary α-Amylase

The Basic Maternal Salivary α-Amylase Model

A four-group model was selected for maternal sAA (See Figure 19). Model selection criteria are presented in Appendix B. The first group was characterized by low baseline levels (intercept = 30.48 U/ml) and no reactivity to the challenge tasks. This group will be referred to as the "low flat" maternal sAA group and consists of 45.9% of the sample. The baseline levels of the second group (33.6% of the sample) were over twice as high as those of the first group (intercept = 78.10 U/ml). This group also did not react to the challenge tasks and will be referred to as the "mid flat" group. The third group comprised 14.8% of the sample and baseline levels were higher than the two previous groups (intercept = 124.13 U/ml). This group showed an 11% increase over baseline by 20-minutes post-tasks and no change between 20- and 40-minutes. This group will be referred to as the "mid increase / flat" group. However, it is important to note that the linear parameter suggests that the change between baseline and 20-minutes is non-significant. Finally, the fourth group had the highest baseline levels (intercept = 192.29) and showed a significant 16% percent increase over baseline by 20-minutes and a significant 6% decrease between 20- and 40-minutes. This last group included 5.7% of the sample and will be called the "high reactivity / recovery" group. Model parameters are reported in Table 10. The AvePP was .87 or higher for each of the four groups.

Figure 19. Trajectories for the four-group model of maternal sAA response to infant challenge tasks.

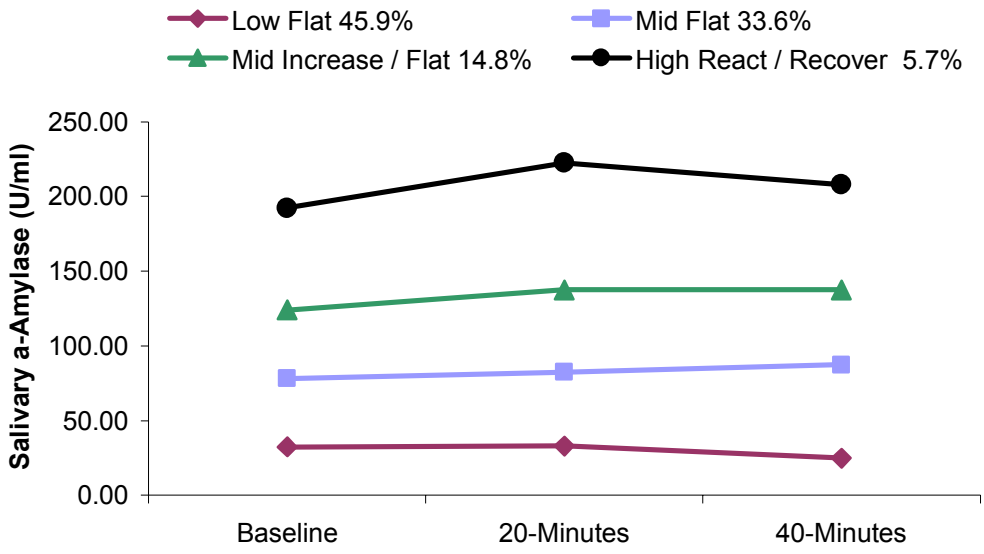


Table 10. Group membership probabilities, model parameters, and AvePP for the four-group model of maternal sAA.

Basic Model – Maternal Salivary *α*-Amylase						
Group	**GM%**	**AvePP**	**Parameter**	**Estimate**	**SE**	**Sig**
Low Flat:	45.91%	.95	Intercept	30.48	2.75	0.00
			Linear	-1.39	64.04	0.98
			Quadratic	77.52	308.20	0.80
Mid Flat:	33.59%	.87	Intercept	78.48	3.78	0.00
			Linear	38.84	79.30	0.62
			Quadratic	40.16	381.04	0.92
Mid Increase / Flat:	14.76%	.91	Intercept	124.13	6.17	0.00
			Linear	199.01	122.74	0.11
			Quadratic	-660.44	598.90	0.27
High React / Recover	5.74%	.98	Intercept	192.29	7.65	0.00
			Linear	526.14	188.55	0.01
			Quadratic	-2238.22	923.87	0.02

Adding Covariates to the Maternal Salivary α-Amylase Model

Several covariates, including hours since the mother had last eaten, sample collection time of day, maternal use of non-steroidal anti-inflammatory medication (NSAIDs), the number of cigarettes the mother had smoked in the last two days, and maternal age, contributed significantly to the four-group model of maternal sAA response to the infant challenge tasks. Given that eating increases sAA levels, it was more likely that a mother would belong to the "high reactivity / recovery" group as compared to the "low flat" group if she had eaten recently, estimate = -0.66, $p < .05$. In line with the increasing diurnal rhythm of sAA, a later time of day at sample collection significantly increased the likelihood that a mother would be in the "mid flat", estimate = 0.17, $p < .01$, "mid increase", estimate = 0.18, $p < .05$, or the "high decline / flat" group, estimate = 0.26, $p < .05$, as compared to the "low flat" group. However, time of day did not distinguish between the three trajectories with higher baseline levels of sAA. The use of NSAIDS made it more likely that a mother would be in the "mid flat", estimate = 1.51, $p < .05$, and the "high decline / flat" sAA groups, estimate = 2.80, $p < .01$, as compared to the "low flat" sAA group. It was marginally more likely that a mother belonged to the "low flat" sAA group as compared to the "high decline / flat" group if she had smoked cigarettes in the last two days, estimate = -1.13, $p = .06$. This is in line with the finding that the aldehydes in cigarette smoke denature the sAA enzyme (Zappacosta et al., 2002). Finally, being older increased a mother's likelihood of being in the "mid flat", estimate = 0.08, $p < .01$, and the "mid increase" group, estimate = .08, $p < .05$, as compared to the "low flat" group. Figure 20 presents the predicted trajectories and group membership probabilities for the four-group maternal sAA model at the mean time since the mothers

last ate, mean time of day at sample collection, mean number of cigarettes smoked, mean maternal age, and when mothers were not taking NSAIDS. Model parameters are presented in Table 11.

Figure 20. Trajectories of maternal sAA response to the challenge tasks remained similar after adding covariates to the model. The trajectories and probabilities for the four-group maternal sAA model with covariates are presented for the mean of hours since the mothers had eaten, time of day at sample collection, cigarettes in the last 48 hours, and maternal age, and for mothers not taking NSAIDS.

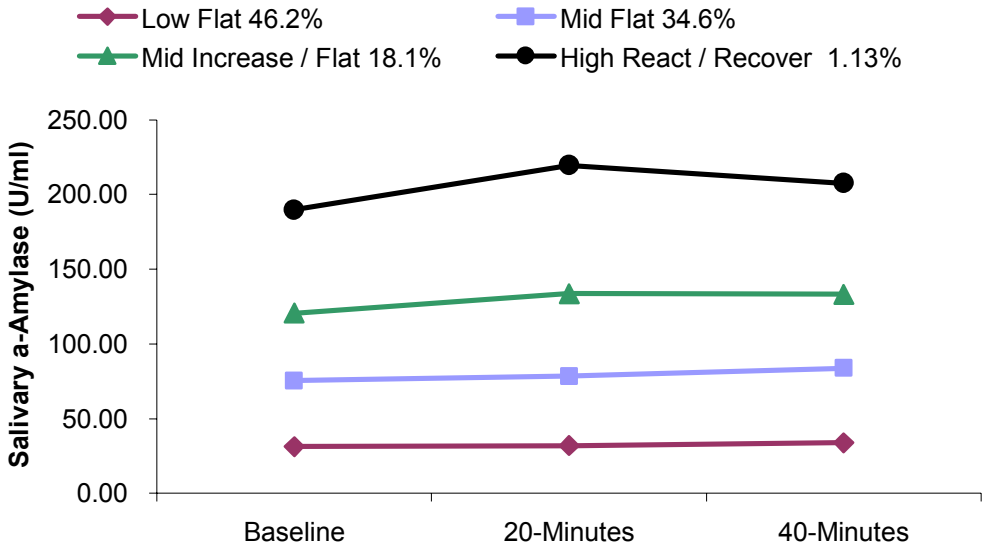


Table 11. Model parameters for four-group maternal sAA model controlling for the number of hours since the mother last had something to eat, time of day at sample collection, NSAID use, cigarettes in the last 48 hours, and maternal age.

Covariate Model – Maternal Salivary *α*-Amylase			
Group	**Estimate**	**SE**	**Sig**
Low Flat			
Intercept	29.15	2.97	0.00
Linear	-1.06	66.65	0.99
Quadratic	80.74	320.57	0.80
Reference Group	-	-	-

Mid Flat:			
Intercept	75.39	4.84	0.00
Linear	16.55	80.18	0.84
Quadratic	128.17	382.91	0.74
Constant	-0.29	0.20	0.16
Hours Since Eating	-0.06	0.04	0.16
Time of Day	0.17	0.07	0.01
NSAID Use (0 = No NSAIDs)	1.51	0.76	0.05
Cigarettes Per Day	-0.15	0.22	0.51
Maternal Age	0.08	0.03	0.01
Mid Increase / Flat:			
Intercept	120.70	6.66	0.00
Linear	198.29	119.43	0.10
Quadratic	-675.00	579.63	0.24
Constant	-0.94	0.32	0.00
Hours Since Eating	-0.04	0.05	0.39
Time of Day	0.18	0.08	0.02
NSAID Use (0 = No NSAIDs)	0.38	1.04	0.71
Cigarettes Per Day	-0.09	0.26	0.73
Maternal Age	0.08	0.04	0.02
High React / Recover			
Intercept	189.89	7.40	0.00
Linear	504.23	177.67	0.01
Quadratic	- 2081.40	860.06	0.02
Constant	-3.71	0.91	0.00
Hours Since Eating	-0.66	0.30	0.03
Time of Day	0.26	0.11	0.02
NSAID Use (0 = No NSAIDs)	2.80	1.01	0.01
Cigarettes in the Last 48 Hours	-1.13	0.60	0.06
Maternal Age	0.02	0.05	0.73

Adding Predictors: Are Infant and Maternal Characteristics Associated with Maternal Salivary α-Amylase Response Profiles?

None of the infant (i.e., reactivity, regulation, and gender) or maternal characteristics (i.e., maternal caregiving behaviors and psychopathology) were found to be related to maternal sAA response profiles in this sample.

Maternal Salivary Cortisol

The Basic Maternal Cortisol Model

A five-group model was selected for maternal salivary cortisol (See Figure 21). Model selection criteria are presented in Appendix B. The first group included 37.5% of the sample and was designated the "low decline 1" group. This group displayed low baseline cortisol levels (intercept = 0.10 μg/dl) and showed significant declines across the assessment period. The baseline levels of the second group were twice as high as those in the first group (intercept = 0.19 μg/dl) and included 35.6% of the sample. This group also declined across the assessment period was thus called the "low decline 2" group. The third group contained 9.4% of the sample and their baseline levels were slightly higher than the second group (intercept = .26 μg/dl). This group did not show any change across the tasks and is labeled the "mid flat" group. Approximately 13% of the sample made up the "mid decline" group. The group declined across the assessment period from a baseline level of 0.38 μg/dl. Finally, the "high decline" group began their decline from a baseline of 0.51 μg/dl. This group contained 4.5% of the mothers in this sample. Model parameters are in Table 12. This model fit the observed data well with an AvePP of .85 or greater in all groups.

Figure 21. Trajectories for the five-group model of maternal salivary cortisol response to infant challenge tasks.

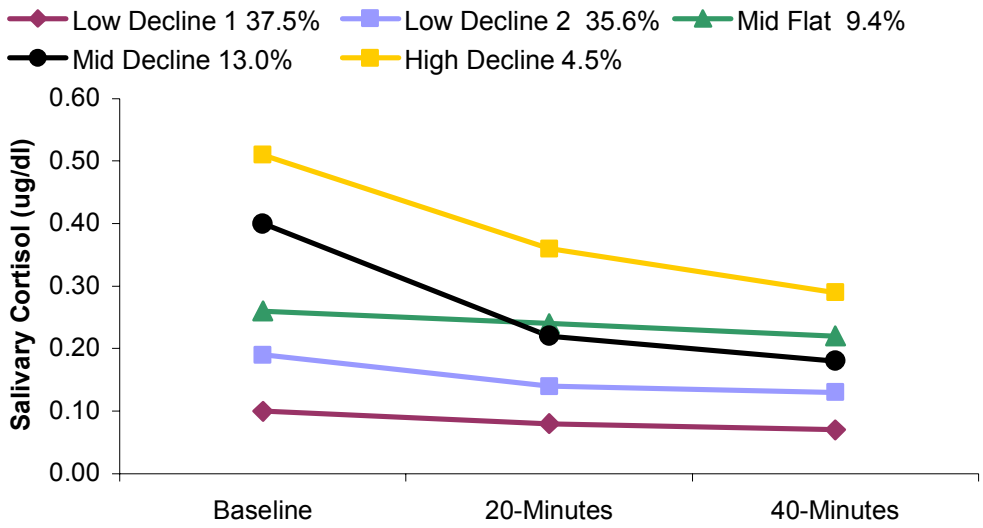


Table 12. Group membership probabilities, model parameters, and AvePP for the five-group model of maternal salivary cortisol.

Basic Model – Maternal Salivary Cortisol

Group	GM%	AvePP	Parameter	Estimate	SE	Sig
Low Decline 1:	37.52%	.93	Intercept	0.10	0.01	0.00
			Linear	-0.31	0.10	0.00
			Quadratic	0.91	0.50	0.07
Low Decline 2:	35.56%	.90	Intercept	0.19	0.01	0.00
			Linear	-0.68	0.11	0.00
			Quadratic	1.88	0.53	0.00
Mid Flat:	9.43%	.85	Intercept	0.26	0.01	0.00
			Linear	-0.14	0.33	0.68
			Quadratic	-0.35	1.37	0.79
Mid Decline:	12.98%	.95	Intercept	0.40	0.01	0.00
			Linear	-2.55	0.20	0.00
			Quadratic	7.16	0.89	0.00
High Decline:	4.51%	.99	Intercept	0.51	0.01	0.00
			Linear	-1.88	0.31	0.00
			Quadratic	3.82	1.50	0.01

Adding Covariates to the Maternal Cortisol Model

Similar to the infant salivary cortisol model, the addition of covariates to the maternal salivary cortisol model altered the shape of one of the trajectories. The group originally designated as the "mid flat" group had displayed a unique pattern as compared to the rest of the study population. However, with the addition of covariates to the model, this group also displayed a decline across the assessment period similar to the rest of the sample. In an effort at presenting a more parsimonious model of maternal cortisol response to the infant challenge, it was decided that four-group model would more clearly present that major patterns in maternal cortisol during the challenge tasks. The four group model for maternal cortisol extracted a "low decline 1" group (17.3%), a "low decline 2" group (39.7%), a "mid decline" group (35.6%), and a "high decline" group, encompassing 7.4% of the sample. Time of day at sample collection, the number of hours since the mother had last eaten, use of hormonal contraceptives, and maternal marital status all significantly distinguished between groups in the four-group model of maternal cortisol. Unlike the other four models, the trajectory with the lowest baseline levels was not the reference group for the maternal cortisol model. The "low decline 2" group instead served as the reference. In line with the diurnal rhythm of cortisol, a later time of day at sample collection made it less likely that a mother would be in the "high decline" group, estimate = -0.60, $p < .01$, and more likely that she would be in the group with the lowest cortisol levels ("low decline 1"), estimate = 0.36, $p < .001$, as compared to the "low decline 2" group. The less time that had passed since a mother had last eaten, the more likely it was that she would belong to the "mid decline" group as compared to the "low decline 2" group, estimate = 0.12, $p < .01$. Mothers taking hormonal

contraceptives (coded as 1) were more likely to be in the "low decline 2" as compared to the "low decline 1" group, estimate = -1.78, $p < .05$. Finally, being married (coded as 1) made it more likely that a mother would be in the "low decline 2" group as compared to the "high decline" group, estimate = -1.29, $p < .05$. Figure 22 presents the predicted trajectories and group membership probabilities at the mean time of day at sample collection, the mean hours since the mothers had last eaten, and for single mothers not using hormonal contraceptives. Model parameters are presented in Table 13.

Figure 22. Following the addition of covariates, the reorganization of groups suggested that the four-group model was a more parsimonious representation of maternal cortisol during the infant challenge tasks. The trajectories and probabilities for the four-group maternal cortisol model with covariates are presented for the mean time of day at sample collection, the mean hours since the mothers had eaten, and for single mothers not using hormonal contraceptives.

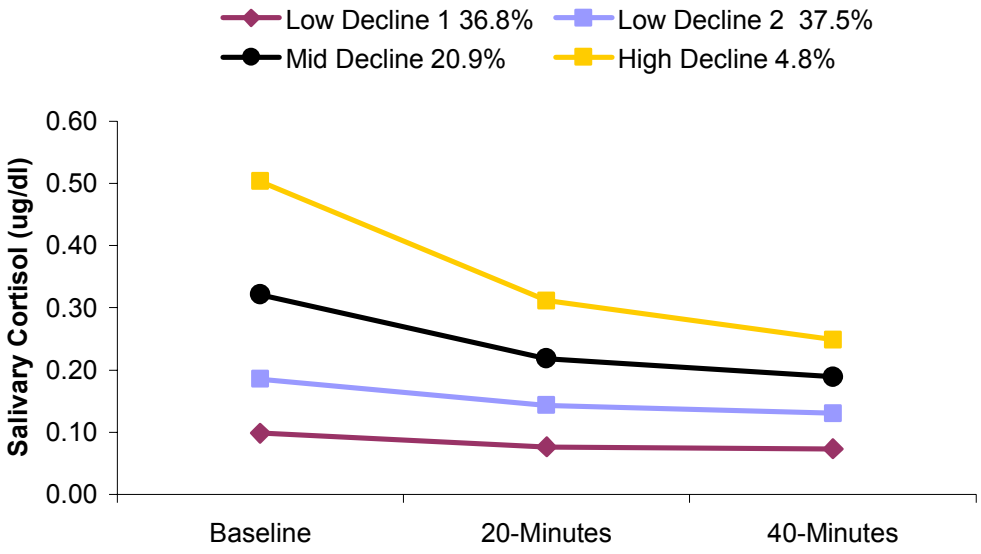


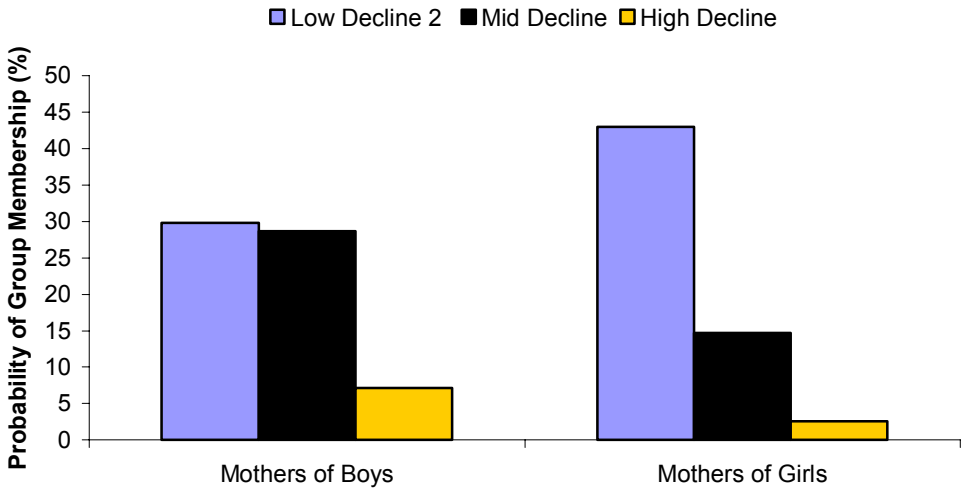
Table 13. Model parameters for four-group maternal cortisol model controlling for time of day at sample collection, the number of hours since the mother last had something to eat, use of hormonal contraceptives, and maternal marital status.

Covariate Model – Maternal Salivary Cortisol			
Group	**Estimate**	**SE**	**Sig**
Low Decline 1:			
Intercept	0.10	0.01	0.00
Linear	-0.32	0.12	0.01
Quadratic	0.96	0.55	0.08
Constant	-0.02	0.33	0.96
Time of Day	0.36	0.08	0.00
Hours Since Eating	0.01	0.05	0.81
Contraceptives (0 = None)	-1.78	0.57	0.04
Marital Status (0 = Single)	-0.03	0.38	0.93
Low Decline 2:			
Intercept	0.19	0.01	0.00
Linear	-0.57	0.12	0.00
Quadratic	1.49	0.57	0.01
Reference Group	-	-	-
Mid Decline:			
Intercept	0.32	0.01	0.00
Linear	-1.40	0.22	0.00
Quadratic	3.71	0.94	0.00
Constant	-0.58	0.32	0.07
Time of Day	-0.04	0.09	0.70
Hours Since Eating	0.12	0.04	0.01
Contraceptives (0 = None)	0.27	0.51	0.60
Marital Status (0 = Single)	-0.57	0.41	0.16
High Decline:			
Intercept	0.50	0.01	0.00
Linear	-2.56	0.27	0.00
Quadratic	6.46	1.28	0.00
Constant	-2.06	0.57	0.00
Time of Day	-0.60	0.20	0.01
Eating	0.02	0.06	0.72
Contraceptives (0 = None)	-0.71	1.14	0.53
Marital Status (0 = Single)	-1.29	0.56	0.02

Adding Predictors: Are Infant and Maternal Characteristics Associated with Maternal Cortisol Response Profiles?

For the four group model of maternal cortisol controlling for time of day, hours since last eating, hormonal contraceptive use, and maternal marital status, infant gender discriminated between the "low decline 2" group and the "high decline", estimate = -1.36, SE = 0.59, $p < .05$, and "mid decline" groups, estimate = -1.04, SE = 0.43, $p < .05$. Mothers of boys were more likely to be in the "mid decline" (28.7%) and "high decline" (7.07%) groups than mothers of girls (14.7% and 2.63% respectively). Thus, mothers of boys were more likely to start the assessment period with higher cortisol levels than mothers of girls. See Figure 23.

Figure 23. Mothers of infant boys have a higher probability of showing the "mid decline" and "high decline" cortisol patterns than mothers of girls.



Infant reactivity and regulation, maternal caregiving behavior, and maternal psychopathology. Neither infant behavior during the challenge tasks, maternal caregiving behavior, nor maternal psychopathology discriminated between maternal cortisol response profiles in this sample.

Attunement in Infant and Maternal Salivary α-Amylase and Cortisol Responses to Infant Challenge

Preliminary Analyses of Physiological Attunement

As a first step to determine whether or not there is attunement between infant and maternal sAA and cortisol responses to infant challenge, partial correlations were computed between infant and maternal baseline sAA levels and reactivity and between infant and maternal baseline cortisol levels and reactivity. To examine attunement between physiological systems within dyads, partial correlations were also computed for the following combinations: infant sAA with maternal cortisol and infant cortisol with maternal sAA. Each of these analyses was controlled for the appropriate covariates listed in Table 4. Within mother-infant dyads, there was a positive association between baseline levels of sAA, r (220) = .12, p =.05 (one-tailed), and an even stronger positive relationship between infant and maternal cortisol levels at baseline, r (230) = .23, p < .01. However, there were no associations between baseline levels of sAA and cortisol within mother-infant dyads. Neither were there significant associations between infant and maternal sAA or cortisol reactivity to the challenge tasks.

Dual Trajectory Model

According to Nagin and Tremblay (2001) and Nagin (2005), use of correlation coefficients to examine relationships between variables of interest does not take advantage of repeated measures designs since they are limited to looking at the relationship between only two variables at a time. In the current study, correlations can only be examined between baseline levels of salivary biomarkers or between change

scores representing biomarker reactivity or recovery. It is for this reason that the use of recovery measures of salivary biomarkers is often underused. In addition, correlations coefficients apply equally to all members of the sample population, whereas for some subgroups, the associations between variables of interest may be stronger than for others (Nagin, 2005). In contrast, the dual trajectory model allows use of all repeated measures in a measurement series by relating membership in identified trajectory groups in one domain of interest (i.e., infant cortisol) to membership in the trajectory groups of another domain of interest (i.e. maternal cortisol). This process also allows identification of linkages between specific subgroups within the larger sample.

Infant and Maternal Salivary α-Amylase

In order to examine attunement between infant and maternal sAA response to infant challenge, a dual trajectory model was estimated specifying a four-group model for infant sAA controlling for infant race, OTC cough-cold stimulant use, infant reactivity, and regulatory behavior during the tasks, and a four-group model for maternal sAA controlling hours since the mothers had last eaten, time of day at sample collection, NSAID use, cigarettes in the last 48 hours, and maternal age. The primary output of interest included the probability of membership in maternal sAA groups given membership in infant sAA groups, the probability of membership in infant sAA groups given membership in maternal sAA groups, and the joint probability of a dyad showing both infant and maternal sAA groups. See Table 14.

Table 14. Probability of membership in maternal sAA groups given membership in infant sAA groups (Panel A – rows sum to 1), probability of membership in infant sAA groups given membership in maternal sAA groups (Panel B – columns sum to 1), and the joint probability of membership in both infant and maternal sAA groups (Panel C – all probabilities sum to 1).

Infant α-Amylase	Maternal α-Amylase			
	Low Flat	Mid Flat	Mid Increase / Flat	High React / Recover
	Panel A - Probability of Maternal sAA Conditional on Infant sAA			
Low Flat	58.40%	24.56%	15.99%	1.05%
Mid Flat	26.66%	51.74%	20.19%	1.41%
Mid Increase	33.30%	55.80%	7.95%	2.95%
High Decline/Flat	28.10%	42.06%	29.84%	0.00%
	Panel B - Probability of Infant sAA Conditional on Maternal sAA			
Low Flat	71.68%	36.96%	51.41%	44.75%
Mid Flat	17.22%	40.98%	34.16%	31.69%
Mid Increase	7.63%	15.68%	4.77%	23.55%
High Decline/Flat	3.47%	6.38%	9.66%	0.00%
	Panel C - Joint Probability of Infant and Maternal sAA			
Low Flat	32.20%	13.54%	8.82%	0.58%
Mid Flat	7.73%	15.01%	5.86%	0.41%
Mid Increase	3.43%	5.75%	0.82%	0.30%
High Decline/Flat	1.56%	2.34%	1.66%	0.00%

There was partial attunement observed between infant and maternal sAA responses to infant challenge. As can be observed in Panel A, the majority of infants showing the "low flat" sAA pattern also had mothers displaying the "low flat" sAA pattern (58.4% of "low flat" infants). Similarly, the majority of mothers with infants displaying the "mid flat" sAA pattern also displayed the "mid flat" pattern (51.7% of "mid flat" infants). Thus, there appears to be an attunement between mothers and infants at these lower sAA levels. For complete attunement, the highest probabilities should be observed on the diagonals of each table. This pattern of high probabilities on the diagonal

was not true for mothers and infants in groups with higher sAA levels. Only 8.0% of the infants showing the "mid increase" pattern had mothers showing the similar "mid increase / flat" pattern, and none of the infants displaying "high decline / flat" pattern had mothers in the "high reactivity / recovery" group. Instead, the majority of the infants showing the "mid increase" (55.8%) and "high decline / flat" patterns (42.1%) had mothers in the "mid flat" group.

Infant and Maternal Salivary Cortisol

To examine attunement between infant and maternal cortisol response to infant challenge, a dual trajectory model was estimated specifying a three-group model for infant cortisol controlling for hours since the infants had last eaten, infant reactivity, and regulatory behavior during the tasks, and a four-group model for maternal cortisol controlling for time of day at sample collection, hours since the mothers had last eaten hormonal contraceptive use, and marital status. The three sets of probabilities for this model are presented in Table 15.

Table 15. Probability of membership in maternal cortisol groups given membership in infant cortisol groups (Panel A – rows sum to 1), probability of membership in infant cortisol groups given membership in maternal cortisol groups (Panel B – columns sum to 1), and the joint probability of membership in both infant and maternal cortisol groups (Panel C – all probabilities sum to 1).

Infant Cortisol	Maternal Cortisol			
	Low Decline 1	Low Decline 2	Mid Decline	High Decline
	Panel A - Probability of Maternal Cortisol Conditional on Infant Cortisol			
Low Flat	40.23%	35.02%	22.02%	2.73%
React / Recover	38.06%	33.83%	20.36%	7.76%
High Decline	7.77%	78.03%	0.00%	14.20%
	Panel B - Probability of Infant Cortisol Conditional on Maternal Cortisol			
Low Flat	84.48%	74.59%	85.82%	52.65%
React / Recover	14.29%	12.88%	14.18%	26.71%
High Decline	1.23%	12.54%	0.00%	20.65%
	Panel C - Joint Probability of Infant and Maternal Cortisol			
Low Flat	32.07%	27.93%	17.55%	2.18%
React / Recover	5.42%	4.82%	2.90%	1.11%
High Decline	0.47%	4.69%	0.00%	0.85%

For the most part, there appears to be no differences between the "low flat" babies and the "reactivity / recovery" babies in their associations with maternal cortisol response patterns. They appear to be distributed as would be expected given the probability of membership in the four maternal cortisol response groups: low decline 1 (38.0%), low decline 2 (37.4%), mid decline (20.5%), and high decline (4.1%). However, the relationship between the infant "high decline" cortisol pattern and the maternal cortisol patterns differs markedly from this pattern. The majority of infants with a "high decline" infant have mothers that show the "low decline 2" pattern (78.0%). In addition, a larger percentage of "high decline" mothers have "high decline" infants than other mothers (20.7%).

Infant Salivary α-Amylase and Maternal Salivary Cortisol

To examine attunement between infant sAA and maternal cortisol responses to infant challenge, a dual trajectory model was estimated specifying a four-group model for infant sAA controlling for infant race, OTC cough-cold stimulant use, infant reactivity, and regulatory behavior during the tasks, and a four-group model for maternal cortisol controlling for time of day at sample collection, hours since the mothers had last eaten hormonal contraceptive use, and marital status. The three sets of probabilities for this model are presented in Table 16.

Table 16. Probability of membership in maternal cortisol groups given membership in infant sAA groups (Panel A – rows sum to 1), probability of membership in infant sAA groups given membership in maternal cortisol groups (Panel B – columns sum to 1), and the joint probability of membership in both infant sAA and maternal cortisol groups (Panel C – all probabilities sum to 1).

Infant α-Amylase	Maternal Cortisol			
	Low Decline 1	Low Decline 2	Mid Decline	High Decline
	Panel A - Probability of Maternal Cortisol Conditional on Infant sAA			
Low Flat	37.93%	36.59%	22.38%	3.10%
Mid Flat	33.81%	50.96%	13.34%	1.89%
Mid Increase	45.29%	19.46%	23.77%	11.48%
High Decline/Flat	43.10%	26.15%	19.95%	10.80%
	Panel B - Probability of Infant sAA Conditional on Maternal Cortisol			
Low Flat	58.02%	55.62%	64.97%	44.07%
Mid Flat	23.28%	34.87%	17.43%	12.07%
Mid Increase	10.03%	4.28%	9.99%	23.64%
High Decline/Flat	8.67%	5.23%	7.62%	20.22%
	Panel C - Joint Probability of Infant sAA and Maternal Cortisol			
Low Flat	21.97%	21.19%	12.97%	1.79%
Mid Flat	8.81%	13.29%	3.48%	0.49%
Mid Increase	3.80%	1.63%	1.99%	0.96%
High Decline/Flat	3.28%	1.99%	1.52%	0.82%

The distribution of infants showing the "low flat" sAA pattern across the maternal cortisol groups reflects the probability of membership in each of the maternal groups: low decline 1 (37.9%), low decline 2 (38.1%), mid decline (20.0%), and high decline (4.1%). While this suggests there is no relationship between the "low flat" infant sAA pattern and maternal cortisol patterns, the other three infant sAA patterns differ in their relationships with the maternal cortisol patterns. The majority of the infants showing the "mid flat" sAA pattern have mothers that display the "low decline 2" pattern (51.0%). In contrast the infants displaying the "mid increase" and "high decline / flat" patterns were most likely to have mothers showing the "low decline 1" cortisol pattern (45.3% and 43.1% respectively). Finally, mothers showing the "high decline" cortisol pattern were more likely than other mothers to have infants displaying "mid increase" (23.6%) or "high decline / flat" sAA patterns (20.2%).

Infant Salivary Cortisol and Maternal Salivary α-Amylase

Finally, to examine attunement between infant cortisol and maternal sAA responses to infant challenge, a dual trajectory model was estimated specifying a three-group model for infant cortisol controlling for hours since the infants had last eaten, infant reactivity, and regulatory behavior during the tasks, and a four-group model for maternal sAA controlling hours since the mothers had last eaten, time of day at sample collection, NSAID use, cigarettes in the last 48 hours, and maternal age. The three sets of probabilities for this model are presented in Table 17.

Table 17. Probability of membership in maternal sAA groups given membership in infant cortisol groups (Panel A – rows sum to 1), probability of membership in infant cortisol groups given membership in maternal sAA groups (Panel B – columns sum to 1), and the joint probability of membership in both infant cortisol and maternal sAA groups (Panel C – all probabilities sum to 1).

Infant Cortisol	Maternal α-Amylase			
	Low Flat	Mid Flat	Mid Increase / Flat	High React / Recover
	Panel A - Probability of Maternal sAA Conditional on Infant Cortisol			
Low Flat	41.99%	35.61%	21.34%	1.05%
React / Recover	64.66%	23.59%	9.97%	1.78%
High Decline	59.14%	40.86%	0.00%	0.00%
	Panel B - Probability of Infant Cortisol Conditional on Maternal sAA			
Low Flat	72.70%	83.04%	92.57%	77.47%
React / Recover	19.24%	9.45%	7.43%	22.53%
High Decline	8.06%	7.50%	0.00%	0.00%
	Panel C - Joint Probability of Infant Cortisol and Maternal sAA			
Low Flat	33.58%	28.48%	17.07%	0.84%
React / Recover	8.89%	3.24%	1.37%	0.25%
High Decline	3.72%	2.57%	0.00%	0.00%

Similar to the relationship with maternal cortisol groups, the infants in the "low flat cortisol" group were distributed as would be expected by the probability of membership in the maternal sAA groups: low flat (46.2%), mid flat (34.3%), mid increase / flat (18.4%), and high reactivity / recovery (1.1%). Interestingly, in a departure from this pattern, the majority of infants displaying cortisol reactivity to the challenge tasks had mothers that showed the "low flat" sAA response pattern. Also noteworthy is the observation that none of the infants displaying the "high decline" cortisol pattern had mothers in the "mid increase / flat" or "high reactivity / recovery" sAA groups.

Summary of Findings Related to Physiological Attunement

In summary, while small to moderate correlations were observed between infants and mothers at baseline levels of sAA and cortisol in the whole group, there was little evidence for linkage between infant and maternal physiological response patterns during the challenge tasks. For the model testing links between infant and maternal sAA was there evidence of partial attunement at lower levels of sAA. However the same was not true for response patterns starting with higher sAA levels. For the three other models, it is clear that the distribution of response patterns across groups does not always follow the progression that would be expected by chance, but there is no clear and easily interpretable pattern of attunement in this sample.

Chapter 8

DISCUSSION

This project sought to determine 1) if infant behaviors (i.e., behavioral reactivity and emotion regulation) and characteristics (i.e. gender) are associated with profiles of sympathetic and adrenocortical stress response to challenge in both mothers and infants, 2) if maternal behaviors (i.e., maternal caregiving behavior) and symptoms of psychopathology (i.e., depression, anxiety) are associated with profiles of sympathetic and adrenocortical stress response to challenge in mother-infant dyads, and 3) to determine if there is attunement between mothers' and their infants' physiological (i.e., sympathetic or adrenocortical) stress response profiles. In general, the findings revealed that infant and maternal behaviors and characteristics were more likely to be associated with infant rather than with maternal physiological responses to the infant challenge. Specifically, the observations that both infant and maternal factors were associated with infant sAA response to challenge suggest that the context of the mother-infant relationship may also have important implications for the development of SNS reactivity to stress. As such, examination of individual differences in sympathetic reactivity will enhance our understanding of the process by which the early environment can have long-term influences on infant health and development. This study also provides support for the existence of physiological attunement in mother-infant dyads as positive associations were observed for baseline levels of both sAA and cortisol. These findings will be discussed with respect to consistency with prior research, applied and theoretical applications, limitations, and future directions for work investigating which aspects of the

mother-infant relationship are most salient to the development of stress physiological systems.

Consistency of the Findings with Prior Research

Overwhelming, the findings of the current study revealed that infant and maternal characteristics were more often associated with infant rather than maternal physiological responses to challenge. Such findings runs counter to the idea that there are bi-directional influences in the mother-infant dyad that serve to regulate both infant and maternal behavior and physiology (Field, 1992). While the literature on infant influences on maternal physiology is sparse, there have been a few studies, which suggest that infant distress cues can induce maternal sympathetic reactivity (Frodi & Lamb, 1980; Wiesenfeld & Klorman, 1978). It was even more surprising that maternal caregiving behaviors and symptoms of psychopathology were unrelated to maternal physiological response profiles. Clinical evidence links maternal depression and anxiety to elevated basal levels of both sympathetic and adrenocortical activity (Stokes, 1995; Gold et al., 1988). In addition, while positive, affectionate caregiving behaviors have been linked to higher maternal cortisol levels (Fleming et al., 1987; 1997; Stallings et al., 2001), atypical maternal behavior is associated with lower baseline cortisol levels (Schechter et al., 2004).

The lack of findings for links with maternal stress physiology may have more to do with the selection of our tasks than with the nature of the mother-child relationship. The series of challenge tasks selected for this study were taken from the Laboratory Temperament Assessment Battery (Goldsmith & Rothbart, 1996) and were designed to examine individual differences in infant behavioral distress to novel and frustrating

stimuli. As such, these tasks were primarily designed to induce infant rather than maternal distress. The mothers in the current study gave permission for their infants to undergo these tasks and had the ability to end the tasks at anytime if they felt the experience was overwhelming the infant. Therefore, our mothers had a great deal of control over this situation, perhaps negating the need for physiological arousal (Henry, 1992). This is reflected in the observation that the task series was a potent stimulator of infant physiological arousal, but few mothers had profiles suggestive of sAA reactivity to the tasks and none of the maternal cortisol profiles reflected adrenocortical activation in response to infant distress.

A dyadic challenge involving both the mother and the infant might be more likely to induce maternal physiological arousal and reveal the bi-directional influences characteristic of the mother-infant relationship. For example, the current protocol might be altered by informing the mother that she will be evaluated on her ability to soothe the child after the completion of the temperament tasks. In this situation, the mother would be challenged by the social evaluation of her ability to calm the child. The Still Face procedure, a paradigm widely used in the study of socioemotional development during infancy, may be another alternative (Tronick, Als, Adamson, Wise, & Brazelton, 1978; Weinberg & Tronick, 1994). Mothers are asked to engage in face-to-face interaction with their infant and then to cease interaction and maintain a blank, non-responsive "still face" for 2 minutes. Exposure to the still face is very upsetting to most infants and they usually respond with behavioral distress (Weinberg & Tronick, 1994; 1996). This paradigm could also be a potent stressor for mothers because each woman must act as the actual source of frustration for her child. She must continue to fulfill this role and hold a

blank face despite her infant's protests. Such a task may serve to challenge both members of the dyad and reveal more of the bi-directional influences inherent to this relationship.

The Influence of Infant Temperament and Gender

As predicted, both infant gender and behavior during the challenge tasks were associated with sympathetic and adrenocortical responses to the challenge in mother-infant dyads. The intensity of infant negative vocalizations (i.e., fussing, crying) and the proportion of time spent using regulatory strategies (i.e., orienting, self-comforting, and avoidence or escape behaviors) during the tasks interacted to predict infant sAA and cortisol responses to infant challenge. It was expected that all behaviorally reactive infants would show sAA reactivity to the challenge tasks, but that regulatory behavior would have little effect on sAA response to challenge. Surprisingly, it was the infants who made few negative vocalizations and spent little time using regulatory strategies that were most likely to show evidence of sAA increases during tasks, while highly reactive infants were more likely to show profiles characterized by low sAA levels and no reactivity.

There is little work on sympathetic responses to stress during infancy to help explain these observations. It is possible that the infants were physiologically distressed by the challenge tasks, but did not show behavioral evidence of this arousal. For example, Buss and colleagues (2004) have reported associations between freezing behavior in threatening situations and faster PEP. Such behavior is also reminiscent of the behavioral and sAA response profile displayed by avoidant infants during the Strange Situation paradigm (Hill, personal communication, February, 2006). These infants

rigidly control their external behavior in order to avoid attracting the attention of their caregivers (Belsky & Cassidy, 1995), but display sAA reactivity to separation and reunion (Hill, personal communication, February, 2006). Mothers of infants later classified as avoidant often behave in ways that are non-contingent on infant cues, too fast paced for the child, and are described as abrupt and interfering (Ainsworth et al., 1978; Belsky et al., 1984; Isabella et al., 1989; Malatesta, Culver, Tesman, & Shepherd, 1989). It is interesting that infants of highly anxious mothers were also likely to show a profile of sAA increase across the challenge task and that these mothers have been characterized as showing less contingent responsiveness, less warmth, and more intrusiveness in their interactions with their infants (Nover et al., 1984; Weinberg & Tronick, 1998; Whaley et al., 1999). It is possible that these low reactive infants of anxious mothers are already showing the beginnings of a behavioral and physiological pattern that will later be classified as avoidant attachment. Alternatively, sAA increases among infants that did not react negatively to the tasks may be linked to positive arousal. Physiological arousal has previously been associated with both positive and negative affective valence (for review, see Cacioppo, Klein, Berntson, & Hatfield, 1993). Preliminary analyses revealed a moderate positive correlation between sAA reactivity and positive behavioral reactivity characterized by smiling, laughing, and approach during the mask and arm restraint tasks. Therefore, infants that were not upset by the challenge tasks may have had a positive experience accompanied by sympathetic arousal.

Infants that showed high behavioral reactivity in response to the challenge tasks were more likely to display both low, non-reactive sAA levels and high cortisol reactivity with recovery. A lack of sAA reactivity among behaviorally reactive infants was not

anticipated. It is possible that the peak for sAA was missed due to our saliva collection strategy. The timing of the peak response for sAA is believed to be approximately 5 to 10 minutes after a stressor (Takai et al., 2004), but our first post-challenge sample was collected at 20-minutes to accommodate the peak for salivary cortisol. In addition, sAA in these infants may have peaked soon after the stressor and quickly returned to baseline levels. According to Henry (1992), challenges that are difficult to cope with quickly progress from sympathetic to adrenocortical activation. As feelings of frustration and defeat rise, levels of the catecholamines norepinephrine and epinephrine are believed to decline while cortisol levels increase. These highly reactive infants may have displayed sAA reactivity prior to our measurement, but the physiological response quickly became dominated by adrenocortical activity. By 20-minutes post-challenge, perhaps the SNS response was no longer observable.

It was also hypothesized that infants that were the most vocal (i.e., intense crying) and spent the least time using regulatory strategies would show the highest cortisol reactivity to challenge. While in general, infants displaying more behavioral reactivity (i.e., fussing, crying) were more likely to show cortisol reactivity to the tasks than low reactive infants, it was the very vocally reactive infants who also spent more time using regulatory strategies that were the most likely to show cortisol reactivity and recovery in response to the tasks. The increased probability of cortisol reactivity in the behaviorally reactive infants was anticipated. Research has repeatedly demonstrated the association between distress to novel and frustrating stimuli and cortisol reactivity during infancy (Donzella et al., 2000; Gunnar et al., 1992; van Bakel & Riksen-Walraven, 2004; Wilson, Megel, Fredrichs, & McLaughlin, 2003; Zimmerman & Stansbury, 2004). However, the

association between the frequent use of regulatory behaviors and cortisol reactivity for behaviorally reactive infants was surprising. Given that regulatory behavior is believed to diminish the need for adrenocortical activation (Stansbury & Gunnar, 1994), it was expected that high regulatory behavior would reduce the likelihood of cortisol reactivity. The different regulatory strategies used by infants in this study (i.e., orienting, self-comforting, avoidance) were summed within and across studies so that they represented the total number of attempts to use any regulatory strategy. However, it has been shown that some regulatory strategies, such as orienting and self-comforting, are associated with decreases in behavioral reactivity, while others, such as avoidance, are actually associated with increases in behavioral reactivity (Stifter & Braungart, 1995). It is believed that avoidance may be associated with heightened negative reactivity because, in the case of the arm restraint, this behavior is directed at the goal of breaking the restraints. When the goal is blocked, increased negative reactivity is the result (Weinberg & Tronick, 1994). Similarly, Lewis and Ramsay (2005) suggest that cortisol reactivity to frustration is observed only when anger does not function to remove goal blockage. Post-hoc analyses (paired t-tests) in the current study revealed greater negative vocalization to the arm restraint as compared to the masks and barrier tasks among vocally reactive infants. Struggling against the restraint was coded as avoidance and further analyses revealed (paired t-tests) that behaviorally reactive infants in this study used avoidance behaviors more often than other strategies (orienting or self-comforting). Thus, the frequent use of avoidance among reactive infants provides one explanation why infants high on behavioral reactivity and regulatory behaviors were more likely to show cortisol reactivity to the challenge tasks in this study.

Gender differences were also expected in the infants' physiological responses to challenge, but this was not observed. Sex differences in both resting and reactive levels of sympathetic and adrenocortical activity have been reported during the neonatal period (Dalmaz & Peyrin, 1982, Davis & Emory, 1995; Nagy et al., 2001). However, several other studies that have reported an absence of gender differences in adrenocortical stress reactivity later in infancy (e.g., Gunnar et al., 1988; Gunnar et al., 1987, for review see Kajantie & Phillips, 2006). It has been suggested that greater adrenocortical activity in males during the neonatal period may be due to greater neurophysiological immaturity in males than females at birth (Davis & Emory, 2005). Cortisol reactivity to stress declines with increasing maturity during infancy (for review, see Gunnar & Donzella, 2002). Therefore, as male and female infants develop during their first year, the sex differences in physiological stress reactivity may disappear, only to re-emerge later in life (Davis & Emory, 2005).

Unexpectedly, infant gender was related to overall levels of maternal cortisol. While all mothers showed declines across the assessment period, mothers of boys were more likely to show cortisol profiles with moderate to high levels of baseline cortisol. To our knowledge, no studies have previously reported differences in maternal cortisol levels by infant gender. It has been observed that male infants are less likely to use emotion regulation strategies and rely more on their mothers for external regulation than female infants (Stifter & Braungart, 1995; Tronick & Cohn, 1989; Tronick & Weinberg, 2000). Perhaps 6 months or more of living with male infants that require more maternal intervention in order to regulate distress resulted in the need for heightened vigilance and consequently, higher baseline activation of the HPA axis for these mothers.

Alternatively, one early study reported that, despite a lack of sex-differences in behavioral distress, mothers of male infants appear to have more difficulty observing their infants display distress than mothers of female infants (Corter & Bow, 1976). If true, mothers of boys may have elevated cortisol levels due to their reduced ability to regulate their own physiological response to normative levels of their infants' distress.

The Influence of Maternal Behavior and Symptoms of Psychopathology

As predicted, maternal caregiving behaviors and symptoms of psychopathology were also associated with infant sAA and cortisol responses to challenge. It was observed that highly detached or withdrawn mothers (i.e., insensitive mothers) were more likely to have infants with low baseline levels of sAA and no reactivity to the challenge tasks than mothers rated low on detachment. To our knowledge, only one other study examined infant sAA response to laboratory stressors (i.e., noise burst and arm restraint). Among 6 month-old infants, those with concurrently depressed mothers, nearly 40% displayed sAA decreases of 10% or more, while only approximately 20% of the infants of non-depressed mothers showed similar decreases in response to the stressors. In addition, approximately 15% fewer of the infants of depressed mothers showed sAA increases of 10% or more than those of non-depressed mothers (approx. 42% as opposed to approx. 58%; Shea et al., 2006). Depressed mothers are often less responsive, more disengaged, and more withdrawn in their interactions with infants than other mothers (Cohn & Campbell, 1992; Bugental et al., 2003). Thus, between these two studies, it appears that there may be a link between detached maternal behavior and reduced sAA levels and reactivity during infancy. Maternal influences on SNS levels and reactivity may be behaviorally mediated. The infants of withdrawn mothers have been observed to

display less affective reactivity to both positive and negative stimuli (Hart et al., 1999). It is possible that a maternal model of restricted affect leads to similar narrowing of affective responses (Hart et al., 1999) and subsequently, reduced physiological reactivity in these infants. Alternatively, genetic or environmental influences *in utero* may explain why infants of detached mothers may show reduced sAA reactivity. Similar to their infants, Shea and colleagues (2006) also observed that their depressed mothers had reduced sAA levels as compared to non-depressed mothers. Similarity in SNS functionality between mothers and infants may reflect transmission of adrenergic system gene variants (Shea et al., 2006), or the direct effects of SNS and HPA axis activity on the developing fetus *in utero* (Diego et al., 2004).

Infants of mothers with a higher number of depression or anxiety symptoms were hypothesized to have elevated baseline levels of both sAA and cortisol. While no associations with maternal depression were observed, infants of highly anxious mothers were more likely to show moderately high sAA levels along with sAA increases across the tasks. While these infants did not show the profile with the highest sAA baseline levels in the sample, 72% of the sample was predicted to show profiles with lower sAA levels. Counter to expectations, these infants of anxious mothers were also much less likely to display the infant cortisol profile characterized by high baseline levels and a steep decline across the assessment. Unexpectedly, neither maternal behaviors nor symptoms of depression or anxiety were associated with maternal physiological response profiles to the infant challenge. The reason for the lack of an association between maternal depression and anxiety symptoms and maternal stress physiology is unknown. There are well-documented associations between clinical manifestations of depression

and anxiety and elevated basal levels of both SNS and HPA axis activity (Stokes, 1995; Gold et al., 1988). A lack of association between maternal depression and infant physiology was also surprising given the previously reported links between maternal depression and reduced behavioral and physiological functioning during infancy (Bugental et al., 2003; Diego, et al., 2004). The instrument used to assess depression and anxiety for the current study was designed to measure symptoms of these psychopathologies within a non-clinical sample (Derogatis, 1993). Perhaps alterations in the functioning of these stress physiological systems would have been observed in a sample of clinically depressed mothers only.

Evidence of Physiological Attunement in Mother-Infant Dyads

Associations between overall levels of infant and maternal sAA and cortisol were expected and this was weakly supported by preliminary analyses that revealed a small to moderate positive associations between infant and maternal baseline levels of sAA and cortisol. In addition, there was evidence of partial attunement between infant and maternal sAA profiles for lower levels of sAA only. Previous studies have observed coherence in heart rate between mothers and infants (Field et al., 1989) and moderate positive correlations between infant and maternal responses to child challenge (Sethre-Hofstad et al., 2002). Thus, there is evidence in the literature of attunement for both sympathetic and adrenocortical activity in mother-infant dyads. In the present study, it is possible that associations were observed for baseline levels of sAA and cortisol only because these levels reflect the history of physiological attunement in mother infant dyads. Field (1992) hypothesizes that the history of interactions and daily stressors faced by the dyad results in affective and physiological synchrony between mother and child.

Overtime, similarity in infant and maternal baseline levels of SNS and HPA axis activity might become more apparent.

It was also expected that were would be attunement between the maternal sAA and infant sAA and cortisol reactivity to the challenge, but there was no evidence to support this hypothesis for this sample. This may have occurred because so few mothers were found to display the profiles characterized by physiological reactivity to the infant challenge. It is possible that attunement in physiological stress reactivity may be context-specific such that it can only be observed under conditions that are arousing for both dyadic partners. As mentioned previously, a different task, such as the Still Face paradigm (Tronick et al., 1978), might be arousing to both partners, thus allowing the observation of attunement in physiological reactivity. Alternatively, attunement may only occur for certain segments of the population. For example, Sethre-Hofstad and colleagues (2002) observed adrenocortical attunement to child challenge only for dyads with a sensitive caregiver. A great deal of work remains to fully elucidate the processes by which physiological attunement evolves in mother-infant dyads as well as the implications of this connection for development.

Applied Implications for Intervention and Methodology

Implications for Intervention Research

Alterations in physiological functioning may act as mediators for the development of behavioral and health problems associated with high-risk early environments (McEwen & Seeman, 1999). Therefore, their appearance may presage the later appearance of poor developmental outcomes. Identification of the physiological

responses to stress that are most often associated with risky mother-infant relationship outcomes could prove useful for intervention studies targeted at improving the quality of mother-infant relationships of at-risk dyads (van den Boom, 1994). In combination with both previous and future research, the physiological response patterns observed in this study could contribute to this identification process. For example, if replicated, the profile of sAA increases in response to stress among infants of highly anxious mothers may prove a useful marker of early risk. However, much work remains to develop a more complete understanding of the associations between high-risk behaviors and patterns of physiological response to stress in mother-infant dyads. Identification of infant and maternal behaviors associated with persistent physiological profiles, such as consistent non-response to potent stressors across multiple assessments, would be an important step in this process. In addition, prospective, longitudinal studies designed to examine the long-term outcomes (e.g., behavioral problems, health, allostatic load) associated with specific types of physiological responses would also be essential before application to intervention studies is possible. If properly identified, interventionists could potentially use physiological response profiles associated with risky infant or maternal behaviors to make early identification of the dyads at greatest risk. Change in these physiological profiles might also be used as a marker for the success of a behavioral intervention. For example, if an intervention targeted at increasing maternal sensitivity resulted in more secure attachments and altered an infant's physiological response, such that the infant no longer displayed a "high-risk" pattern, this would be even greater evidence of the success of the intervention. While such avenues for research seem promising, much preliminary research remains to be completed before this promise can be fulfilled.

Implications for Stress Research Methodology

The present study also hoped to address two major issues currently faced by stress researchers through the use of growth mixture modeling. First, the field of stress research needs to move beyond focusing on physiological responses to stress at the group level towards examining individual differences in physiological responses to stress. Growth mixture models used in the present study aimed to address this issue through identification of distinct profiles of sAA and cortisol response to challenge in mother-infant dyads (Nagin, 1999; 2005). For each of the four dependent variables (infant sAA and cortisol; maternal sAA and cortisol), three or four unique responses to the stressor were identified and predictors were used successfully to distinguish between these response profiles. However, these response profiles did not always seem to reflect the wide-varying individual differences observed in sAA and cortisol responses to the challenge. For example, investigation of maternal sAA reveals that 45% of mothers showed sAA increases of 10% or more in response to the challenge tasks and this was reflected in a significant increase in maternal sAA between baseline and 20-minutes at the group level for these mothers. In contrast, the growth mixture model of maternal sAA only extracted a single profile displaying reactivity between baseline and 20-minutes and only 5.7% of the mothers were estimated as belonging to this subgroup. All of the remaining profiles were characterized by a lack of reactivity, but large differences in baseline levels of sAA. Such an observation leads to the conclusion that these models are more strongly influenced by the value of the intercept (i.e., baseline) than by the shape of the change over time. Therefore, it can be suspected that mothers were grouped according to their baseline levels of sAA regardless of how they responded to the tasks.

The result was three groups with no mean change in response to the tasks. As a result of these observations, it is difficult to conclude that the growth mixture models used in this study should be applied to examine individual differences in stress reactivity. Perhaps application to studies focused on the diurnal rhythm of the HPA axis would be a better use of these models due to their emphasis on individual differences in waking levels of cortisol (Clow, Thorn, Evans, & Hucklebridge, 2004).

Another problem currently faced by the field of stress research is the need for concurrent examination of multiple physiological responses to stress. The dual trajectory model (Nagin & Tremblay, 2001) was forwarded as a potential solution for this problem. Originally, it had been hoped that these models would allow for concurrent examination of both SNS and HPA axis activity within individuals. However, closer examination of these models revealed that they were most appropriately applied to relating trajectories of two outcome measures for which there is *a priori* reason to believe that they are related. In effect, these models improve upon the correlation coefficient by providing information about associations between specific profiles and for specific segments of the population (Nagin, 2005). As such, these models were not appropriate for concurrent examination of sAA and cortisol responses to stress since it was not expected that they would be related based on prior research (Granger, Kivlighan, et al., 2006; Nater et al, 2005; 2006; Rohleder et al., 2005). However, these models were appropriate for the investigation of physiological attunement between infants and mothers as preliminary analyses revealed positive correlations between infants and their mothers for both sAA and cortisol levels. It may yet be worthwhile to apply these models to a study employing a stressor designed to induce stress reactivity in both members of the mother-infant dyad. Attunement in

reactivity profiles may not have been observed in the current study due to the focus of the task on the infant. Application of these models to a more dyadic stressor may reveal more interesting associations between infant and maternal physiological responses to stress.

Theoretical Implications for Biosocial Models of Behavioral and Physiological Regulation

The findings of this study suggest that our understanding of the function of and associations between behavioral and physiological regulation may need to be refined and enhanced. Stansbury and Gunnar (1994) produced a seminal work organizing findings relating emotion regulation strategies and adrenocortical reactivity to stress. In general, they suggested that emotion regulation strategies serve to reduce the need for adrenocortical reactivity. However, the current study hints that all regulatory strategies are not created equal. Considering the use of orienting, self-comforting, and avoidance behaviors as equally likely to reduce cortisol responses to stress resulted in an unexpected positive association between regulatory behavior and cortisol reactivity. A closer look revealed that avoidance behaviors, which were most common during the arm restraint, are actually associated with greater behavioral reactivity in the literature (Stifter & Braungart, 1995) and with greater cortisol reactivity in this sample. Similarly, Zimmermann and Stansbury (2003) found that self-comforting behaviors (e.g., rubbing, non-nutritive sucking, rocking) were positively associated with cortisol reactivity and suggested that other strategies, such as distraction or instrumental behaviors, might be more effective at reducing cortisol reactivity because they attempt to change a distressing

situation. A more fine-grained analysis of the regulatory behaviors most effective at reducing adrenocortical activity during infancy would be beneficial to our understanding of early influences on the development of the HPA axis. For example, do regulatory behaviors moderate the association between behavioral reactivity and adrenocortical reactivity and do relationships with these behaviors change across early development?

A related finding of the current study revealed an interaction between behavioral reactivity and regulation in the prediction of infant sAA reactivity to stress. Little is known about associations between regulatory strategies and SNS reactivity during infancy. Eriksen and colleagues (1999) suggest that even in the presence of coping, there will be a brief period of initial SNS arousal following a stressor and that the magnitude of this response will be similar whether or not it is followed by adrenocortical activation. Additionally, the adult literature has reported that SNS responses to stress do not seem to habituate on successive presentations of stressors (Gerra et al., 2001; Schommer et al., 2003). The findings of the present study suggest that idea that the SNS is unaffected by emotion regulation may need to be revised, especially during infancy. Examination of the development of SNS responses to stress in relation to emotion regulatory strategies may be a promising direction for future research.

In addition to examining the links between behavioral regulatory strategies and regulation of stress physiological systems, a better understanding of the short-term functionality of different patterns of physiological regulation (e.g., reactivity and recovery versus a lack of response) may help us to elucidate the processes by which different individuals develop stress pathologies that are damaging in the long-term. McEwen and Seeman (1999) have suggested four mechanisms through which

environmental assault or inadequate physiological regulation in stress responsive systems might lead to the development of allostatic load. They suggest that a normative response is activated in reaction to a stressor and then is appropriately "turned off" when the stressor has passed. In contrast, if an individual is faced with multiple, novel stressors, they may not have an opportunity to down-regulate the activity of these systems. Such may be the case with children living in the chaotic environments associated with early poverty (Evans, 2004). Alternatively, inadequate regulation of physiological systems may develop as the result of exposure to adverse early environments. The result may be a lack of habituation to repeated stressors (i.e., constant family conflict), a prolonged physiological response due to a lack of return to baseline levels following a stressor, or an inadequate physiological response to potent stressors. Future research in this area needs to confirm the existence these four mechanisms in early development in an effort to begin to understand the process by which early adversity can get "under the skin".

In particular, hypo-reactivity to stress may require additional attention in the literature. Much of the work on the development of stress responsive physiological systems during early childhood has focused on the reactivity of the HPA axis (Gunnar & Vazquez, 2001). However, research has increasingly revealed that adverse early environments are also capable of producing suppression of the activity of the HPA axis (i.e., hypocortisolism) and this phenomenon may be more common during early childhood than previously believed (Gunnar & Vazquez, 2001). Hypo-reactivity to stress appears to be associated with a variety of adverse physiological and psychological disorders including PTSD, chronic fatigue syndrome, fibromyalgia, rheumatoid arthritis, asthma, externalizing behavior problems, substance abuse disorders, and antisocial

behavior in adults (for reviews see, Heim, Ehlert, & Hellhammer, 2000; Gunnar & Vazquez, 2001; Susman, 2006).

The findings of the current study imply that hypo-reactivity of the SNS could also be a relatively common phenomenon among infants from low-income and impoverished families. The proportion of the infants displaying cortisol response profiles characterized by low cortisol levels and a lack of reactivity was fairly large at approximately 70-80% of the sample. Similarly, approximately 80-85% of the sample was estimated to show sAA response profiles with low to moderate sAA levels and no reactivity. The lack of physiological response in the majority of subjects was especially surprising given evidence that the majority of infants (85%) responded to the challenge tasks with at least some negative behavioral reactivity. To our knowledge, this study is among the first to examine individual differences in sAA responses to challenge during infancy. While much work remains to determine if sAA reactivity during infancy reflects SNS reactivity to stress as it does in older children and adults, the large numbers of infants with both low sAA levels and a lack of reactivity in the current study implies it may be worthwhile to consider a lack of sympathetic reactivity to stress as another marker of early adversity. In support of the link between early adversity and attenuated sAA reactivity, maternal detachment was observed to be associated with a hypo-responsive profile of sAA for infants in the present study. Shea and colleagues (2006) also found reduced sAA reactivity among infants of concurrently depressed mothers.

Based on observations that antisocial behavior patterns appear to be particularly associated with attenuation of both SNS and HPA axis responses to stress, Susman (2006) recently proposed that is the confluence of genetic and biological vulnerabilities,

brain development, stressful early environments marked by unpredictability, and early learning that results in the hypo-reactivity of these physiological systems and subsequent behavior problems. However, much work remains to develop a better understanding of the processes by which different adverse early rearing environments and biological vulnerabilities result in wide-ranging differences physiological responses to stress. Where does the divergence occur that leads some children raised under adverse conditions to show exaggerated physiological reactivity and internalizing behavior problems and others, raised under similarly adverse conditions, to manifest attenuated physiological reactivity and externalizing behavior problems? Prospective, longitudinal may be necessary to identify those children that consistently display the four patterns of physiological regulation highlighted by McEwen and Seeman (1999) and to determine the biological and environmental influences associated with each of these patterns of physiological regulation.

Limitations to the Present Study

Few infant or maternal factors were associated with maternal physiology in the current study. As previously suggested, the challenge tasks used in this study were specific to the infant, rather than focused on both members of the dyad, whereas a task designed to elicit physiological reactivity from both mother and infant, such as paradigm that incorporates social evaluation for the mother, may have revealed more interesting associations with maternal physiology or demonstrated greater attunement in infant and maternal physiological responses to stress. For example, maternal soothing following a dyadic challenge could provide rich information on the functioning of the dyad. Lewis

and Ramsey (1999) previously demonstrated that maternal soothing did not influence infant adrenocortical or behavioral responses to distress. Replicating this finding and determining if maternal behavior at the time of the stressor has the potential to influence maternal physiological stress responses would be extremely informative. It is likely that maternal success or failure at soothing her child would be more salient for maternal stress physiology than infant distress alone. Similarly, no measures of maternal cognitive, behavioral, or affective responses to the infant distress were included in the current study design. It has become increasingly clear that it is difficult to interpret the meaning of physiological responses to stress without a least some measures of behavioral or psychological responses to the same stressor. Observational coding or self-report of maternal response to infant distress might have revealed some interesting associations with maternal physiology that we were not able to capture with our distal measures of maternal caregiving behavior and psychopathology. More detailed assessment of maternal response to infant distress might have aided interpretation of the individual differences we did observe in maternal physiological reactivity.

The timing of saliva collection with regard to sAA also represented another limitation to the current study. The study was designed for the assessment of infant and maternal cortisol reactivity to infant challenge and the saliva samples were collected for optimal measurement of this hormone. Assay of sAA was added later in an effort to examine multiple measures of physiological reactivity to stress. Optimal timing of saliva samples to capture peak reactivity of sAA is approximately 5 to 10 minutes following the termination of a stressor (Granger, Kivlighan, et al., 2006). Therefore, we may have missed the peak for sAA response in some individuals. As such, while this study

demonstrates that sAA is both reactive to stress in this age group and that sAA is related to both infant and maternal behaviors and characteristics, the generalizability of this information is limited and needs to be replicated in future studies.

In addition to these design concerns, there were a few analytical limitations to current study. First, in retrospect, the selection of growth mixture models for the current analyses may not have been optimal to answer the questions of interest. As mentioned previously, the models used may have relied more heavily on baseline levels of sAA and cortisol as opposed to change over time for group selection, reducing their usefulness in studies of stress reactivity. In addition, the use of these models required examining the effect of each predictor individually rather examining multiple predictors in a single model. An alternative approach, such as structural equation modeling might have been more appropriate to address the questions of interest and would have reduced the total number of statistical tests used in this study. It is well known that as the number of statistical tests increases, there is a concomitant increase in the probability of Type I error (Cupples, Heeren, Schatzkin, & Colton, 1984; Curtin & Schultz, 1998). Given that only significant associations were reported in the results and no adjustments, such as the Bonferroni correction, were applied, it is likely that at least some of these associations occurred by chance.

Future Directions for Research

There is mounting evidence for the existence of maternal-infant synchrony between a variety of infant and maternal physiological systems. Evidence of synchrony has been observed between infant and maternal sleep cycles (McKenna et al., 1994), heart rate (Field et al., 1989), vagal tone reactivity (Bornstein & Seuss, 2000), cortisol

reactivity (Sethre-Hofstad et al., 2002), and for baseline levels of cortisol and sAA in the current study. The fact that physiological links have been observed between mother and child across such a wide variety of physiological systems suggests that this may be a ubiquitous phenomenon of mother-infant relationships. While we have very little knowledge to date of the implications of this synchrony for normative development, physiological attunement may represent a previously unexplored marker of mother-infant relationship quality. It is also possible that individual differences in the degree of linkage between mother and infant may have important implications for maternal ability to sensitively respond to her child and for child development. For example, it has even been suggested that attunement in mother-infant sleep cycles may have implications for reducing infant mortality (McKenna et al., 1994). As a first step towards understanding the meaning of maternal-infant physiological synchrony, identification of normative developmental trends and deviations from these trends in synchrony within a variety of physiological systems is essential. The evidence suggests that biobehavioral synchrony (i.e., infant movement with maternal heart rate and SNS activity) may even begin prior to birth (DiPietro et al., 2004; 2006). It is possible that this prenatal synchrony prepares both the infant and mother to engage in both behavioral and physiological synchrony during the postnatal period. Future work should focus on determining if there is a link between prenatal and postnatal synchrony, as well as examining how individual differences in behavioral and physiological synchrony during the first years of life are related to developmental outcomes. Additionally, researchers interested in gender differences related to parenting behavior might consider examining dyadic stress physiology and physiological synchrony in father-infant dyads as well. Previous research

has suggested that the antecedents of father-infant attachment relationships differ from those of mother-infant attachment relationships (Braungart-Rieker et al., 2001). Examination of both mother-infant and father-infant physiological attunement in the same families would provide interesting information regarding differences the nature in mother-infant and father-infant relationships, as well as a test of whether or not prenatal biobehavioral synchrony increases the likelihood of postnatal synchrony.

Other potentially interesting studies could follow from the study of physiological responses to stress in mother-infant dyads. Determination of the stability of physiological responses to stress over repeated occasions in mother-infant dyads could provide information about the stability or flexibility infant and maternal physiological responses to stress overtime. Such longitudinal research might be useful for identification of dyads at greatest risk for poor outcomes, as well as determination of whether a single observation of dyadic stress reactivity is a useful measure of stress reactivity in mother-infant dyads. Finally, there is a need for studies designed to address questions related to the coordination or dissociation of SNS and HPA axis responses to stress early in development. Including measurements of both stress responsive physiological systems is a step in the right direction, but studies need to be designed with the appropriate research designs and methods for this problem. New means for modeling the complex interplay between these two systems early in development might be a necessary step for advancing hormone-behavior research.

Concluding Remarks

In conclusion, this study sought to determine if infant and maternal behaviors and characteristics influenced both infant and maternal sympathetic and adrenocortical

reactivity to infant challenge. Surprisingly, infant and maternal factors more heavily influenced infant sAA and cortisol responses rather than maternal physiological responses to infant distress. A second goal of this project was to examine attunement between infant and maternal physiological responses to stress. While evidence of attunement was found between baseline levels of sAA and cortisol, no attunement was observed between infant and maternal reactivity to infant challenge. Both observations demonstrate the importance of a dyadic context for the expression of behavioral and physiological responses to stress in both members of the mother-infant dyads. This study was unique in its concurrent investigation of multiple stress physiological systems, as well as in its examination of maternal physiological response to infant distress. To our knowledge, this is the first study to report links between infant temperament, maternal caregiving behavior, and maternal anxiety with infant sAA response to stress. These findings emphasize the importance of examining the impact of early environmental contexts on multiple stress physiological systems. Finally, the majority of mother-infant dyads in this ethnically diverse sample were low-income or impoverished and living in very rural and isolated areas. In contrast, the bulk of the studies addressing infant and maternal physiological responses to challenge have been conducted with Caucasian, middle class samples. Examination of questions related to the development of stress responsive physiological systems under conditions of social and ethnic diversity should expose researchers to a wider variety of individual differences mother-infant relationship quality. Perhaps this will enhance our understanding of how this early relationship shapes the development of stress responsive physiological systems with implications for health and well being across the lifespan.

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APPENDIX A

NC Recruiting Script

Good morning/afternoon. My name is _____. I work with the Family Life Project. Is now an OK time to talk? **[If 'yes' continue; if 'no' ask:** *when is a better time for me to return today?***].** *The Family Life Project is a federally funded project that is taking place in Sampson, Wayne and Wilson counties. The goal of the Family Life Project is to better understand children's early development and family life in these counties and counties like them. Have you received a copy of our brochure? (if not, give one) Here is also a small gift, a magnetic picture frame, for you and your new baby.*

We are talking to every mother who gives birth in this hospital about the Family Life Project. Families who are interested in participating and who are selected will earn $450 in gift certificates over the first three years of their child's life. Specifically, families that are selected for this project will be visited in their homes at times that are convenient for them. At each visit we collect information about family life and about the new baby. We provide families with a $50 gift certificate each time that we visit. In the past, most families have enjoyed participating in projects like this.

In order to become eligible for this project, I would like to ask you some questions that should take about 5-10 minutes to complete. By answering those questions, your name would be put into a lottery. If your family was selected, we would call you in about 4 weeks and give you more information about the project. At that time, we would also schedule the first home visit.

Do you have any questions about what I have described or about the project in general? Would you like for your family to be considered for this project?

[If yes…*Great! I'll ask some questions about your family situation, like where you live and what types of state and federal services your family may receive. We ask these questions to make sure that families from all different backgrounds are included in the project. Of course, your answers are confidential and will not be shared with people not associated with the project.* **Proceed w/ Screening form**]

[If no, say: *OK, I understand that families are very busy with the arrival of a new baby. Would you be willing to answer 8 quick questions so that we can describe which families did not have interest in our project?*

If yes, ask 8 'refusal' items on recruitment screening form that are designated with an asterisk '*'.
If no, say: *OK. Thanks for your time. Good luck with your new baby.*]

[Below is the text and cutoff values for item 19 of the Recruitment Screening form. Determination of the correct cutoff value depends on answers to items 17 & 18 of the form. Record answer to this on that form].

*OK, please consider all the sources of income that are available in your household. This includes both your income, as well as regular income contributions from other people both in and outside of your household. This may include earnings from jobs, public assistance received from local, state, or the federal government, profits from investments or things that are sold, as well as any other money--like child support payments-- that your household receives from other sources on a regular basis. Taking these different sources of income into account, would you say that your combined household income is above or below…/***Read or write on card the dollar amount that applies to this family based on the number of adults and children that were reported in items 17 and 18 of the Recruitment Screening form]**

Size of family unit	# Related children under age 18	Cutoff
2 (householder under 65 yrs)	1	$24,415
2 (householder over 65 yrs)	1	$24,323
3	1	$28,511
3	2	$28,539
4	1	$37,133
4	2	$35,921
4	3	$36,045
5	1	$44,699
5	2	$43,331
5	3	$42,271
5	4	$41,625
6	1	$50,877
6	2	$49,829
6	3	$48,823
6	4	$47,329
6	5	$46,443
7	1	$58,673
7	2	$57,417
7	3	$56,543
7	4	$54,913
7	5	$53,011
7	6	$50,925
8	1	$65,789
8	2	$64,605
8	3	$63,567
8	4	$62,095
8	5	$60,225
8	6	$58,281
8	7	$57,787
9+	1	$78,827
9+	2	$77,779
9+	3	$76,899
9+	4	$75,453
9+	5	$73,465
9+	6	$71,667
9+	7	$71,221
9+	8+	$68,477

NC SCREENING FORM Recruiter #1 ID: _____ #2 ID:_____
Today's date: ____/____/_____ mmddyyyy

LOCATION & RESULTS OF RECRUITMENT
1. **Hospital**: 1. Sampson Regional 2.Wayne Mem 3.Wilson Med 4. Birth Records 5. High-Risk

2. **Outcome**: (Check whether mom was contacted or not; then check additional details as necessary)

Mother was contacted (clarify result of contact by choosing **one of the options** below)

Agreed to become eligible	Learned that no contact should have occurred (e.g., language, adoption), no Qs asked
Mom completely refused	Mom refused but answered screening Qs
Parental/legal guardian refused	Parental/legal guardian consent never arrived (attempt follow-up calls before checking)
Mother contacted at inconvenient time and later checked out; efforts to call at home failed	

Mother was NOT contacted (clarify reason for no contact by choosing **all of the options that apply** below)

Denied access b/c of HIPAA	Baby placed for adoption
Family did not live in target county	Baby deceased
Mother did not speak English	Other_____
Mother checked out of hospital prior to contact; efforts to call at home within 7 days failed	

SCREENING/DEMOGRAPHIC Qs (Check/circle **one** response; NA = not applicable; DK = don't know; R=refuse)

*3. **What county do you live in?** 1.Sampson 2.Wayne 3.Wilson 4.Other

*4. **What is the primary race of your baby?** White Black Asian Am Indian Pac Island

*5. **Would you say that your baby is Hispanic/Latino/Latina?** (Y) (N)

*6. **Does anyone in your household use or receive any of the following: Medicaid, welfare, food stamps or an EBT card for groceries, WIC (also called Women Infant & Children), Free/Reduced lunch program at school, or NC Children's health insurance (also called NC Health Choice)?** (DK) (R) (Y) (N)

*7. **Did you graduate from high school or get a GED?** (R) (Y) (N)

8. **If someone other than you is considered head of your household did that person graduate from high school or get a GED?** (NA) (Y) (N)

*9. **Do you expect to move out of the state in the next 3 years (including military)?** (Y) (N)

*10. **Is English the primary language that is spoken in your home?** (Y) (N)

11. **Do you already have a child in this study?** (Y) (N)

12. **What was your baby's date of birth? (mm/dd/yyyy)**
____/____/_____

13. **What was your baby's due date? (mm/dd/yyyy)** (DK) ____/____/_____

14. **Are you 18 years or older?** (Y) (N)

*15. **What is your date of birth (mm/dd/yyyy)?** ____/____/_____

16. **If you are under age 18, do you live with your parent or legal guardian?** (N**A**) (Y) (N)

17. **Including yourself, how many people 18 years or older live in your household?** _____

18. **Including your new baby, how many people under 18 years live in your household?**

_____

19. (see Recruitment script). **Is household income above or below cutoff?** (R) (**D**K) (**A**bove) (**B**elow)

CONTACT/IDENTIFYING INFORMATION

Now, I would like to collect some information that we could use to contact you if you are selected to be invited into the project. May I please have your full name, current home address and telephone numbers where I could contact you.

Is primary contact Bio Mom: Yes No If not, relationship to baby: _____

20	Mom/PC first name:	
21	Mom/PC middle name:	
22	Mom/PC maiden name:	
23	Mom/PC last name (if applicable):	___NA
24	Baby first name:	___Undecided
25	Baby last name:	___Undecided
26	Mom/PC street address, including apt #:	
27	Mom/PC city, state, zipcode:	
28	Home phone (enter # or choose NA):	( ) _____ - _____ ___NA
29	Work phone: (enter # or choose NA)	( ) _____ - _____ ___NA
30	Cell phone (enter # or choose NA):	( ) _____ - _____ ___NA
31	What name is home phone listed under?	___NA ___Refused __DK
32	Preferable days to call	__S __M __T __W __R __F __S __any
33	Preferable times to call:	___morning __afternoon __evening __any

Finally, we know that having a baby is a time of great change for families. I wonder if you would please give me the names and phone numbers of two other people who do <u>not</u> live with you but would know where you could be located, if we were unable to contact you using the information you just provided. If possible, we would like for at least one of these people to be a family member.

34	First contact name:	
35	First contact relationship to baby:	
36	First contact phone number #1	( ) ____ - _____ ___home ___work ___cell
37	First contact phone number #2	( ) ____ - _____ ___home ___work ___cell __NA
38	Second contact name:	
39	Second contact relationship to baby:	
40	Second contact phone number #1	( ) ____ - _____ ___home ___work ___cell
41	Second contact phone number #2	( ) ____ - _____ ___home ___work ___cell __NA

PA Recruiting Script

Good morning/afternoon. My name is _____. I work with the Family Life Project. Is now an OK time to talk? **[If 'yes' continue; if 'no' ask:** *when is a better time for me to return today?***].** *The Family Life Project is a federally funded project that is taking place in Blair, Cambria, and Huntingdon counties. The goal of the Family Life Project is to better understand children's early development and family life in these counties and counties like them. Have you received a copy of our brochure? (if not, give one) Here is also a small gift, a magnetic picture frame, for you and your new baby.*

We are talking to every mother who gives birth in this hospital about the Family Life Project. Families who are interested in participating and who are selected will earn $450 in gift certificates over the first three years of their child's life. Specifically, families that are selected for this project will be visited in their homes at times that are convenient for them. At each visit we collect information about family life and about the new baby. We provide families with a $50 gift certificate each time that we visit. In the past, most families have enjoyed participating in projects like this.

In order to become eligible for this project, I would like to ask you some questions that should take about 5-10 minutes to complete. By answering those questions, your name would be put into a lottery. If your family was selected, we would call you in about 4 weeks and give you more information about the project. At that time, we would also schedule the first home visit.

Do you have any questions about what I have described or about the project in general? Would you like for your family to be considered for this project?

[If yes…*Great! I'll ask some questions about your family situation, like where you live and what types of state and federal services your family may receive. We ask these questions to make sure that families from all different backgrounds are included in the project. Of course, your answers are confidential and will not be shared with people not associated with the project.* **Proceed w/ Screening form**]

If no, say: *OK, I understand that families are very busy with the arrival of a new baby. Would you be willing to answer 8 quick questions so that we can describe which families did not have interest in our project?*

If yes, ask 8 'refusal' items on recruitment screening form that are designated with an asterisk '*'.

If no, say: *OK. Thanks for your time. Good luck with your new baby*.][Below is the text and cutoff values for item 19 of the Recruitment Screening form. Determination of the correct cutoff value depends on answers to items 17 & 18 of the form. Record answer to this on that form].

OK, please consider all the sources of income that are available in your household. This includes both your income, as well as regular income contributions from other people both in and outside of your household. This may include earnings from jobs, public assistance received from local, state, or the federal government, profits from investments or things that are sold, as well as any other money--like child support payments-- that your household receives from other sources on a regular basis. Taking these different sources of income into account, would you say that your combined household income is above or below… **[Read or write on card the dollar amount that applies to this family based on the number of adults and children that were reported in items 17 and 18 of the Recruitment Screening form]**

Size of family unit	# Related children under age 18	Cutoff
2 (householder under 65 yrs)	1	$24,415
2 (householder over 65 yrs)	1	$24,323
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6	3	$48,823
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6	5	$46,443
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7	5	$53,011
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9+	1	$78,827
9+	2	$77,779
9+	3	$76,899
9+	4	$75,453
9+	5	$73,465
9+	6	$71,667
9+	7	$71,221
9+	8+	$68,477

PA SCREENING FORM Recruiter #1 ID: _____ #2 ID:_____
Today's date: ____/____/_____ mmddyyyy

LOCATION & RESULTS OF RECRUITMENT
1. **Hospital**: 1. Altoona 2. JC Blair 3. Conemaugh Medical Center 4. Birth Records 5. High-Risk

2. **Outcome**: (Check whether mom was contacted or not; then check additional details as necessary)

Mother was contacted (clarify result of contact by choosing **one of the options** below)

Agreed to become eligible	Learned that no contact should have occurred (e.g., language, adoption), no Qs asked
Mom completely refused	Mom refused but answered screening Qs
Parental/legal guardian refused	Parental/legal guardian consent never arrived (attempt follow-up calls before checking)
Mother contacted at inconvenient time and later checked out; efforts to contact at home failed	

Mother was NOT contacted (clarify reason for no contact by choosing **all of the options that apply** below)

Denied access b/c of HIPAA	Baby placed for adoption
Family did not live in target county	Baby deceased
Mother did not speak English	Other_____
Mother checked out of hospital prior to contact; efforts to call at home within 7 days failed	

SCREENING/DEMOGRAPHIC Qs (Check/circle one response; NA = not applicable; DK = don't know; R=refuse)

*3. **What county do you live in?** 1.Blair 2.Cambria 3.Huntingdon 4.Other

*4. **What is the primary race of your baby? White Black Asian Am Indian Pac Islander**

*5. **Would you say that your baby is Hispanic/Latino/Latina?** (Y) (N)

*6. **Does anyone in your household use or receive any of the following:** (DK) (R) (Y) (N)
the Access Medical Card (also called Medicaid), Gateway health insurance, Healthy Beginnings, welfare, food stamps or an EBT card for groceries, WIC (also called Women Infant & Children), Free/Reduced lunch program at school, or PA Children's health insurance (also called CHIP)?

*7. **Did you graduate from high school or get a GED?** (R) (Y) (N)

8. **If someone other than you is considered head of your household did that person graduate from high school or get a GED?** (NA) (Y) (N)

*9. **Do you expect to move out of the state in the next 3 years (including military)?** (Y) (N)

*10. **Is English the primary language that is spoken in your home?** (Y) (N)

11. **Do you already have a child in this study?** (Y) (N)

12. **What was your baby's date of birth? (mm/dd/yyyy)** ____/____/_____

13. **What was your baby's due date? (mm/dd/yyyy)** (DK) ____/____/_____

14. **Are you 18 years or older?** (Y) (N)

*15. **What is your date of birth (mm/dd/yyyy)?** ____/____/_____

16. **If you are under age 18, do you live with your parent or legal guardian?** (N**A**) (Y) (N)

17. **Including yourself, how many people 18 years or older live in your household?** _____

18. **Including your new baby, how many people under 18 years live in your household?**

_____

19. (see Recruitment script). **Is household income above or below cutoff?** (R) (**D**K) (**A**bove) (**B**elow)

CONTACT/IDENTIFYING INFORMATION

Now, I would like to collect some information that we could use to contact you if you are selected to be invited into the project. May I please have your full name, current home address and telephone numbers where I could contact you.

Is primary contact Bio Mom: Yes No If not, relationship to baby: _____

20	Mom/PC first name:	
21	Mom/PC middle name:	
22	Mom/PC maiden name:	
23	Mom/PC last name (if applicable):	___NA
24	Baby first name:	___Undecided
25	Baby last name:	___Undecided
26	Mom/PC street address, including apt #:	
27	Mom/PC city, state, zipcode:	
28	Home phone (enter # or choose NA):	( ) _____ - _____ ___NA
29	Work phone: (enter # or choose NA)	( ) _____ - _____ ___NA
30	Cell phone (enter # or choose NA):	( ) _____ - _____ ___NA
31	What name is home phone listed under?	___NA ___Refused __DK
32	Preferable days to call	__S __M __T __W __R __F __S __any
33	Preferable times to call:	___morning __afternoon __evening __any

Finally, we know that having a baby is a time of great change for families. I wonder if you would please give me the names and phone numbers of two other people who do <u>not</u> live with you but would know where you could be located, if we were unable to contact you using the information you just provided. If possible, we would like for at least one of these people to be a family member.

34	First contact name:	
35	First contact relationship to baby:	
36	First contact phone number #1	( ) ____ - _____ ___home ___work ___cell
37	First contact phone number #2	( ) ____ - _____ ___home ___work ___cell __NA
38	Second contact name:	
39	Second contact relationship to baby:	
40	Second contact phone number #1	( ) ____ - _____ ___home ___work ___cell
41	Second contact phone number #2	( ) ____ - _____ ___home ___work ___cell __NA

APPENDIX B

Table 1. Model selection criteria (BIC and Probabilities of Group Membership) for infant sAA response to challenge tasks are presented. While the BIC favored 5 or 6 groups, these models contained subgroups with less than 4% of the sample. Therefore, a 4-group model was selected for infant sAA.

Model Selection – Infant Salivary *α*-Amylase

Model	BIC (N = 810)	BIC (N = 284)	Group #	Group Membership (%)
1 Group	-3880.40	-3878.30	1	100.00%
2 Groups	-3728.10	-3723.90	1	82.96%
			2	17.04%
3 Groups	-3692.14	-3685.85	1	62.65%
			2	26.35%
			3	11.00%
4 Groups*	-3687.30	-3678.92	1	60.57%
			2	24.49%
			3	8.56%
			4	6.37%
5 Groups	-3665.07	-3654.89	1	58.21%
			2	24.26%
			3	2.48%[a]
			4	8.98%
			5	6.07%
6 Groups	-3665.18	-3652.61	1	24.21%
			2	57.52%
			3	2.02%[a]
			4	1.08%[a]
			5	9.10%
			6	6.06%

[a] The percentage of group membership falls below 4% of the sample (N=~11 individuals).
*Model selected for the current analysis.

Table 2. Model selection criteria (BIC and Probabilities of Group Membership) for infant cortisol response to challenge tasks are presented. While the BIC favored 6 groups, this model contained subgroups with less than 4% of the sample and extracted redundant groups. Therefore, a 4-group model was selected for infant sAA.

Model Selection – Infant Salivary Cortisol

Model	BIC (N = 818)	BIC (N = 284)	Group #	Group Membership (%)
1 Group	317.73	319.85	1	100.00%
2 Groups	401.21	405.44	1	83.99%
			2	16.01%
3 Groups	494.16	500.50	1	77.00%
			2	16.77%
			3	6.23%
4 Groups*	501.96	510.42	1	71.01%
			2	5.01%
			3	17.88%
			4	6.11%
5 Groups	499.78	510.36	1	67.66%
			2	5.48%
			3	19.10%
			4	1.67%[a]
			5	6.09%
6 Groups	506.44	519.13	1	18.76%
			2	5.00%
			3	59.18%
			4	7.71%
			5	3.69%[a]
			6	5.65%

[a] The percentage of group membership falls below 4% of the sample (N=~11 individuals).
*Model selected for the current analysis.

Table 3. Model selection criteria (BIC and Probabilities of Group Membership) for maternal sAA response to challenge tasks are presented. While the BIC favored 5 or 6 groups, these models contained subgroups with less than 4% of the sample. Therefore, a 4-group model was selected for infant sAA.

Model Selection – Maternal Salivary *α*-Amylase

Model	BIC (N = 847)	BIC (N = 284)	Group #	Group Membership (%)
1 Group	-4600.49	-4598.31	1	100.00%
2 Groups	-4425.18	-4420.81	1	76.34%
			2	23.66%
3 Groups	-4343.27	-4336.71	1	55.53%
			2	37.00%
			3	7.46%
4 Groups*	-4322.45	-4313.71	1	45.91%
			2	33.59%
			3	14.76%
			4	5.74%
5 Groups	-4304.87	-4293.94	1	46.00%
			2	34.34%
			3	14.54%
			4	2.17%[a]
			5	2.95%[a]
6 Groups	-4300.31	-4287.20	1	44.67%
			2	33.00%
			3	15.12%
			4	3.11%[a]
			5	3.74%[a]
			6	0.36%[a]

[a] The percentage of group membership falls below 4% of the sample (N=~11 individuals).
*Model selected for the current analysis.

Table 4. Model selection criteria (BIC and Probabilities of Group Membership) for maternal cortisol response to challenge tasks are presented. While the BIC favored 6 groups, this model contained subgroups with less than 4% of the sample. In addition, the 5-group model was the first to extract the theoretically interesting "mid-flat" group. Therefore, a 5-group model was selected for infant sAA.

Model Selection – Maternal Salivary Cortisol

Model	BIC (N = 847)	BIC (N = 284)	Group #	Group Membership (%)
1 Group	774.36	776.54	1	100.00%
2 Groups	990.48	994.86	1	75.81%
			2	24.19%
3 Groups	1059.2	1065.76	1	28.05%
			2	62.90%
			3	9.05%
4 Groups	1089.63	1098.37	1	39.67%
			2	17.30%
			3	35.63%
			4	7.40%
5 Groups*	1105.47	1116.40	1	37.52%
			2	35.56%
			3	9.43%
			4	12.98%
			5	4.51%
6 Groups	1122.85	1135.96	1	2.52%[a]
			2	15.31%
			3	4.75%
			4	35.36%
			5	38.19%
			6	3.29%[a]

[a] The percentage of group membership falls below 4% of the sample (N=~11 individuals).
*Model selected for the current analysis.

Vita

Katie T. Kivlighan

Katie T. Kivlighan was born in Hayward, CA on December 24, 1978. She received her bachelor's degree in Biology with a concentration in Biological Psychology from the College of the Holy Cross in 2001 and commenced graduate study in pursuit of a doctoral degree at Pennsylvania State University in 2002. Her major program of study is Biobehavioral Health with a minor in Human Development and Family Studies. Her major research interests include the development of stress responsive physiological systems in the context of family and social relationships, the attunement of stress physiology in close social relationships, and gender differences in social behavior and physiology. After the completion of her doctorate, the author will begin a postdoctoral research position in the Bloomberg School of Public Health at Johns Hopkins University and plans to pursue a career as a research academic.