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

BIOBEHAVIORAL RESPONSES TO CAREGIVING
IN VERY LOW BIRTH WEIGHT PRETERM INFANTS

A Thesis in
Biobehavioral Health

by
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Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

May 2005
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ABSTRACT

Life-sustaining medical care in the neonatal intensive care unit (NICU) may cause discomfort and stress in very low birth weight (VLBW) preterm infants. Moreover, repeated exposures to unmitigated stress may contribute to illness and poor developmental outcomes. This study examined linkages between biological and behavioral responses to caregiving as predictors of health outcomes in preterm infants. The specific aims of the study were: (a) to examine hypothesized relationships between neuroendocrine responses, vagal tone, and behavioral responses within the context of nursing caregiving in the NICU, (b) to test whether early patterns of hormonal, heart rate, and behavioral responses in the first few days of life are predictors of health outcomes for very low birth weight infants at the time of discharge, and (c) to identify maternal perceptions of the NICU environment and the effects of these perceptions on maternal-infant interaction and subsequent parenting beliefs. The hypotheses were: 1 (a) there will be direct positive relationships between cortisol reactivity and autonomic parameters, (b) there will be direct positive relationships between cortisol reactivity and behavioral responses, (c) there will be direct positive relationships between heart rate variability (HRV) and behavioral responses, 2 (a) infants with higher cortisol responses to handling will have poorer health outcomes than infants with lower cortisol reactivity, (b) infants with lower HRV at rest will have poorer health outcomes than infants with higher heart rate variability, 3 (a) infants with higher behavioral distress will have poorer health
outcomes than infants with lower distress, (b) infants with higher cortisol levels post-handling (at 60 minutes) will have poorer health outcomes than infants with lower cortisol levels post-handling, (a) mothers reporting higher stress will be less responsive to their infants than mothers with lower stress, and (b) mothers who are less responsive to their infants will rate themselves lower in parenting competency than mothers with high responsiveness. Thirteen preterm infants were enrolled in this prospective, observational study, with each infant serving as her/his own control. On day 4 - 5 of life, morning plasma samples were obtained for assessment of cortisol levels from an existing umbilical catheter while simultaneously measuring heart and respiratory rates, and oxygen saturations at baseline and 30 and 60 minutes post-handling during standard nursing care. Activity, state, and behavioral stress signals were quantified using the NIDCAP® observational method. Resting HRV was measured on day five and one month postnatally. Health outcomes data were collected via chart audit. Maternal questionnaires and behavioral interaction ratings were administered during the first week of hospitalization and one month postnatally. The results of the analyses showed that cortisol reactivity was highly positively associated with visceral cues ($p = 0.03$) during handling and tachypnea ($p = 0.04$) post-handling. HRV was highly positively correlated with motor, facial, and attentional cues. Recovery (60 min) cortisol was highly positively associated with duration of ventilation ($p = 0.03$), duration of oxygen ($p = 0.02$), and length of stay ($p = 0.005$). Those infants with greater high frequency HRV (vagal tone) achieved oral feeding earlier ($p = 0.048$) and had improved health outcomes including: shorter
ventilation (p = 0.03), lower incidence of retinopathy (p = 0.03), and shorter length of stay (p = 0.04). Infants demonstrating expression of facial distress cues (brow bulge, eye squeeze) had a higher incidence of nosocomial sepsis (p = 0.04), longer duration of oxygen therapy (p = 0.02), and longer length of stay identified by correlation and regression analyses (p = 0.02). Mothers of study infants reported high general stress levels during early hospitalization; however, maternal stress levels did not predict nurse-rated competency of mothers at one month of age. Mothers rated by NICU nurses as more competent and sensitive to their infants had higher self-ratings of competency in parenting at one month of age. All infants exhibited hormonal and behavioral reactivity to handling. The majority quieted and had lower cortisol levels at one hour post-handling in response to appropriate pacing of care and containment. Those infants with persistent tachypnea and higher cortisol levels post-handling had higher respiratory morbidity. Behavioral distress responses (facial and visceral cues) were associated with higher rates of nosocomial sepsis and longer oxygen duration; these indicators represented a uniquely vulnerable subgroup, more challenged by environmental stimulation. In contrast, more robust infants demonstrating high motor and attentional behaviors had greater high frequency HRV and improved outcomes. This study adds to the accruing evidence that vagal tone represents an important homeostatic mechanism that may be an independent measure of stress vulnerability. In addition, cue-based caregiving was crucial in promoting infant stability. Further investigation with a larger sample of newborns is required to confirm the clinical significance of these findings.
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ACKNOWLEDGEMENTS

This thesis is the culmination of many years of planning, discussing, and revising of a research idea that originated from my NIDCAP® training and certification with Virginia Passero under the direction of Heidelise Als and Jean Gardner Cole at Boston City Hospital. This endeavor could not have been accomplished without the help and prayers of so many. Above all, I would like to acknowledge the Higher Power that directed my path. In addition, I would like to offer thanks to my entire extended family for their continued love and support during the many years of this process. I am grateful to my husband Keith and my son Jared for love and support through difficult times, and to my sister Lori whose prayers carried me through many challenging moments.

I would like to offer sincere thanks to all my committee members for their direction during all phases of the process. Deep gratitude is extended to Dr. Liz Susman for the many years of support and mentoring she provided. She offered encouragement and wisdom at every step of this journey. Dr. Keith Marks provided scientific oversight to the project on-site and encouragement when recruitment was difficult. Dr. Sheila West imparted valuable expertise in the area of heart rate variability measurement, and provided support, direction, and encouraging words throughout the process. Dr. Doug Granger offered expertise with cortisol sampling and Dr. Charles Palmer provided me with time to conduct the project and offered expertise with use of the videotaping equipment. Kelly Klaus and Marisa Censani donated their time to help with data collection.
I would like to offer a special thanks to Jodi Heaton, Shannon Seiner Anthony, and Lisa Grove for all the help they provided with course scheduling, teaching assistantships, and the valuable insight they provided. Gratitude also is extended to Marsha Haack, Virginia Schiefelbein, and Samantha Dockray for assistance with SPSS. Deep gratitude is extended to Kim Walker who provided assistance with preparation of tables and formatting, and to my colleagues and friends in nursing and Biobehavioral Health who offered encouragement.

This dissertation was funded in part by grants from the Pediatric Endocrinology Nursing Society and the General Clinical Research Center (GCRC), Hershey Campus, NIH-MO1-RR10732. I would like to thank Rebecca Jenkins for assistance with the application, and Shirlynn Motilla and staff of the GCRC for processing and storage of cortisol assays. A special thanks is extended to Dr. Larry Demers and Christopher Hamilton of the Core Endocrinology Laboratory for providing high quality analyses of the assays.

In addition, I would like to acknowledge the nurses, nurse practitioners, and physicians of the Neonatal Intensive Care Unit at Penn State Children’s Hospital for their help with study recruitment and the provision of high quality developmentally focused care to the infants. Finally, I offer gratitude to the mothers who so graciously donated their time to participate, and the babies who taught us so much.
Chapter 1: Introduction

Introduction

Preterm birth (a live birth prior to 37 completed weeks of pregnancy) is the second leading cause of mortality for all infants in the United States and is the number one cause of death for African American newborn infants (Hoyert, Freeman, Strobino, & Guyer, 2001). It is estimated that 12 percent of all infants born in the United States are more than 4 weeks preterm, and approximately one percent are born more than 12 weeks preterm (Arias, MacDorman, Strobino, & Guyer, 2003). The risk of infant death is 6 times greater for low birth weight infants (less than 2500 grams or 5 ½ pounds) and 96 times greater for very low birth weight infants (less than 1500 grams or 3 ½ pounds) when compared with infants of normal birth weight (Hoyert, Freeman, Strobino, & Guyer, 2001). Preterm birth and the associated low birth weight status accompanying preterm birth are central predictors of infant mortality.

There has been a steady decline in the overall infant mortality rate in the United States since 1980. This decline has been linked predominantly to improvements in obstetrical and neonatal care. Examples of innovations in neonatal care that have contributed to decreasing mortality of very low birth weight (VLBW) infants include: establishment of high level neonatal intensive
care units, exogenous surfactant administration, gentler strategies for providing artificial ventilation, and more complete nutritional care.

Despite the dramatic improvements in survival for VLBW preterm infants, the morbidities for these fragile infants remain high. Preterm infants require many weeks to months of vigilant care in the neonatal intensive care unit (NICU) with associated hospital costs on average from $30,000 to $100,000 (National Center for Health Statistics, 2000). The technology-driven environment of the NICU is harsh in comparison with the uterine environment the infant was required to leave prematurely. Even during an age of developmental sensitivity to NICU design, preterm infants are exposed to bright lights, high sound levels, and disruption of sleep with frequent handling and administration of multiple, often painful procedures. These experiences are assumed to cause distress in infants too immature to adapt and cope with such high environmental demands.

Common stressful procedures for VLBW infants include standard nursing caregiving practices and common medical interventions (e.g. physical exams, endotracheal suctioning, gastric tube insertion, and heel prick blood sampling). Stevens and colleagues (1999) demonstrated that preterm infants could be exposed to as many as 134 procedures in the first two weeks of life. The VLBW preterm infant with immature brain and vascular structures is especially vulnerable to the effects of a high-tech, high-touch environment.

The immature brain is particularly vulnerable to fluctuations in blood pressure (Perlman et al., 1985), a potential consequence of stressful procedures. An area known as the germinal matrix layer, prominent from 25-34 weeks
gestation, is a periventricular structure that contains a rich vascular network without supportive stroma. Significant fluctuations in systemic and cerebral circulations can result in bleeding arising from this fragile “capillary network” with a resulting periventricular-intraventricular hemorrhage (PIVH) (Klaus & Fanaroff, 2001). The magnitude of PIVH is dramatic as the most frequent type of brain injury that occurs in the premature newborn is caused by PIVH or related sequelae such as periventricular hemorrhagic infarction, posthemorrhagic hydrocephalus, or concomitantly occurring periventricular leukomalacia or diffuse hypoxic-ischemic injury (Klaus & Fanaroff, 2001). Although the overall incidence of PIVH has declined in recent years, the improving survival of very small premature infants suggests that PIVH will continue to be a problem in years to come.

The potential deleterious effects of repeated exposures to stressors on immature brain organization and function are other areas of concern for the preterm infant. There is a growing body of evidence to suggest that repeated exposures to the numerous diagnostic and therapeutic procedures required during the infant’s hospitalization might contribute to long-term neurodevelopmental sequelae, such as impaired cognition and memory, and behavioral and attentional problems in childhood (Wallace & McCarton, 1997). A significant issue is that there is little research to show the impact of medical caregiving on neurobehavioral development of fragile preterm infants during this critical period of postnatal development and potential immediate and long-term consequences.
The current study is a first-step in advancing the literature by examining biological and behavioral responses of very low birth weight infants to caregiving. The specific aims were:

(1) to examine hypothesized relationships between neuroendocrine responses, vagal tone, and behavioral responses within the context of nursing caregiving in the neonatal intensive care unit.

(2) to test whether postnatal patterns of hormonal, heart rate, and behavioral responses in the first few days of life are predictors of health outcomes in very low birth weight infants at the time of discharge.

(3) to identify maternal perceptions of the NICU environment and the effects of these perceptions on maternal-infant interaction and subsequent parenting beliefs.

Specific hypotheses will be tested within each of these aims in a sample of 13 very low birth weight preterm infants. This study is designed to generate findings as a first step in a very new and important area of research.
Chapter 2: Literature Review

The following is a review of the literature with the purpose of creating a foundation for a study examining the hormonal, heart rate, and behavioral responses of the preterm, very low birth weight infant to handling within the context of a nurse-infant, caregiving interaction. To offer the reader the necessary background to consider the theoretical model, a brief description of the stress system first is presented, including the relationships between stress, as measured by the hypothalamic-pituitary-adrenal (HPA) axis, and the known impact of excessive glucocorticoid production on fetal development and postnatal disease processes.

A description of stress is followed by a discussion of cortisol measurement with special considerations for infant assay determination. Subsequently, heart rate responsivity is described with an emphasis on heart rate variability and the applicability of this non-invasive technique as a predictor of neonatal health outcomes. Next, a framework for individualized developmental care is presented, along with key concepts related to assessment of neurobehavioral regulation and behavioral responses. Then, the special needs of parents of preterm infants are addressed as well as how early maternal-infant attachment contributes to successful parenting of the preterm infant. In the final section, a description of the biobehavioral model and the relevance of the current investigation are advanced.
The Stress System

Dr. Hans Selye, a renowned endocrinologist and pioneer in stress research, described “stress” as the non-specific response of the body to any demand placed on it (Selye, 1956). Selye labeled agents or experiences that cause stress "stressors", and believed these agents to arise from the organism’s external, internal, and psychosocial environment. According to Selye, organisms show a systemic response of resistance to a stressor, and in the early phase this enhances system functioning. In animal studies (Selye, 1974; Smith & Selye, 1979), Selye observed that the same stressor produced unique responses and disorders in organisms related to the adaptive capability or “conditioning factors” required for systemic regulation.

More recently, scientists have advanced the field of stress research to demonstrate that brief controlled periods of stress (eustress) enhance organism functioning, while protracted, uncontrolled periods of stress (distress) that exceed an individual’s adaptive capacities result in stress-related illnesses (McEwen & Stellar, 1993). Moreover, stress hormones have been recognized for their complex role in emotional and cognitive processes. The specific physiological sequelae of high exposures to stress have been identified in both animal and human models.
Components of the Stress System

The central components of the stress system include corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) neurons of the paraventricular nuclei of the hypothalamus along with CRH neurons and other nuclei of the medulla, catecholaminergic neurons of the locus ceruleus, cell groups of the medulla and pons, and receptors in the hippocampus (Lovello & Thomas, 2000). The hypothalamic-pituitary-adrenal (HPA) axis and efferent sympathetic/adrenomedullary system comprise the peripheral limbs of the stress system (Stratakis & Chrousos, 1997). The HPA and central components of the stress system support behaviors with required metabolic fuels to respond to thoughts and emotions.

When faced with a potentially life-threatening situation, the brain’s stress response goes into action to focus attention, enhance fear and fight-or-flight response, while at the same time inhibiting behaviors such as feeding, sex, and sleep in order to improve the chance of survival (Sternberg & Gold, 1992). This highly complex system acts to protect the body by responding to internal and external stressors in order to achieve stability for the organism.

Corticotropin-releasing hormone (CRH)

Corticotropin-releasing hormone (CRH) is a 41 amino acid peptide that stimulates the secretion of adrenocorticotropic hormone (ACTH) and beta-endorphin from the adenohypophysis of the pituitary gland. CRH is synergized with arginine-vasopressin (AVP) to stimulate ACTH release (Griffin & Ojeda, 1996). CRH is a key releasing hormone shared by the central nervous and
immune systems. This neuroendocrine-immune system relationship is characterized by the immune-hypothalamic-pituitary-adrenal axis (Chrousos & Gold, 1992; Granger, Granger, & Granger, in press). Cytokines, in particular interleukin-1, which are produced by antigenically challenged macrophages, act on the hypothalamus to stimulate the secretion of CRH (Granger, Granger, & Granger, in press; Sapolsky, 1997). CRH in turn, stimulates the pituitary gland to secrete ACTH, the adenohypophyseal hormone that controls the function of the adrenal cortex.

This large peptide hormone is produced in specific loci of the brain and in the anterior and intermediate lobes of the pituitary gland (Griffin & Ojeda, 1996). The secretion of ACTH is augmented by noxious stimuli (stresses) and inhibited by glucocorticoids through the classic negative feedback regulation. ACTH acts on the adrenal cortex to elicit glucocorticoid secretion, including cortisol, the best-known hormone of the stress response.

**Glucocorticoids**

Cortisol, a potent immunoregulator and anti-inflammatory agent is stimulated by ACTH and is a steroid hormone that increases the rate and strength of heart contractions, sensitizes blood vessels, and affects many metabolic functions in order to prepare the body to adapt to a stressful situation (McEwen & Stellar, 1993). The hippocampus has a large number of both glucocorticoid and mineralocorticoid receptor sites and has become recognized as the primary site of negative feedback for cortisol regulation (Lovello & Thomas, 2000; Gunnar & Cheatham, 2003). It is noteworthy that the
hippocampus is a key structure of the limbic system important for emotional and cognitive processes.

**Interacting Systems and Health Outcomes**

The CNS and its interaction with the HPA axis affect the combination of neurotransmitters and neuropeptides within the neurons of the ANS (Stratakis & Chrousos, 1997). The ANS also innervates immune organs, such as the thymus, lymph nodes, and spleen thereby helping to control inflammatory responses throughout the body. The immune system is responsible for neutralizing potentially dangerous toxins, facilitating repair of damaged tissues, and disposing of abnormal cells (McEwen, 1998). When the immune system escapes regulation, autoimmune/inflammatory diseases, or immune deficiencies result.

In addition to interaction with the immune system, the stress system interacts with the reproductive axis, somatotropic axis, thyroid axis, and metabolic and gastrointestinal systems. The interactions between these systems play an important role in organism allostasis, or the ability to maintain stability (McEwen, 1998). When system imbalances occur the potential for allostatic load, the wear and tear on organs and tissues is high, resulting from chronic overactivity or underactivity of the stress system (McEwen & Stellar, 1993). A number of pathologic disease states have their etiologic roots in dysregulation of the stress system. This system is now understood in relation to pregnancy with resulting disease pathology in infants.

A relatively new field of research, coined “fetal origins of adult diseases” is concerned with linking cortisol-induced fetal growth restriction with adult
cardiovascular diseases, type II diabetes and insulin-resistance, depression, and end-stage renal disease (Watterberg, 2004). The posited theory is that high fetal cortisol and intra-uterine growth restriction result in permanent changes in total organ cell numbers through inhibition of cell replication and markedly decreased total DNA.

Maternal infection during pregnancy with chorioamnionitis, produces proinflammatory cytokines (interleukin-1, and interleukin-6) that stimulate HPA activation and active cortisol production in the fetus (Bolt et al., 2002). In addition, both animal and human studies provide evidence that excessive exposure to glucocorticoids passively during fetal life (secondary to high maternal cortisol levels from maternal malnutrition or stress that cross the placenta) results in neuroendocrine axis changes in the fetus (Wadhwa et al., 1996) and lasting effects in newborns including: low birth weight, lower organ weights, and HPA axis suppression, also known as adrenal insufficiency.

Adrenal insufficiency is a moderately common condition for infants in the neonatal intensive care unit (NICU). Relative adrenal insufficiency in the presence of serious illness occurs with an expected frequency of 3-56% (Fernandez, Schrader, & Watterberg, 2005). Recent studies in sick newborns demonstrate that adrenal insufficiency (basal cortisol < 15 mcg/dl) in term to near-term infants is associated with hypotension (Fernandez, Schrader, & Watterberg, 2005). In very low birth weight infants, hypoadrenal response to ACTH stimulation is associated with hypotension (Efird, et al., 2005), cardiac dysfunction (Watterberg, 2002), respiratory insufficiency, and a higher incidence of chronic
lung disease at one month of age (Watterberg & Scott, 1995). Chronic lung disease in premature infants is a major contributor to longer hospital stays and chronic respiratory illnesses post discharge.

Recent literature on the treatment of adrenal insufficiency with low-dose hydrocortisone replacement therapy has shown promising results. Hydrocortisone treatment was effective in the treatment of refractory hypotension and reduced the need for vasopressor support with dopamine (Efird et al., 2005; Fernandez, Schrader, & Watterberg, 2005). Further randomized controlled clinical trials are needed to determine the efficacy and safety of hydrocortisone replacement prior to widespread prophylactic treatment of cortisol deficiency in newborns. In addition, studies are needed to identify typical patterns of cortisol response to stimulation with caregiving in very low birth weight infants.

**Biomarkers of Stress**

Cortisol is the most commonly obtained biological marker used to detect and quantify stress responses in neonates. Cortisol is the hormonal end product of activation of the hypothalamic-pituitary-adrenal (HPA) system. Most of the cortisol in the circulation is bound to protein, cortisol-binding globulin, and about ten percent remains unbound or “free” in the circulation. The unbound fraction is that which is biologically active. Cortisol can be measured in plasma and saliva, and its metabolites detected in urine (Granger et al., 2003). Recent evidence suggests that newborns and infants before 3 months of age do not have basal diurnal patterns like adults (de Weerth, Zijl, & Buitelaar, 2003; Spangler, 1991), but pre-stress challenge cortisol is correlated with behavioral state of the
preceding 30-minute period prior to sampling (Banks et al., 2001; Gunnar et al., 1996; Huysman, Hokken-Koelega, De Ridder, & Sauer 2000; Larson, White, Cochran, Donzella, & Gunnar, 1998; Peters, 1998, 1999). The intensity and duration of crying has been correlated with increased cortisol levels in newborns.

Salivary sampling of cortisol is gaining widespread popularity in behavioral and medical research. Salivary determination is the preferred method of collection as it more directly reflects the unbound cortisol, because binding globulins in the blood interfere with total/free ratio of cortisol in blood assays (Casey & MacDonald, 1997; Kivlighan et al., 2004; Shirtcliff, Granger, Schwartz, & Curran, 2001). In addition, the non-invasive nature of salivary determination makes it an ideal sampling method.

However, in newborns there are situations that require special consideration. First, it is common to have residual maternal blood (from passage through the birth canal) in the infant’s mouth or regurgitation of swallowed maternal blood for up to 24-48 hours after birth. Secondary to large differences in concentration between blood and salivary markers, even traces of blood in the saliva affect the integrity of quantification of salivary biomarkers (Kivlighan et al., 2004; Shirtcliff, Granger, Schwartz, & Curran, 2001). Second, residual breast milk or formula in the infant’s mouth may contain maternal or bovine hormone-like substances that interfere with antibody binding and alter immunoassay results (Magnano, 1989). Therefore, the best conditions for facilitating accurate salivary determination in newborns would include: assuring that the mouth is clear of residual blood and or milk, and minimizing the risk of regurgitation by
waiting a minimum of two hours after a completed feeding before obtaining the sample.

To obtain salivary samples in infants, a dental cotton roll is placed in the cheek and, once removed, saliva is aspirated from the moistened cotton by plunging the roll through a needleless syringe into a collection vial. The sample is then frozen at –80°C until radio- or enzyme- immunoassay (RIA or EIA) is performed (Magnano et al., 1992; Schwartz, Granger, Susman, Gunnar, & Laird, 1998). Until recently, standard RIA kits for cortisol required 100-500 microliters of clear saliva, making assay determination in very low birth weight infants impossible. Because recent technological improvements in assay determination assure accuracy with as little as 25 microliters of saliva, it is now feasible to obtain samples in preterm infants (Granger, Schwartz, Kivlighan, Nelson, Whembolua, & Singer, in press). However, to obtain even such small quantities is nearly impossible in extremely low birth weight infants.

Stress Reactivity

Stress reactivity is a term used to define the extent to which one or more of the biological indices (e.g. cortisol, heart rate) change in response to an acute stressor or intervention. This reactivity may be indexed in relationship to the stressor or intervention and expressed as a change from baseline (Granger et al., 1996; Ponirakis, Susman, & Stifter, 1998; Zakowski, 1992,) or change after the stressor or intervention. There is substantial evidence to suggest that intraindividual change in cortisol level in response to a novel stressor is a more sensitive indicator of HPA axis functioning than a mean cortisol level (Granger et
Relationships between cortisol and emotional-behavioral regulation have been shown to impact: emotionality in toddlers (Susman, Schmeelk, Ponirakis, & Gariepy, 2001), social competence in children (Granger et al., 1996; Blair, Peters, & Granger, 2004), and conduct disorder and depression in adolescents (Dorn, Susman, & Petersen, 1992; McCool & Susman, 1994; Susman et al., 1997; Susman & Pajer, 2004). In infants, increased HPA activation has been correlated with the intensity and duration of crying in response to a stressor (Gunnar, 1992; Gunnar, Broderson, Nachmias, Buss, & Rigatuso, 1996), and novelty to procedure (Grunau, Weinberg, & Whitfield, 2004; Kennan, Grace, & Gunthorpe, 2003). These findings suggest that the neuroendocrine system is responsive to stressors from birth onward.

**Sympathetic Arm of the Autonomic Nervous System**

During hypothalamic activation of the sympathetic branch of the autonomic nervous system, the adrenal medulla releases increased amounts of acetylcholine, epinephrine, norepinephrine, as well as a variety of neuropeptides. In addition, the sympathetic arm of the autonomic nervous system (ANS) innervates cells of the gastrointestinal system, and salivary glands (Stratakis & Chrousos, 1997). In ventilated preterm infants with respiratory distress syndrome high baseline levels of catecholamines (Chan et al., 1994) and rises in plasma epinephrine and norepinephrine levels have been shown with procedures such as chest physiotherapy and endotracheal suctioning (Goldman and Koren, 2002). Catecholamine levels can be measured in saliva or blood using an enzyme-based immunoassay; however, catecholamines in saliva take about one hour to
reflect stress-induced changes (Rohleder et al., 2004). Recent advances in quantitative enzyme kinetics demonstrate the ability to measure the salivary protein, alpha-amylase (sAA) a strong surrogate marker of norepinephrine in 10 microliters of saliva (Granger, Kivlighan, & Blair, in press; Nater et al., 2005; Rohleder et al., 2004). Biomarkers of each of the central components of the stress system are now detectable in saliva.

The measurement of stress biomarkers provides key information reflective of the HPA and sympatho-adrenomedullary (SAM) systems. When combined with other autonomic parameters, such as heart rate variability, a more complete assessment of the stress system is represented and may be useful to prognosticate health outcomes. In the present study, cortisol, heart rate and respiratory parameters were measured at rest and following caregiving to determine responsiveness and recovery from handling.

**Heart Rate Responses: Measurement Issues**

The clinical importance of heart rate responsivity was first described in 1965 when Hon and Lee noted that fetal distress was preceded by changes in heart beat (AHA, 1996). The technique of evaluating fetal well-being in response to the uterine environment became known as heart rate variability (HRV). Heart rate variability (HRV) has become a recognized term to describe instantaneous variations in heart rate as well as interval changes in beat-to-beat variability (AHA, 1996). In addition to important applications in maternal-fetal status, studies to date have shown heart rate variability (HRV) to be an important prognosticator of neonatal well-being (Griffin et al., 2004;
Increased HRV reflects adequate functioning of neural control mechanisms and high stress adaptability (Porges, 1992). In contrast, attenuated HRV has been associated with pathological disease states including hypoxemia, periventricular-intraventricular hemorrhage, perinatal asphyxia, and acute sepsis.

The most common methods to evaluate heart rate variability in neonates are time domain and frequency domain analyses. The time domain analysis of HRV is the calculation of interval length variations between heartbeats as measured by distance between R waves on the electrocardiogram (ECG) (Brownley et al., 2000; Doussard-Roosevelt et al., 2001). This is commonly referred to as interbeat interval (IBI) variation. HRV reflects the autonomic nervous system’s influence on the cardiovascular system. The main physiological mechanisms contributing to HRV are respiration, baroreceptor reflex, and thermoregulation.

HRV is comprised of short-term variability and long-term variability. Short-term variability is measured by calculating the standard deviation of the sequential R-wave to R-wave interval (R-R)i difference over one minute; whereas long-term variability is the standard deviation of all (R-R)i over one minute (Sahni, 2000). A preprocessor calculates the intervals between R waves and computes HR and HRV minute-to-minute. Quadrant analysis is a technique to quantify the occurrence of increases, decreases, and absence of change in successive (R-R)i (Sahni, 2000). Application of this technique
has been used to study the maturational development of the autonomic nervous system regulation of heart rate in preterm infants.

Another important time-domain technique utilizes adaptive polynomial filtering to assess the magnitude of vagal-respiratory, respiratory sinus arrhythmia (RSA) and non-respiratory influences on heart rate variability. The method separates the heart rate signal from the respiratory signal to create a smooth template of slow respiratory oscillations (Brownley et al., 2000; Doussard-Roosevelt et al., 2001). Maturation of RSA follows myelination of vagal fibers, thus RSA is a function of gestational age (Doussard-Roosevelt et al., 2001). When RSA is being used as an indicator of vagal maturation using spectral technique, it is imperative that the heart rate is at least two times greater than the respiratory rate to avoid erroneous measurement. (Valimaki & Rantonen, 1999). Except for isolated events of apnea in preterm infants, when in normal sinus rhythm, the heart rate should always be more than two times greater than the respiratory rate. Another important factor in accuracy of the measurement of RSA is the number of data points, ideally a minimum of 4000 data points should be obtained through continuous monitoring (Valimaki & Rontonen, 1999). These data points can be achieved by obtaining 30-40 minute segments of data with minimal artifact.

The infant’s behavioral state is an important confounder to consider when measuring heart rate and heart rate variability. Simultaneous behavioral state assessment is necessary to document the infant’s state of sleep versus arousal during the physiological monitoring of heart rate and respiratory rate (Doussard-
Roosevelt, Porges, & McCleey, 1996). Overall, HRV is affected by motor activity, a parameter that distinguishes between active and quiet sleep.

Spectral power analysis is a frequency domain measure that assesses the underlying system control of the cardiac rhythm. It is a non-invasive modality using digitally recorded heart rate via the electrocardiogram to quantify autonomic activity and assess cardiovascular response (Oberlander, & Saul, 2002). The graphic representation of the power spectral function is plotted against the bandwidth frequency (Rosen et al., 2000). Commercial computer program packages are becoming increasingly available to compute the quantitative analyses of the oscillations of the biosignals.

In 1822 a French mathematician, Fourier, showed that a periodic signal could be converted into a set of harmonic sinusoidal and co-sinusoidal waves (Valimaki & Rantonen, 1999). This principle, known as the fast Fourier transform, converts the periodic (R-R)i signal into sine functions by plotting density of variability against frequency (Rosenstock, Cassuto, & Zmora, 1999; Rosen et al., 2000). Each of the different frequencies is expressed as a function of their relative power, or power spectrum. The applicability of this technique to engineering and clinical psychology has been enhanced by the development of fast digital computers.

The bandwidth frequencies of spectral analysis divide into three ranges: very low frequency (VLF) bandwidth (0.003 - 0.03 Hz), low frequency (LF) bandwidth (0.03 - 0.15 Hz), and high frequency (HF) bandwidth (0.15 - 1.0). Each of these bandwidths represents an underlying physiological mechanism that
affects HRV. The VLF bandwidth represents peripheral vascular resistance fluctuations caused by vasomotor thermoregulation and renin-angiotensin system. The LF bandwidth represents sympathetic and parasympathetic nervous system regulation mediated by baroreceptors. The HF bandwidth is modulated by the parasympathetic nervous system and is the result of respiratory activity and respiratory sinus arrhythmia (Rosen et al., 2000; Sahni, et al., 2000; Valimaki & Rantonen, 1999). The HF bandwidth is representative of cardiac vagal tone.

The measurement of vagal tone has been identified as a method to index both the stress response and the individual’s vulnerability to stress. The parasympathetic nervous system, known to regulate internal state, facilitate digestion, and conserve energy by slowing heart rate, also serves as an index of homeostasis. Under most situations, the greater the range of phasic increases and decreases in neural efferent output via the vagus nerve to the heart, the healthier the individual (Porges, 1992). In sum, vagal suppression of sympathetic system activation is important for sympathovagal balance and stress attenuation.

**Clinical Utility of HRV**

The utility of HRV measurement as a prognosticator of outcome for critically ill newborns has been the theme of several recent reports in the medical literature. In a case-series study design of four newborns with congenital diaphragmatic hernia, two of which required life-support with extracorporeal membrane oxygenation (ECMO), HRV measurements were obtained daily to document differences and similarities between the two infants that required ECMO and the two that did not (Verklan & Padhye, 2004). The authors reported
infants who demonstrated consistent increasing total spectral energies had better outcomes than those who did not. Three of the neonates with very low total power values had difficult intensive care courses, and all eventually succumbed. The infant that survived had a consistent energy gain across bandwidths during recovery and at the time of discharge had 70% of the total power contained in the HF bandwidth. The proposed mechanism of higher physiological stability was that the predominance of parasympathetic system influence (as reflected in the HF bandwidth, or cardiac vagal tone) was important in maintaining neurophysiologic homeostasis and balance against sympathetic system output secondary to stress overload or stress vulnerability.

The use of HRV measurement to evaluate maturational development of the autonomic nervous system and related behavioral development has been the focus of several investigations. Sahni and colleagues (2000) obtained 6-hour continuous ECG recordings and simultaneous minute-by-minute behavioral activity measurements in 61 growing low birth weight infants, at 31 - 38 weeks postconceptional age. During quiet sleep, HRV increased in relation to age, and high frequency spectral power band changes paralleled those seen with time domain measures of HRV. The maturational changes with age (decreased heart rate, increased heart rate variability, and increased interbeat interval change) were consistent with maturation of the autonomic cardio-regulatory system.

In a longitudinal study of 20 very low birth weight infants, investigators obtained weekly recordings of mean heart rate and cardiac vagal tone at 33, 34, and 35 weeks gestational age while in the NICU. The neonatal physiological
measure of RSA was correlated with social competence on the behavior child checklist at school age. (Doussard-Roosevelt, McClenny, & Porges, 2001). The amplitude of RSA directly reflects cardiac vagal tone, representing the ability of the central nervous system (CNS) to regulate autonomic cardiovascular function. Self-regulatory abilities of the CNS are developing at this same critical period of development and are important in the establishment of affect and social engagement.

In a study of 16 preterm infants residing in the NICU, the infants had HRV determinations made on five consecutive days. There was a significant inverse relationship between severity of clinical condition and vagal tone (Porges, 1992). Preterm infants, with lower vagal tone until 35 - 37 weeks of gestation, have a limited capacity to regulate their internal state and are challenged by the environmental demands to regulate body temperature, and adapt to the high environmental sensory burden of the NICU.

In a multi-center study by Griffin et al., 2004, 685 infants were enrolled and had continuous ECG monitoring upon admission to the NICU. A heart rate characteristic score (HRC) was computed using the number and frequency of heart rate decelerations, and inter-beat-interval variability. Thirty-seven infants in this prospective study died. The major study findings were: 1. HRC index was highly associated with death in the succeeding 7 days, and 2. the cumulative HRC was highly associated with neonatal in-house mortality (r = 0.8, p = 0.001).

In addition to its usefulness in mortality estimates, recent interest in the use of HRV as a diagnostic tool for the detection of sepsis in newborns has been
reported by Griffin et al. (2001). In a four-year prospective study researchers measured normalized heart rate for 5 days before and 3 days after sepsis, sepsis-like illness, and random controls. Infants in the sepsis and sepsis-like illness groups had reduced baseline heart rate variability and decelerations in heart rate that worsened within 24 hours prior to the clinical manifestations of illness in the infant.

The measurement of HRV provides unique physiological information that can be used to complement other physiological parameters to measure the stress response and prognosticate health outcomes. HRV provides information on the integrity of cardiac neuroregulatory activity, especially modulation of heart rate through the parasympathetic system. When HRV is combined with HPA stress biomarkers and behavioral assessments, the fullness and complexity of the stress response can be evaluated. In the current study, heart rate variability was measured during the first week of life and again at one month of age to evaluate cardiac vagal tone and to predict health outcomes.

**Behavioral Responses**

*Sickness Behaviors*

The regulation of the immune system CRH- secreting neurons of the hypothalamus sends fibers to regions in the brain stem that help to regulate the sympathetic nervous system. Stimulation of the locus ceruleus leads to behavioral arousal, fear, and enhanced vigilance. By recruiting CRH-secreting neurons, cortisol-mediated restraint of the immune response is activated, as well as induction of behaviors that assist in recovery of injury or illness (Granger,
Through sympathetic and HPA systems activation there is an increased production of catecholamines and glucocorticoids facilitating the conversion of energy from glycogen and protein stores. The energy is necessary for the proliferation of cellular responses associated with immune system activation (Granger, Granger, & Granger, in press; Maier & Watkins, 1998). Immune suppression may play a role in attenuation of other stress-sensitive systems to prevent “overshooting” (Gunnar, 1992). Cortisol acts to suppress immune system activity and inflammatory processes.

Because the pathways for this immune-brain interaction are in place, it seems plausible that many of the same “sickness behaviors” as seen in older children would be observable in high-risk newborns. Patterns of sickness behavior in infants are subtler than in older children and adults. Common manifestations of illness in newborns include: lethargy, temperature instability, diminished motor activity, irritability, hyperglycemia, poor feeding and/or feeding intolerance. Of these “sickness behaviors” irritability and feeding intolerance are often the first to be manifested. Unfortunately, because these symptoms (fussiness and spitting up) are part of the continuum of normal infant behavior these symptoms may often go unrecognized as “sickness behavior”. In addition, because the infant’s temperamental attributes contribute significantly to patterns of sucking, feeding behavior, and irritability/mood (Medoff-Cooper & Schraeder, 1982), it may be much more difficult to discern “sickness behavior” from “behavioral style” in the temperamentally challenging infant.
Psychobiological manifestations of illness in the preterm infant may be less apparent than with the full-term infant as the preterm infant’s behavioral repertoire is not fully mature. Because preterm infants have immature thermoregulatory abilities, hypothermia and temperature instability are more common than fever. Lethargy, diminished tone, and loss of motor activity may be observed as the first indicators of “sickness” or “stress overload” in the preterm infant. These behaviors are adaptive as the inactivity and loss of alertness serve to minimize attentive interactions with caregivers in order to conserve the energy required for restorative processes.

If the acute phase stress response continues unimpeded, increased breakdown of protein, fat, and carbohydrate, unregulated temperature balancing, and metabolic derangements including metabolic acidosis, hypoglycemia, hyperglycemia, and electrolyte imbalance can result (Goldman & Koren, 2002). The subtle cues of distress are important as they serve a protective role and alert the care provider that the infant has a limited capacity to handle further stress.

The limited evidence to date on the biobehavioral stress response system in preterm infants, compared to term infants, suggests that responsiveness to procedures and environment may be heightened due to underdevelopment of adaptive regulatory mechanisms resulting in increased arousal to stress in preterm infants. This heightened arousal, coupled with the vast stressors (tactile, proprioceptive, vestibular, auditory, and visual) created by the neonatal intensive care unit (NICU) environment may lead to significant multi-system strain and the potential for serious long-term illness in the neonates.
Caregiver Interaction and Environmental Influences

The Synactive Theory of Infant Development, proposed by Als, (1982) (Figure 1) describes the preterm infant’s development as involving interacting subsystems (autonomic, motor, state and interactional) outwardly characterized by specific behaviors and responses to stimuli. Infants born prematurely have not yet developed to the point that their initial inborn adaptive processes can effectively cope with the increased demands in their extra-uterine environment, hence, system disruptions can produce life threatening disorganization. In
addition, stressful environmental demands can affect and distort normal developmental processes leading to unwanted developmental pathways.

According to Als (1986) the initial extra-uterine environment should: (a) be made highly similar to the intrauterine environment the infant had to prematurely leave, and (b) then slowly and progressively be elaborated at a rate appropriate to the infant’s capabilities. Developmentally supportive care promotes auto-regulation, protects the infant from stress, and assists the infant to maintain stability and control. The guiding principle of developmental care is to facilitate infant interaction based on astute behavioral observation and to proceed with the interaction at a rate appropriate to decrease disorganization, conserve energy, and support self-regulation and stability within a nurturing environment that includes the family. Supporting these aspects of development should promote overall stability and infant health.

An approach for caregiving using the Newborn Individualized Developmental Care and Assessment Program (NIDCAP®) (Als, 1982) protects the infant from stress by promoting self-regulation, while environmental modifications such as dim lighting, incubator covers, and noise control reduce stressful stimuli (Als, 1982; Browne, 2004; Graven et al., 1992; Lawhon, G., 1997; Tribotti & Stein, 1992; White-Traut et al., 1994). An “in-tune” caregiver should provide supportive developmental positioning to promote fetal posture along with supportive handling and pacing of caregiving to prevent additional stress to the already compromised infant (DiPietro, Cusson, Obrian, Caughy, & Fox 1994; Horns, 1998; Peters, 1999; Ward-Larson, Horn, & Gosnell, 2004). Gentle touch,
non-nutritive sucking, flexed positioning, hands-on containment (supportively cradling with the hands), and nesting are interventions that help the preterm infant to maintain neurobehavioral stability and thermoregulatory control. In addition, periods of undisturbed rest are essential for growth and tissue healing.

Promotion of natural sleep-wake rhythms may be facilitated by cycling lights off for periods to induce longer phases of deeper sleep for the infant (Brandon, Holditch-Davis, Belyea, 2002; Brown, 2004; Thomas, 1995). When procedures must be done, gently assisting the preterm infant to an awake state by soft voice and gentle touch followed by containment and positioning, help the infant to manage stress and behavioral disorganization (Field, 1990; Harrison et al., 1996; Harrison, Roane, & Weaver, 2004; Lester, 1990; Modrin-Talbott, Harrison, Groer, & Younger 2003; White-Traut & Nelson, 1988, Zahr & Balian, 1995). Sedation and analgesia should be used as indicated for necessary procedures to ameliorate pain and stress (AAP, 2000; Anand, 1998; Acharya, Annamali, Taub, & Field, 2004; McNair, Ballantyne, Dionne, Stephens, & Stevens, 2004; Stevens, et al., 1999). If stress is left unhindered, the expense of biological adaptation and coping for the preterm infant may be costly as adaptive regulatory systems are underdeveloped and metabolic expenditures are high.

Using a NIDCAP® approach requires that a weekly neurobehavioral assessment be made by a NIDCAP® trained developmental specialist during a caregiving procedure to assess the infant’s competencies and vulnerabilities with care. The NIDCAP® specialist summarizes the infant’s neurobehavioral
responses and makes recommendations to structure caregiving to support the infant’s strengths and reduce self-regulatory vulnerability (Als et al., 2004). Support and recommendations are also given to parents. A nurturing and supportive environment that includes families is proposed to conserve energy and promote self-regulation of the autonomic, motor, and state subsystem functioning (Als et al., 2004; Brown, 2004; Lott, 1989; Merenstein, 1994; Peters, 1999; Peters 2001; VandenBerg, 1993). This developmentally supportive care of infants combined with support and encouragement for families, fosters a holistic approach to caregiving.

**Evidence Supporting Developmental Care in Preterm Infants**

There is a growing body of evidence indicating that individualized developmental care programs reduce stress and impact health outcomes for preterm infants. Als (1994) randomly assigned 38 singleton, medically fragile, preterm infants to experimental and control groups. The experimental group received individualized developmental care by nurses educated in infant behavior and caregiving for at least one NICU shift per 24 hours. After assessments of stress induced and regulatory behaviors, nurses promoted infant stability and competence with flexed positioning, supportive holding, structured sleep, feeding based on sleep/wake cycles, and parent support in nurturing and infant care. The control group received standard primary nursing care using incubator shielding, crib side chairs for parents, and 24-hour visitation.
The individualized developmental care intervention group showed significantly improved medical outcomes with decreased need for ventilation and supplemental oxygen, decreased intraventricular hemorrhage and pneumothoraces, less severe chronic lung disease, improved weight gain, earlier oral feedings, shorter hospital stays, and younger age at discharge. The experimental group also showed significantly improved neurodevelopmental behavior at two weeks of age and had improved mental and psychomotor developmental index scores at nine months adjusted age. Reducing stress improved both short-term and long-term health outcomes.

In a follow-up experimental study by Buehler, Als, Duffy, McAnulty and Liederman (1995), 24 low-risk preterm infants were randomly assigned to experimental individualized developmental care and standard care control groups within a NICU. Twelve healthy full term infants also served as a control group. At two weeks post-term, the experimental subjects showed autonomic, motor, and state regulation and attentional functioning (demonstrated by electrophysiology) comparable to the full-term controls and significantly better than the preterm controls. The authors concluded that individualized developmental care promoted normal neurodevelopmental functioning, improved lower frontal lobe activity and reduced attentional problems previously associated with preterm infants.

Other investigators have replicated similar findings using the NIDCAP® intervention model. Fleisher and associates (1995) used a similar randomized control \((n = 18)\), experimental \((n = 17)\) research design to investigate the effects
of an individualized developmental care program administered by specially trained personnel to very low birth weight (VLBW) infants throughout the duration of their hospital stay in a NICU. Similar to the findings by Als et al., 1994, Fleisher showed that the experimental group infants had fewer days on ventilator support, tolerated feedings earlier, and had increased weight gain, and fewer days in hospital. In addition, the reduction in hospital stay produced a cost savings of $128,670 per treatment baby.

In 1997, this same group of researchers expanded their research to investigate the impact of developmental care versus standard nursery care on narcotic and sedative requirements in VLBW infants (Heller, Constantinou, VandenBerg, Benitz, & Fleisher 1997). They found significantly lower sedation requirements among the most severely ill study infants receiving developmental care, and proposed that a reduction in stress had decreased the sedation requirements.

In a Swedish investigation by Westrup and colleagues (2000), researchers used a randomized, controlled trial to evaluate the effects of NIDCAP® (n = 12) or conventional care (n = 13) during NICU hospitalization on short-term outcome measures in VLBW infants. Infants in the intervention group were cared for in a separate room by specially trained nurses. Recommendations for developmental interventions were based on formal weekly NIDCAP® observations. The results of the study were that the NIDCAP® intervention group infants had: a shorter duration of ventilation (NS), and statistically significant decreases in supplementary oxygen duration. There also were trends of increased weight gain
and head growth, and younger age at discharge in the experimental group, although these findings were not statistically significant.

Most recently, researchers have employed strategies to directly measure the impact of NIDCAP® on neurobehavior, electrophysiology, and brain structure. Als and colleagues (2004) reported the findings of a randomized clinical trial on 30 preterm infants between 28-33 weeks gestational age at birth. Experimental group infants (n = 16) were enrolled in NIDCAP® care within 72 hours of birth and continued on the program throughout the duration of hospitalization. The experimental group infants showed: significantly better neurobehavioral functioning at 2 weeks and 9 months adjusted age (Prechtl Neurologic Examination, Assessment of Preterm Infant behavior (APIB), and Bayley II- Scale of Infant Development), enhanced coherence by EEG between frontal and occipital brain regions, and greater anisotropy of white matter using magnetic resonance with diffusion tensor imaging (suggesting higher white matter development). Correlation of the brain functional measures and structural measures showed that improved behavioral regulation was associated with more mature frontal structural development of the brain.

Consistent findings from the above studies indicate that developmental care interventions result in: improved short-term growth outcomes, decreased respiratory support, lowered incidence of chronic lung disease, decreased length and cost of hospital stay, and improved neurodevelopmental outcomes. Randomized, controlled clinical trials on developmental care have shown that an individualized developmental care approach decreases morbidity and
significantly improves post-discharge neurodevelopmental behavior of preterm infants (Als et al., 2004; Als et al., 1994; Becker, Grunwald, Moorman, & Stuhr, 1993; Buehler, Als, Duffy, McAnulty & Liederman, 1995). Moreover, none of the studies showed any harmful effects on the treatment group infants.

Although systematic reviews on developmental intervention programs suggest potential benefits of such programs (Lotas, & Walden, 1996), and no harmful effects, critics argue that only a few studies to date have used high quality randomized trials with consistent outcome measures (Symington & Pinelli, 2002). Further research is necessary on the impact of the NIDCAP® approach on parent involvement and parent outcomes. In addition, research is needed to evaluate the usefulness of NIDCAP® behavioral response measures with physiological parameters to identify characteristic patterns of stress response in preterm infants. The current study is one of the first studies to examine relationships between NIDCAP® behavioral responses and HRV as predictors of stress vulnerability and health outcomes in VLBW preterm infants.

Effects of Environment on Brain Structure and Function

Brain-behavior studies in animal models have helped to elucidate the role of early postnatal environment on endocrine responsiveness and later social development (Hood, Dreschel, & Granger, 2003). Meaney and colleagues (1988) studied the effects of an early postnatal environmental manipulation on behavioral and endocrine responses in newborn male Long-Evans rats. Neonatal rats in the experimental group were subjected to the stress of handling and
separation from mothers for fifteen minutes daily during the first three weeks of life. Subsequently, after the handling episode, both mothers and rat pups were returned to their cages without further environmental manipulation. The control litters were left undisturbed in their cages. Findings of this research were that the handled rat pups at all ages secreted less glucocorticoids in response to stress and had lower basal glucocorticoid levels than non-handled rats. The physiological mechanism underlying the lower glucocorticoid response was a permanent increase in glucocorticoid receptor (GR) concentration within the hippocampus in the handled rat pups.

The hippocampus is a critical region in the brain for glucocorticoid feedback inhibition over hypothalamic CRH synthesis. Glucocorticoids damage the neurons indirectly by disrupting energy metabolism, thus compromising the ability of the neuron to survive metabolic changes. Hypersecretion of glucocorticoids causes neuronal cell death within the hippocampus leading to a degenerative cognitive impairment. The non-handled aged rats showed higher spatial memory deficits as well as increased neuronal cell loss in comparison to the handled aged rats (Meaney et al., 1988). The diminished rate of hippocampal neuron loss in the aged handled rats is secondary to the lower cumulative lifetime exposure to glucocorticoids.

Investigations by Francis and associates (1999), Ladd et al. (2000), and Liu and colleagues (1997) provide further elucidation to the physiological mechanisms that impact stress in the newborn rat. In these studies, it was demonstrated that handling of rat pups resulted in changes in the mother-pup
interaction. Mothers of handled pups had shorter, but more frequent nesting bouts and demonstrated increased licking and grooming of pups with less passive posturing during nursing. Furthermore, offspring of the high licking-grooming mothers showed increased glucocorticoid feedback sensitivity and decreased hypothalamic CRH expression mediated by increased glucocorticoid receptor (GR) expression, suggesting a non-genomic behavioral transmission of individual differences across generations of rats.

Increased hippocampal GR mRNA expression, increased central benzodiazepine receptor levels in the central and basolateral nuclei of the amygdala, and decreased corticotropin-releasing factor mRNA in the paraventricular nucleus of the hypothalamus occurred in the offspring of high licking-grooming mothers (Francis et al., 1999; Ladd et al., 2000). Tactile stimulation through maternal licking and grooming regulates pup physiology and affects central nervous system development. More specifically, the effects of maternal care on the development of the stress system are thought to be mediated through changes in the levels of expression of specific genes in the brain that regulate stress response.

In an experiment by Francis, Diorio, Plotsky, & Meaney (2002) researchers set out to determine the effects of reversibility of behavioral, hormonal, and glucocorticoid receptor expression of maternal separation on offspring in Long-Evans rats. The rats were exposed to either handling (H) or maternal separation (MS) daily for the first two weeks of life. HPA responses to stress were significantly greater in MS control rats compared with H rats. At the
time of weaning, peri-pubertal control rats were reared in standard housing while the environmentally enriched rats were housed in larger cages with interconnecting burrows and novel toys. There were no group differences in HPA responses to stress among animals raised in the environmental enrichment program. In addition, the control MS rats were more fearful than those raised in the environment enrichment program. Thus, environmental enrichment reversed the effects of maternal separation on both HPA and behavior responses to stress. The authors posit a functional reversal of the neural and behavioral effects of early life adversity caused by maternal separation and HPA activation occurred because of environmental enrichment. There were no changes on the permanent effects of increased hypothalamic corticotropin-releasing factor (CRF) gene expression.

In a human study, Isaacs et al. (2000) compared a group of 11 adolescents who were former very low birth weight (VLBW) preterm infants born at less than 30 weeks gestation with age-matched controls, former term infants with normal neonatal courses. The purpose of the study was to explore the relationship between memory deficits and neuropathology using cognitive testing, parent questionnaires, and quantitative magnetic resonance imaging. The results showed that the children who had been VLBW babies had deficits in every day memory, by cognitive testing and parental report, and had a striking deficit in mathematics ability, especially numerical operations. In addition, in the former preterm, VLBW group there was a lower bilateral hippocampal volume
(p = 0.002), despite normal total intracranical volume and normal head circumference. Certainly, the exact mechanism for this finding is unknown. The authors speculate that inadequate nutrition may have been a causative factor. But, taken together with the findings of animal studies, it seems reasonable to postulate that the mechanism could involve the impact of early life experience on an immature stress system with related impact on brain structure and function.

**Maternal Stress**

The unexpected birth of a preterm infant is characterized by incomplete prenatal preparation and sudden separation from the newborn (Klaus, Kennel and Klaus, 1995). The newborn’s admission to the NICU leads to parental stress; fatigue, financial worries, separation and isolation from home and family, and may adversely affect the development of the parent-infant relationship (Beaton, 1984; Fife, 1985; Franck, Cox, Allen, & Winter, 2004; McGettigan et al., 1994; Montgomery, 1983; Scharer & Brooks, 1994; Snowdon & Kane, 1995; Vasquez, 1995). During such a situational and maturational crisis, stress may increase as personal and family roles become shifted (Battles, 1988; Tiller, 1995; Tiedje and Darling-Fisher, 1996; Vehvilainen-Julkunen, 1995; Walker and Montgomery, 1994). This stressful family situation may be communicated to the infant via the mother’s parenting style.

Stress also increases when the mother’s self-concept is diminished, as when parenting a preterm infant (Miles, 1989). Self-concept, the degree to which an individual perceives herself as being in control of forces affecting life, is
difficult for mothers to achieve during a preterm infant’s hospitalization (Pearlin, Lieberman, Menaghan, & Mullan, 1981; Miles & Holditch-Davis, 1995). Most mothers will not have the knowledge and skill to understand and deal with the special developmental and functional problems of prematurely born infants, and their anticipatory thoughts and related emotions are likely to make effective behavior towards their preterm infant even more difficult for them (Minde, Whitelaw, Brown, & Fitzhardinge (1983). Teaching mothers to read the “behavioral language” of their preterm infant can strengthen parental mastery and self-confidence, leading to a sense of control, competence, and empowerment (Brown, 2004; Dodds-Azzopardi & Chapman, 1995; Sokol, 1995; Miles, Holditch-Davis, & Shepherd, 1998). Stress may also be ameliorated through personal coping skills and social support (Kemp & Page, 1986; Ward, 2001). Additionally, family social networks, which normally serve as a major source of social support, are instrumental in helping parents to cope with the stress of their preterm newborn’s hospitalization.

Maternal attachment, beginning in pregnancy and continuing after birth, is an interactive process characterized by observable, quantifiable behaviors of maintaining close contact and experiencing gratification with the infant (DeWolff & van Ijzendoorn, 1997; Klaus, Kennel & Klaus, 1995; Mercer & Ferketich, 1990; Mercer & Ferketich, 1994). The resulting emotional bonds motivate a life-long commitment to caring for the child and are important in the development of psychobiological organization in infancy (Spangler et al., 1994). Maternal sensitivity and responsiveness to the infant promote a secure attachment by
enhancing security that the infant’s needs are satisfied (Gunnar, Broderson & Nachmias, 1996). With a premature birth, the infant is less able to participate in the attachment process (Harrison & Magill-Evans, 1996). The infant's appearance and behavior may be confusing and a source of stress for mothers (Brunssen & Miles, 1996). Subsequently, mothers may not learn to read the infant’s subtle cues and react appropriately to comfort their infant.

Although many view the transition to motherhood as a natural life-stage, it is not an instinctive process, especially for mothers of preterm infants. New mothers may lack experience and knowledge about handling and caring for their preterm infant and often rely solely on the support and education provided by the neonatal team (Carter-Jessop & Yoos, 1994, Kenner and Lott, 1990). These health care professionals are viewed as experts in the care of preterm infants and their families and are essential links to the establishment of successful parenting. It is imperative for health professionals to empower families through active caregiving and involvement in decision-making for their preterm infant.

Parenting Competency

In contrast to the ideal, parents may perceive little support for their parenting role during their preterm infant’s hospitalization in the NICU. Families of very low birth weight (LBW) infants are especially vulnerable to isolation and ineffective interactions as they have infants with unique health care needs who are often more temperamentally difficult, and present particular challenges for successful parenting (Brown & Fitsharding, 1983; Gennero, 1996; Medoff-Cooper
& Schraeder, 1982; Miles & Holditch-Davis, 1995; Minde, Whitelaw, Browne & Fitzhardinge, 1983; Shields-Poe & Pinelli, 1997). Parents may be coping with their own feelings of anxiety, grief, fear, helplessness, guilt, and depression (Field, 1998; Miles, 1989; Vasquez, 1995). These emotions may inhibit successful interactions with their infants.

Several teams of ethnographic researchers have investigated the dynamics of parent and health care provider communication in the NICU setting (Bass, 1991; Brunssen & Miles, 1996; Kenner & Lott, 1990; Scharer & Brooks, 1994). Common themes expressed by parents included: concerns about insufficient information regarding the infant’s medical condition and prognosis, lack of involvement in decision-making, the harshness of the NICU environment, lack of information on attachment and parenting, and frustration over inadequate preparation for discharge.

Mothers and fathers of preterm infants need spousal support, information from health care providers, and social support in order to master the parenting role (Kemp & Page, 1986; Kenner & Lott, 1990). Veteran parent supports through one-to-one parent interactions and parent led groups have been shown efficacious in facilitating parent-infant interaction and successful parenting (Lindsay et al., 1993; Roman et al., 1995). Supportive parenting is developed through interactions with others.

Family-centered care (also known as family-focused, family driven, family-friendly, and family-allied care) is a philosophy of care that requires mutuality in decision-making and goal setting between health care providers and families.
The hallmark is flexibility whereby the definition for “family-centered” is co-created with each new family-professional relationship (Winton and Bailey, 1997). The “Principles for Family-Centered Care,” established by a parent and physician group, are guidelines that promote involvement and advocacy for parents (Harrison, 1993). Family-centered interventions are especially needed to support the efforts of young parents, and grandparents to become active caregivers (Smith-Battle, 1997). According to Field, 1990, “the most cost-effective interventions for the ICU neonate may be the education of parents and the facilitation of their early interactions with infants” (as cited in DiPietro et al., 1994, p.61). In addition, strategies that include parents in providing care and comfort to their infants like massage, and skin-to-skin holding help parents to feel more involved and important to their infant’s recovery (Bond, 2002; Field, Hernandez-Reif, & Freedman, 2004). By promoting parental advocacy within a family-centered developmental model of care, parents are more likely to feel welcome and involved in the caregiving, and thus more confident in the provision of care and successful in their parenting role.

Preterm infants are especially vulnerable to stress as they have multiple and repeated exposures to vast amounts of noxious environmental stimuli at a time when they are not yet developmentally capable of successful adaptation. Accumulating evidence suggests that chronic stress leads to significant morbidity and prolonged hospitalization for the sick infant. Although the effects of stressors on neuroendocrine arousal, cardiovascular reactivity, and behavioral-cognitive effects have been demonstrated independently, there is a paucity of research on
the interactions of these response systems. In order to understand the complexity of the newborn stress response, it is essential to explore relationships among hormonal, cardiovascular, and behavioral indices of stress.

**Summary**

As previously stated, the interaction of HPA axis functioning with heart rate and behavioral patterns of response presents a comprehensive model for the study of the preterm stress response in the NICU setting. Identifying relationships between physiological and behavioral indicators of stress would provide important information for application to clinical practice. Clinicians with proficiency in the assessment and interpretation of stress indicators would be more likely to identify and support the infant’s adaptive capabilities to environmental stimuli, thereby, reducing metabolic expenditures and supporting growth, and recovery from illness. Furthermore, testing of supportive developmental approaches during caregiving is needed to evaluate the effectiveness of these interventions in ameliorating stress in preterm infants, facilitating positive maternal-infant interactions, and promoting subsequent parenting competency. Such knowledge is crucial to the understanding of the benefits of developmental interventions in improving outcomes for the tiniest and most vulnerable patients and their families.
Biobehavioral Model of Neonatal Stress Response

Although much knowledge has been gained regarding the interpretation of biological and behavioral responses to stress in preterm infants independently, the complexity of the interrelatedness of these responses within the context of the infant-caregiver interaction requires further investigation. The current study is based on a biopsychosocial model to investigate these patterns of relationships and the relationship between these patterns and infant health outcomes and parenting competency.

*Figure 2. Conceptual Model of Infant Reactivity to Nurse Caregiver Interaction and Infant and Parenting Outcomes*
The aims of the longitudinal study described are: (a) to examine hypothesized relationships between neuroendocrine responses, vagal tone, and behavioral responses within the context of nursing caregiving in the neonatal intensive care unit, (b) to test whether early patterns of hormonal, heart rate, and behavioral responses in the first few days of life are predictors of health outcomes for very low birth weight infants at the time of discharge, and (c) to identify maternal perceptions of the NICU environment, knowledge of infant responses, and the effects of these perceptions on maternal-infant interactions and subsequent parenting beliefs. The model depicted in Figure 2 shows the constructs to be examined in this study. Caregiver interaction is hypothesized to affect infant reactivity (cortisol, autonomic parameters, and behavioral responses), which, in turn, will predict infant health outcomes at the time of discharge (duration of oxygen therapy, duration of ventilation therapy, gestational age at full enteral feedings, gestational age at full oral feedings, and length of hospital stay).

It is likely that mothers that are supported within a developmental model of interaction with their preterm infant are more likely to facilitate the infant’s adaptive capacities to handle stress in the NICU setting, thereby, leading to improved infant outcomes. It is also plausible that providing developmental instruction through caregiver modeling may alleviate maternal stress and thus promote a more supportive and nurturing interaction with the infant, facilitating perceptions of competency in parenting.
Hypotheses

The cross-sectional hypotheses for this investigation are: 1 (a) there will be direct positive relationships between cortisol reactivity and autonomic parameters, (b) there will be direct positive relationships between cortisol reactivity and behavioral responses, (c) there will be direct positive relationships between HRV and behavioral responses. The longitudinal hypotheses are: 2 (a) infants with higher cortisol responses to handling will have poorer health outcomes than infants with lower cortisol reactivity, (b) infants with lower heart rate variability at rest will have poorer health outcomes than infants with higher heart rate variability, 3 (a) infants with higher behavioral distress will have poorer health outcomes than infants with lower distress, b) infants with higher cortisol levels post-handling (at 60 minutes) will have poorer health outcomes than infants with lower cortisol levels post-handling, 4 (a) mothers reporting higher stress will be less responsive to their infants than mothers with lower stress, and (b) mothers who are less responsive to their infants will rate themselves lower in parenting competency than mothers with high responsiveness.
Chapter 3: Methods

Setting

The study setting was a 31 bed level III tertiary referral Neonatal-Intensive Care Unit, located within the academic medical center of The Pennsylvania State University Children’s Hospital, in south central Pennsylvania. In January 1997, the implementation of a formal staff developmental care education program was given to all members of the neonatal team. The program was provided by an outside consulting team of nurses, physicians, and physical therapists with expertise and certification in newborn developmental care practice. Ongoing staff education and an active developmental care committee team approach have continued in this NICU to maintain a standard of care that provides a developmentally supportive environment for infants and their parents. The Institutional Review Boards (IRBs) of The Pennsylvania State University, University Park Campus and College of Medicine (Appendix A1 & A2) and The General Clinical Research Center (GCRC) (Appendix B) Hershey Campus approved the study. Informed consent was obtained from the mother of each study infant (Appendix C). The study was conducted at the Penn State University Children’s Hospital, Neonatal Intensive Unit (NICU) from January through December 2004 (See Timetable, Appendix D1). An undergraduate nursing student and medical student served as research assistants for the study.
Recruitment

The clinical head nurses, neonatologists, and neonatal nurse practitioners assisted in the identification of eligible infants and their mothers. From January 2004 through December 2004, infant subjects meeting the inclusion criteria and their mothers were recruited within the first 48 hours of admission to the neonatal intensive care unit (NICU). Mothers of infants eligible for participation were given a full verbal explanation regarding the study by the principal investigator (PI). The mother's support person and or family members were encouraged to participate in the consent process. At the conclusion of this discussion, the mother was asked to review the written consent form and discuss any concerns about participation with her support person.

A follow-up meeting was scheduled with the mother and support person within 24 hours of the initial meeting to answer questions and to complete the consent process. After maternal consent was obtained, a copy of the signed consent form and research abstract were placed in the infant’s crib side chart and a signed copy was given to the mother, as required by IRB policy.

Sample

Thirteen very low birth weight preterm infants born at The Penn State Milton S. Hershey Medical Center and admitted to the Neonatal Intensive Care Unit (NICU) of Penn State Children’s Hospital were included in the present study. The infant inclusion criteria for participation in the study were: singleton birth, postmenstrual age of less than 31 weeks gestation, and an existing umbilical catheter for the purpose of blood sampling. Umbilical catheters were neither
inserted nor kept in place because of the study. Infants were excluded from study participation because of congenital (chromosomal, malformational, or deformational) anomalies, known intraventricular-periventricular hemorrhage greater than a grade II hemorrhage, administration of narcotics and or sedatives to the infant prior to postnatal day 4 of life, reported maternal use of cocaine or heroine, or maternal illness preventing the ability to obtain informed consent. All infants were studied during the immediate intensive care phase between 4 and 5 postnatal days of life and were followed prospectively throughout the duration of their neonatal intensive care unit stay.

**Description of the Infant Sample**

The descriptive statistics of the study infants are presented in Tables 1 and 2. The sample consisted of 13 very low birth weight preterm infants with a mean postmenstrual age of 28 weeks gestation, range 22.5 to 30.6 weeks. The mean birth weight was 954 grams, range 446 grams (15.7 ounces) to 1530 grams (3 pounds, 5.9 ounces). Twelve babies (92%) were born to Caucasian mothers and 1 baby (8%) was born to an African American mother. Nine mothers (69%) received antenatal steroids (betamethasone) within 24 hours of delivery. Ten of the infants were born by cesarean delivery and 3 delivered vaginally. All of the infants had diagnoses of prematurity and mild to moderate respiratory distress. Although only three of 13 infants were on ventilators at the time of the observation, 10 of 13 infants required ventilator therapy during their NICU course. One infant died at 11 days of age. This subject was included for cortisol,
autonomic, and behavioral measures, but was not alive for inclusion at discharge (health outcomes) analyses.

*Table 1. Infant Characteristics at Birth (N = 13)*

<table>
<thead>
<tr>
<th>Infant Characteristic</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male (6)</td>
<td>Fem (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational Age (weeks)</td>
<td>22.5</td>
<td>30.6</td>
<td>28.0</td>
<td>2.72</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>446</td>
<td>1530</td>
<td>954</td>
<td>388</td>
</tr>
<tr>
<td>SNAP</td>
<td>0</td>
<td>20</td>
<td>8.54</td>
<td>5.88</td>
</tr>
</tbody>
</table>

*SNAP = Score for Neonatal Acute Physiology*

*Table 2. Infant Characteristics at Discharge (N = 12)*

<table>
<thead>
<tr>
<th>Infant Characteristic</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraventricular Hemorrhage</td>
<td>0</td>
<td>3</td>
<td>.54</td>
<td>.967</td>
</tr>
<tr>
<td>Nosocomial Sepsis</td>
<td>0</td>
<td>4</td>
<td>1.23</td>
<td>1.30</td>
</tr>
<tr>
<td>Days on Ventilation</td>
<td>0</td>
<td>60</td>
<td>12.67</td>
<td>21.28</td>
</tr>
<tr>
<td>Days on Oxygen</td>
<td>0</td>
<td>153</td>
<td>54.58</td>
<td>48.39</td>
</tr>
<tr>
<td>Retinopathy of Prematurity</td>
<td>0</td>
<td>5</td>
<td>1.25</td>
<td>1.87</td>
</tr>
<tr>
<td>Length of Hospital Stay (days)</td>
<td>31</td>
<td>159</td>
<td>71.5</td>
<td>38.27</td>
</tr>
<tr>
<td>Adjusted GA at discharge</td>
<td>34.5</td>
<td>46</td>
<td>37.87</td>
<td>2.88</td>
</tr>
</tbody>
</table>

*GA = Gestational Age*
Description of the Maternal Sample

The descriptive statistics for the mothers who participated in the study are presented in Table 3. The thirteen sample mothers participating in the study had a mean age of 25.4 (4.3) years, 92% received adequate prenatal care, 77% delivered by cesarean mode, 77% had involved partners, all denied use of alcohol or illicit drugs during the pregnancy, and 39% admitted to smoking during the pregnancy. One mother reported having a past history of alcohol addiction, and one mother reported occasional use of marijuana up to the week before delivery, however, her urine toxicology and the infant’s urine toxicology were negative within 72 hours of birth. For 85% of mothers this was their first infant requiring neonatal intensive care.

Table 3. Maternal Characteristics (N = 13)

<table>
<thead>
<tr>
<th>Maternal Characteristic</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>19</td>
<td>33</td>
<td>25.38</td>
<td>4.27</td>
</tr>
<tr>
<td>Partner involved</td>
<td>0</td>
<td>1</td>
<td>0.77</td>
<td>0.44</td>
</tr>
<tr>
<td>Gravida</td>
<td>1</td>
<td>9</td>
<td>3.00</td>
<td>2.67</td>
</tr>
<tr>
<td>Parity</td>
<td>0</td>
<td>8</td>
<td>1.85</td>
<td>2.67</td>
</tr>
<tr>
<td>Prenatal Care (PNC)*</td>
<td>0</td>
<td>2</td>
<td>1.92</td>
<td>0.28</td>
</tr>
<tr>
<td>Antenatal Steroids</td>
<td>0</td>
<td>1</td>
<td>0.31</td>
<td>0.48</td>
</tr>
<tr>
<td>Smoked during pregnancy</td>
<td>0</td>
<td>1</td>
<td>0.70</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*PNC was coded:
0 = no prenatal care,
1 = partial prenatal care,
2 = complete prenatal care
Measures

Cortisol

Blood samples (100 µl) were collected from an existing umbilical catheter for cortisol at baseline and at 30 and 60 minutes after the initiation of handing for morning (between 7a.m. – 11:50 a.m.) nursing care (see Procedural Timeline Figure 3). All specimens were labeled with a code identification number and transported to the Core Endocrine Laboratory at The Milton S. Hershey Medical Center, College of Medicine. Samples were centrifuged and plasma was stored at –80° C. Plasma samples were analyzed in duplicate using a highly sensitive immunoassay, Solid-Phase Radio-immuno-assay® manufactured by Diagnostic Products Co., LA, CA. The demonstrated functional sensitivity was 1.0 µg/dl, Interassay Precision was: Mean = 3.2 µg/dl CV = 9.3%, Mean = 22.9 µg/dl CV = 6.5%, and Intrassay Precision: Mean = 3.1 µg/dl CV = 4.8%, Mean = 20.0 µg/dl CV = 3.0%.

Salivary samples were not collected because in a pilot attempt to obtain saliva from a very low birth weight (VLBW) infant, the application of a small cotton wick into the buccal mucosa caused the infant to gag, and have a vagal response (drop in heart rate). In another attempt to aspirate saliva using a short blunt cut intravenous tube attached to a syringe, it was impossible to obtain the required 25 µl of saliva for cortisol analysis. The blood samples that were obtained via indwelling umbilical catheters did not cause the infants any distress.
Autonomic Parameters

As per standard neonatal intensive care unit procedure, all study participants were maintained on Hewlett Packard- Merlin (Waltham, MA USA) neonatal cardiorespiratory monitors with continuous and trend recording capabilities. Surface electrocardiogram (ECG) and transthoracic impedance
respiratory waveforms were obtained using standard lead II bipolar chest lead placement. On day 4 - 5 of life during the standard morning (between 7a.m. - 11:50 a.m.) nursing care (physical assessment, diaper change, reposition, replacement of boundaries) continuous measurements of infant autonomic parameters: heart rate (HR), respiratory rate (RR), oxygen-hemoglobin saturation (O₂ Sat) (Nellcor 3000) were recorded. Using the trend monitor function, the mean values of HR, RR, O₂ Sat values were computed for each minute and recorded throughout the pre-actual- and post-observation phases.

On day 4 - 5 of life and again at one-month postnatal age, 45 - 60 minutes of ECG R-wave data were obtained for intervals during periods of quiet/deep sleep in the late afternoon or early evening hours. To assure that these data were collected during periods of undisturbed rest, behavioral observations of sleep state and movement were simultaneously recorded. In addition, to minimize the artifact known to occur with random movements, the data acquisition time was scheduled to be performed 30 - 60 minutes after the last handling episode and during a time when there was minimal activity around the infant’s crib space. If the infant was on enteral feedings, the ECG recordings were obtained 45 minutes to 1 hour after the feeding was completed.

Using a portable calibrated data acquisition system (National Instruments) the electrocardiogram (ECG) data were obtained from the synchronous R wave output of the Merlin monitor at a sampling frequency of 1000 Hz. Through an analogue-digital converter, this output was logged into a data acquisition program (BioBench, National Instruments, Austin, TX) and stored in a password protected
database file. The waveforms were later digitized and analyzed using a
customized software package to perform heart rate variability (HRV) spectral
analysis (MindWare HRV module, MindWare Technologies LTD, Westerville,
OH). The analysis software was configured to compute the spectral outputs in
time mode at 120-second segments of data (see Figure 4). In the first 4 subjects
there were technical difficulties with the configuration of the ECG sensitivity and
output to the data acquisition program, resulting in data loss and data exclusion
for these subjects for analyses involving HRV.

**Figure 4:** Example of pre-selected segment of ECG data for spectral analysis with
MindWare HRV software showing identification of R waves for spectral analysis.

The MindWare HRV software uses the fast Fourier transform technique to
compute the spectral frequency outputs. The frequency parameters for spectral
analysis were set according to the method established by Andriessen et al. (2003)
for preterm neonates as follows: very low frequency (VLF) band range: 0.003 - 0.029 u/msec\(^2\), low frequency (LF) band range: 0.03 - 0.199 u/msec\(^2\) and the high frequency (HF) band range: 0.2 - 2.0 u/msec\(^2\). Each pre-selected segment was reviewed to assure that the data were artifact free and that the R-wave was properly identified for each cardiac cycle. The LF and HF power were expressed as normalized units (e.g. nLF = LF/ total power X 100%) and the LF/HF ratio was calculated to estimate sympathovagal balance. Because the fast Fourier transform method is known to have a limited accuracy of power estimation in the VLF frequency range with segments of short duration, only the LF, HF, and LF/HF ratio were used as the variables of interest for the analyses.

**Infant Behavior**

Simultaneous real-time behavioral data were recorded using a tripod stabilized Sony 700X HandyCam\textsuperscript{®} audio/digital video recorder camera. Infant sleep/wake cycles, facial activity, stress signals (finger splay), and motor responses were recorded during a ten-minute baseline, during the nurse-infant interaction, and at thirty minutes post-interaction. The behavioral observation was recorded on day 4 - 5 of life at the same time cortisol and autonomic parameters (HR, RR, O\textsubscript{2} saturations) were measured.

The Newborn Individualized Developmental Care and Assessment Program (NIDCAP\textsuperscript{®}) by Als, 1986 was used as a framework for the observation and coding of behavioral data during the infant-nurse interaction. The NIDCAP\textsuperscript{®} observation includes use of the NIDCAP\textsuperscript{®} check sheet, or The Naturalistic
Observation of the Newborn (Appendix E1) to record the presence or absence of 85 different behaviors comprising autonomic reactivity, motor function, state of sleep/wakefulness, and self-regulatory and interactive capabilities (Als, 1986). All observations were recorded in 2-minute increments using the NIDCAP® check sheet. Using the behavioral data sheets, the newborn’s behavior was later quantified for total frequency per domain into each of three subsystems: autonomic behaviors, motor behaviors, and state behaviors (Pressler & Hepworth, 2002). The principal investigator and research assistant video recording the observation also logged field notes on behavioral stress responses (motor, state, attentional, and facial activity) to extraneous environmental stressors (noise, personnel movement around the crib space) as these were happening outside the wide-angle view of the video camera. These field notes were later transcribed onto the NIDCAP® check sheet for quantification. The interrater agreement for the observational field notes was 92%.

For all infants, the observed nurse-infant interaction included: a brief physical exam with auscultation of heart and breath sounds, changing the infant’s diaper and providing skin care, and repositioning the infant with the aid of supportive positioning devices to facilitate containment and “nesting” within boundaries. The nurse’s use of developmental care comforting techniques including: non-nutritive sucking, hands on containment- firm supportive placement hands on the infant’s head and back, use of soft voice to awaken infant prior to handling, allowing recovery “time-outs”, facilitative tucking-supporting the infant in a flexed posture, placement of soft boundaries around the
infant, and re-covering of the incubator to promote a darker, quieter environment were documented on the NIDCAP® check sheet.

Maternal Stress

A packet of questionnaires containing a Parent Survey (Appendix E2), a Parent Stress Scale: NICU (PSS: NICU) (Miles, Funk, & Carlson, 1993) (Appendix E3), and a Family Environment Scale (FES) (Moos, 1984) (Appendix E4) was administered to mothers during the first week of admission to the NICU to determine: baseline assessments of developmental care knowledge and use, perceptions regarding stress and family and staff relationships, and social support. Completed questionnaires and audit forms were stored in a locked cabinet in the office of the principal investigator.

The Parental Stressor Scale: NICU (PSS-NICU) is a 50 item self report scale used to assess parental anxiety and reactivity to stressors in four dimensions: (a) infant appearance/behavior (21 items), (b) parental role: parents interaction with the baby (11 items), (c) environment: sights and sounds in the NICU (5 items), and (d) relationships: staff behavior and communications (13 items). The parent is asked to rate each item on a 5-point Likert-type scale from “not experienced” (0) or “not stressful” (1) to “extremely stressful” (5).

The mean subscale scores are calculated for each dimension, as well as the mean total stress score representing the overall stress level. In North American studies, the scale has demonstrated high test-retest reliability, with reported Cronbach’s alpha coefficients ranging from .73 to .92 for the subscales and .87 for the total scale, and construct validity with statistically significant correlations (Miles,
Funk, & Carlson, 1993; Miles, Funk, & Kasper, 1992; Shields-Poe & Pinelli, 1997).

For this study, the alpha coefficient ranged from .81 - .91.

Family Environment Scale (FES) (Moos, 1984) is an instrument designed to evaluate the social and environmental characteristics of families. It measures the domains of Relationship, Personal Growth, and System Maintenance. *Relationship* assesses the degree of commitment, help, and support family members provide for one another and the extent to which family members are encouraged to act openly and express feelings directly. *Personal Growth* appraises the extent to which family members are assertive and self-sufficient, and the degree of interest and participation in political, social, intellectual, cultural and recreational activities. Form R, a shortened version with 40 items, measures an individual’s perceptions of their conjugal or nuclear family environments. The respondent scores each item according to whether it is true or false for her family using a five-point scale (strongly agree, agree, neutral, disagree, and strongly disagree). The authors and other researchers have established content, construct, and concurrent validity for the instrument. The Cronbach’s alpha for each of 10 subscales ranges from .61 to .78, and test-retest reliability is reported as ranging from .73 - .86 (Sawin and Harrigan, 1995). For this sample the alpha coefficient ranged from .72 - .92. The results of the mean composite scores for the Family Environment Scale (FES) are shown in Figure 5.
Maternal Sensitivity and Parenting Competency

At one month of age, a pre-scheduled videotaping session was performed of mother and infant during a regularly scheduled contact time in the evening. The nurse assigned to provide care for the infant rated the maternal-infant interaction using the B-CHAPS (Boston City Hospital Assessment of Parental Sensitivity) (Appendix E5). Additionally, at one month of age (or at the time of discharge if that preceded the one month of age time period) mothers were asked to complete the NICU Parental Beliefs Scale (PBS) (Appendix E6) a questionnaire designed to assess maternal perceptions of parenting. These ratings were done simultaneously.
The **Boston City Hospital Assessment of Parental Sensitivity (B-CHAPS)** is a behavioral-interaction instrument evaluating the mother’s sensitivity to baby cues and responses and competence in caregiving. Internal consistency is reported as .61 - .87 and inter-rater reliability as .57 - .72. (Cole, 1986). The authors of the **Parental Beliefs Scale** are currently analyzing test-retest reliability and construct validity. The alpha coefficient for this sample was .79 - .82.

**Health Data**

The principal investigator completed the **Score for Neonatal Acute Physiology (SNAP)**, a measure of severity of illness (Richardson, Gray, McCormick, Workman, & Goldman, 1993), for each infant from chart data on the third postnatal day of life. The mean and standard deviation (SD) SNAP score at 72 hours of age for the study sample was 8.5 (5.9) with a range of scores from 0 - 20. A one-way analysis of variance to compare for differences between males and females showed there were no differences between groups, F (1,12) = 0.28, p = 0.60. A three category variable was created to group cases by range of predicted mortality (PM) as suggested by the authors of the SNAP (Richardson et al., 1993): SNAP 0 - 9 (PM = 0 - 33%), SNAP 10 - 19 (PM = 6 - 42%), and SNAP > 19 (PM = 52 - 68%). It is noteworthy that the infant with a SNAP of 20 in the present study died at 11 days of age secondary to complications from extreme prematurity (acute oliguria and arrhythmia secondary to a high serum potassium). The frequency distribution of SNAP grouping for this sample is
presented in Figure 6, suggesting mild to moderate mortality risk for the remaining 12 infants of the sample.

*Figure 6. Frequency Distribution of SNAP by severity group*

<table>
<thead>
<tr>
<th>SNAP Group</th>
<th>Number of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>snap=0-9</td>
<td>7</td>
</tr>
<tr>
<td>snap=10-19</td>
<td>5</td>
</tr>
<tr>
<td>snap=20-30</td>
<td>1</td>
</tr>
</tbody>
</table>

*Chart-audits* (Appendix E7) on involvement and developmental care instruction given by nurses were completed prospectively each week during the infant’s hospitalization. All 13 mothers completed survey packets during the first week to 10 days of their newborn’s admission to the NICU. Fifty-four percent of mother respondents reported feeling very involved in their infant’s care, 39% reported feeling somewhat involved in their infant’s care, and 8% reported not feeling involved in their infant’s care. Mother respondents reported receiving instruction on the following standard developmental care principles within the first week of NICU admission: gentle touch (77%), talking softly (62%), supportive positioning (31%), midline positioning (0%), comforting (46%), holding (39%),
swaddling (15%), non-nutritive sucking (15%), kangaroo care (15%), containment (31%), nesting (15%), decreasing noise (39%), dimming lights (23%), covering the incubator (46%), infant states and behavioral cues (8%).

Infant outcomes were recorded weekly on the chart audit form including: the amount of time on ventilator support, the duration of oxygen therapy, the time to advancement of enteral feedings, the postmenstrual age (gestational age at delivery plus days since birth) at the time of advancement to oral feedings in the amount of 150 ml per kilogram per day, the frequency and severity of nosocomial infections, the incidence and severity of retinopathy of prematurity, and the number of days of hospital stay.

**Statistical Analyses**

Data were entered into the Statistical Package for the Social Sciences (SPSS®), version 11.5 database by the principal investigator and a research assistant. Descriptive statistics (frequencies, means, medians and scatter plots) were computed for all parent and infant variables. All variables were checked for outliers and normality before analysis. Pearson Correlation Coefficients were used to measure the strength of association between continuous variables of interest, and Spearman Rank Coefficients were used to compare relationships between non-parametric categorical variables. For all analyses, alpha was set at 0.05, and two-tailed tests were used. Hierarchical regression was done to assess the relative contribution of (predictors) change in cortisol, HRV, and behavioral stress cues to infant morbidity variables (ventilation, oxygen therapy, and length of hospital stay).
Chapter 4: Results

The results of the descriptive and hypothesis testing analyses are presented in this section. The descriptive statistics showing the characteristics: mean, standard deviation, range and/or frequency of the demographic variables first are presented. Next, the results of the primary analyses are presented for each hypothesis including: correlational analyses of the infants’ cortisol level and reactivity, autonomic parameters (heart and respiratory rates), and behavioral responses to caregiving. Then, the correlation analyses for the relationships between the infant responses and outcome variables are presented followed by hierarchical regression. Next, the correlation analyses of the maternal variables (stress, family environment, maternal sensitivity and parenting competency) are presented, which show the relationship between maternal sensitivity and parenting competency. Last, the results of the secondary analyses are presented, which show the relationships between morbidity and infant health outcomes and infant patterns of response to nurse caregiver handling.
Primary Analyses

Cortisol

The mean (SD) baseline plasma cortisol level for the total sample was 16.9 (7.7) mcg/dl. There were no significant differences in baseline cortisol levels between groups related to: mode of delivery (vaginal versus cesarean) ($p = 0.14$), level of acuity by SNAP index ($p = 0.37$), presence of chorioamnionitis in the mother ($p = 0.95$), gestational age at birth less than 28 weeks ($p = 0.09$), or presence of extremely low birth weight status ($\leq 750$gms) ($p = 0.47$). Male infants had lower baseline cortisol levels 14.6 (2.6) mcg/dl than females 19.2 (10.4) mcg/dl, but this difference was not statistically significant ($p = 0.33$). In infants whose mothers received antenatal steroids (betamethasone), the mean baseline cortisol was lower 13.1(4.0) mcg/dl than those who did not receive antenatal steroids 22.6 (11.8) mcg/dl, but this difference was not significant ($p = 0.29$).

To index the change in cortisol in response to caregiving, difference scores were computed by subtracting the $T_1$ cortisol (Prestimulus) from the $T_2$ cortisol (30 minutes after initiation of care) and subtracting $T_2$ from the $T_3$ cortisol (60 minutes after initiation of care). A total change score also was computed subtracting $T_1$ cortisol from $T_3$ cortisol. In addition, area under curve (AUC) at ground (AUC g) and change from ground to sample 2 (AUC 2), change to sample 3 (AUC 3), and change for all (AUC all) were calculated using the method described by Pruessner, Kirschbaum, Meinlschmid, & Hellhammer (2003). The levels for cortisol and calculations for AUC appear in Table 4.
Table 4. Plasma Cortisol Values (mcg/dl) (N = 13)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁ (Prestimulus)</td>
<td>6.2</td>
<td>36.0</td>
<td>16.9</td>
<td>7.7</td>
</tr>
<tr>
<td>T₂ (30 min after care)</td>
<td>7.3</td>
<td>35.4</td>
<td>15.3</td>
<td>7.7</td>
</tr>
<tr>
<td>T₃ (60 min after care)</td>
<td>5.2</td>
<td>37.1</td>
<td>12.5</td>
<td>8.0</td>
</tr>
<tr>
<td>Difference T₂ minus T₁</td>
<td>-5.2</td>
<td>4.7</td>
<td>-1.0</td>
<td>2.9</td>
</tr>
<tr>
<td>Difference T₃ minus T₂</td>
<td>-3.3</td>
<td>3.2</td>
<td>-2.9</td>
<td>3.5</td>
</tr>
<tr>
<td>Difference T₃ minus T₁</td>
<td>-10.7</td>
<td>3.2</td>
<td>-3.8</td>
<td>4.2</td>
</tr>
<tr>
<td>AUC ground</td>
<td>312.5</td>
<td>1812.5</td>
<td>694.04</td>
<td>384.17</td>
</tr>
<tr>
<td>AUC 2</td>
<td>-397.5</td>
<td>142.5</td>
<td>-135.63</td>
<td>147.41</td>
</tr>
<tr>
<td>AUC 3</td>
<td>-2147.5</td>
<td>-369.5</td>
<td>-1151.63</td>
<td>495.91</td>
</tr>
<tr>
<td>AUC all</td>
<td>-3947.5</td>
<td>-697.5</td>
<td>-1998.29</td>
<td>868.77</td>
</tr>
</tbody>
</table>

AUC = area under curve

AUC ground = (( (2nd sample + 1st sample) * time of first sample ) /2 ) + 
            ( ( (3rd sample + 2nd sample) * time of second sample ) /2 ).

AUC₂ = AUCincrease_sample2 = AUCground –
       (Time₁baseline * time of sample 2-time of sample 1).

AUC₃ = AUCincrease_sample3 = AUCground –
       (Time₁baseline * time of sample 3-time of sample 2).

AUC all = AUCincrease_all_samples = AUCground -
         ((Time₁baseline * time of sample 3-time of sample 2).
Hypothesis 1a: Cortisol Levels in Relation to Autonomic Parameters

The hypothesis was that there would be direct positive relationships between cortisol reactivity and autonomic parameters. To describe the general pattern of relationships, bivariate correlations were computed between cortisol reactivity and autonomic responses (heart and respiratory responses to care and several parameters of HRV). Scatter plots for significant correlations are presented in Appendix D2.

The results of the bivariate correlations between cortisol reactivity and autonomic parameters are shown in Table 5. There was a strong negative association between cortisol response \((T_2-T_1)\) and the pre-handling respiratory rate \((r (12) = -0.76, p = 0.004)\). Infants with a greater change in cortisol had lower baseline respiratory rates. In addition, the cortisol response \((T_2-T_1)\) was positively correlated with the peak change in respiratory rate from baseline through post-care (60 minutes after the initiation of caregiving) \((r (12) = 0.59, p = 0.044)\). Cortisol AUC 2 was negatively correlated with heart rate at 30 minutes after the initiation of handling \((r (12) = -0.63, p = 0.03)\) and with resting mean heart rate \((r (7) = -0.70, p = 0.05)\). Cortisol reactivity was positively associated with the LF/HF ratio \((r (7) = 0.68, p = 0.06)\), the sympathovagal balance measure of HRV. These findings demonstrate direct relationships between cortisol reactivity and autonomic parameters.
Table 5. Cortisol Reactivity and Autonomic Parameters (N = 13)

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Correlation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^1)Cortisol T2-T1 → Resp Rate T1</td>
<td>-.756</td>
<td>.004***</td>
</tr>
<tr>
<td>(^1)AUC 2 → Resp Rate T1</td>
<td>-.687</td>
<td>.014**</td>
</tr>
<tr>
<td>(^1)Cortisol T2-T1 → Change RR</td>
<td>.589</td>
<td>.044**</td>
</tr>
<tr>
<td>(^1)AUC 2 → Heart rate T2</td>
<td>-.634</td>
<td>.027**</td>
</tr>
<tr>
<td>(^1)AUC 2 → Mean resting heart rate+</td>
<td>-.703</td>
<td>.052*</td>
</tr>
<tr>
<td>(^1)Cortisol T2-T1 → LF/HF (HRV)+</td>
<td>.682</td>
<td>.063*</td>
</tr>
</tbody>
</table>

\(^1\)Pearson Product Moment Correlation
AUC2 = area under curve for change from baseline to sample 2
+ N = 8

Hypothesis 1b: Cortisol Levels in Relation to Behavioral Responses

The hypothesis was that there would be direct positive relationships between cortisol reactivity and behavioral responses. The results of the bivariate correlations between cortisol reactivity and NIDCAP® behavioral responses are shown in Table 6. There was a direct positive association between cortisol reactivity and behavioral stress cues, such that infants with higher cortisol levels demonstrated a higher frequency of visceral stress cues (gag, hiccup, yawn) \((r (12) = 0.62, p = 0.03)\), facial distress cues \((r (12) = 0.64, p = 0.02)\), and motor stress cues \((r (12) = 0.60, p = 0.04)\) in response to handling during nurse caregiving. These findings support the hypothesis.
Table 6. Cortisol and Behavior (N = 13)

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Correlation Coefficient (r)</th>
<th>p-value (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T^1Cortisol T2-T1 → Visceral cues</td>
<td>.625</td>
<td>.030**</td>
</tr>
<tr>
<td>T^1AUC 2 → Visceral cues</td>
<td>.557</td>
<td>.060*</td>
</tr>
<tr>
<td>T^1AUC 2 → Facial cues</td>
<td>.644</td>
<td>.024**</td>
</tr>
<tr>
<td>T^1AUC all → Motor stress cues</td>
<td>.600</td>
<td>.039**</td>
</tr>
</tbody>
</table>

^1Pearson Product Moment Correlation

| AUC 2 = area under curve change from baseline to second sample |
| AUC all = area under curve change from baseline for all samples |

Heart Rate Variability Change from 4 - 5 Days to 1 Month

Due to difficulties with the data acquisition program (Biobench) as previously described, only a sub-sample of 8 infants had “clean” data suitable for heart rate variability (HRV) spectral analysis. The results of the power spectral analysis of the R-R interval series using fast Fourier transform analysis software (MindWare HRV) on day 4 - 5 of life (Observation Rest = Ob rest) are presented in Table 7. The mean (SD) HRV indices for the sample were: heart rate 149 (13) bpm, interbeat interval (IBI) 403 (33) msec, LF power 7.7 (7.0) u/msec^2, HF power 3.3 (3.6) u/msec^2, Total power 11 (10.5) u/msec^2, nLF 71(7)%, nHF 28.8 (7)%, and LF/HF ratio 2.7(1.1).
Table 7. HRV Spectral Power Values (Ob Rest) (N = 8)

<table>
<thead>
<tr>
<th>R-R interval series</th>
<th>Range</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Heart Rate (bpm)</td>
<td>35.23</td>
<td>134.33</td>
<td>169.56</td>
<td>149.45</td>
<td>12.70</td>
</tr>
<tr>
<td>Mean IBI (msec)</td>
<td>92.82</td>
<td>353.85</td>
<td>446.67</td>
<td>403.91</td>
<td>32.76</td>
</tr>
<tr>
<td>LF power (u/msec$^2$)</td>
<td>23.21</td>
<td>0.54</td>
<td>23.75</td>
<td>7.739</td>
<td>6.96</td>
</tr>
<tr>
<td>HF power (u/msec$^2$)</td>
<td>11.66</td>
<td>0.26</td>
<td>11.92</td>
<td>3.30</td>
<td>3.64</td>
</tr>
<tr>
<td>Total power (u/msec$^2$)</td>
<td>34.87</td>
<td>0.80</td>
<td>35.67</td>
<td>11.04</td>
<td>10.53</td>
</tr>
<tr>
<td>Normalized LF pwr (nLF %)</td>
<td>20.51</td>
<td>62.48</td>
<td>82.99</td>
<td>71.19</td>
<td>7.04</td>
</tr>
<tr>
<td>Normalized HF pwr (nHF %)</td>
<td>20.51</td>
<td>17.01</td>
<td>37.52</td>
<td>28.81</td>
<td>7.04</td>
</tr>
<tr>
<td>LF/HF Ratio</td>
<td>3.21</td>
<td>1.67</td>
<td>4.88</td>
<td>2.70</td>
<td>1.09</td>
</tr>
</tbody>
</table>

The results of the power spectral analysis of the R-R interval series using fast Fourier transform analysis software (MindWare HRV) at one month of age (Mo Rest) are presented in Table 8. The mean (SD) for the heart rate variability (HRV) indices for the sample were: heart rate 148(9) bpm, interbeat interval (IBI) 406(25) msec, LF power 25(18.0) u/msec$^2$, HF power 8.8(9.4) u/msec$^2$, Total power 34(26.7) u/msec$^2$, nLF 78(9)%, nHF 22(9)%, and LF/HF ratio 4.3(2.3).
Table 8. HRV Spectral Power Values (Mo Rest) (N = 8)

<table>
<thead>
<tr>
<th>R-R interval series</th>
<th>Range</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Heart Rate (bpm)</td>
<td>28.22</td>
<td>131.62</td>
<td>159.84</td>
<td>148.33</td>
<td>9.01</td>
</tr>
<tr>
<td>Mean IBI (msec)</td>
<td>80.49</td>
<td>375.38</td>
<td>455.87</td>
<td>405.93</td>
<td>25.48</td>
</tr>
<tr>
<td>LF power (u/msec$^2$)</td>
<td>49.41</td>
<td>4.39</td>
<td>53.80</td>
<td>24.98</td>
<td>18.10</td>
</tr>
<tr>
<td>HF power (u/msec$^2$)</td>
<td>29.20</td>
<td>0.54</td>
<td>29.75</td>
<td>8.81</td>
<td>9.39</td>
</tr>
<tr>
<td>Total power (u/msec$^2$)</td>
<td>78.61</td>
<td>4.94</td>
<td>83.55</td>
<td>33.79</td>
<td>26.74</td>
</tr>
<tr>
<td>Normalized LF pwr (nLF %)</td>
<td>24.58</td>
<td>64.40</td>
<td>88.98</td>
<td>77.87</td>
<td>8.80</td>
</tr>
<tr>
<td>Normalized HF pwr (nHF %)</td>
<td>24.58</td>
<td>11.02</td>
<td>35.60</td>
<td>22.13</td>
<td>8.80</td>
</tr>
<tr>
<td>LF/HF Ratio</td>
<td>6.26</td>
<td>1.81</td>
<td>8.07</td>
<td>4.26</td>
<td>2.26</td>
</tr>
</tbody>
</table>

Paired samples T-tests were computed to compare differences between heart rate variability (HRV) at observation rest (Ob Rest) on day 4 - 5 of life and HRV at one month (Mo Rest) of age. Complete longitudinal data for comparison from both measurement times were only available on 6 subjects. The analysis demonstrated an increase in HF power spectral output from 3.6 to 9.1 u/msec$^2$ ($t(5) = 1.8, p = 0.13$), and an increase in total power from 12.1 to 36.3 u/msec$^2$ ($t(5) = 2.5, p = 0.05$).
Hypothesis 1c: Heart Rate Variability (HRV) in Relation to Stress Behaviors

The hypothesis was that there would be direct positive relationships between HRV and behavioral responses. The results of the bivariate correlations between HRV at rest on day 4 - 5 (Ob Rest) and behavioral responses at day 4 - 5 are shown in Table 9. HRV indices were highly positively associated with behavioral stress cues from the NIDCAP ® behavioral composites. Infants with higher low frequency (LF), high frequency (HF), and total power spectral output had higher gross motor movement (arms/legs), higher attentional signaling (fuss, cry) and greater facial distress cues (brow bulge, eye squeeze).

Table 9. HRV (Ob Rest) and Behavioral Responses (N = 8)

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Correlation Coefficient (r)</th>
<th>p-value (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF power → Motor cues</td>
<td>.817</td>
<td>.013**</td>
</tr>
<tr>
<td>HF power → Motor cues</td>
<td>.794</td>
<td>.019**</td>
</tr>
<tr>
<td>Total power → Motor cues</td>
<td>.814</td>
<td>.014**</td>
</tr>
<tr>
<td>LF power → Attentional cues</td>
<td>.727</td>
<td>.041**</td>
</tr>
<tr>
<td>HF power → Attentional cues</td>
<td>.715</td>
<td>.046**</td>
</tr>
<tr>
<td>Total power → Attentional cues</td>
<td>.727</td>
<td>.041**</td>
</tr>
<tr>
<td>LF power → Total stress cues</td>
<td>.867</td>
<td>.005***</td>
</tr>
<tr>
<td>HF power → Total stress cues</td>
<td>.846</td>
<td>.008***</td>
</tr>
<tr>
<td>Total power → Total stress cues</td>
<td>.865</td>
<td>.006***</td>
</tr>
<tr>
<td>LF power → Facial cues</td>
<td>.741</td>
<td>.035**</td>
</tr>
<tr>
<td>HF power → Facial cues</td>
<td>.734</td>
<td>.038**</td>
</tr>
<tr>
<td>Total power → Facial cues</td>
<td>.743</td>
<td>.035**</td>
</tr>
</tbody>
</table>

** Pearson Product Moment Correlation

***p<.01, **p<.05, *p<.10
Hypothesis 2a: Cortisol Reactivity and Health Outcomes

The hypothesis was that infants with higher cortisol responses to caregiving would have poorer health outcomes than infants with lower cortisol reactivity. To identify patterns of cortisol response to caregiving, mean cortisol values were compared in relation to the timing of caregiving. The mean (SD) cortisol levels after the initiation of care by nurses (see Figure 7) were 15.3 (7.7) mcg/dl (30 minutes) and 12.5 (8.0) mcg/dl (60 minutes) respectively. The serial mean values of cortisol before and at 30 and 60 minutes after care are depicted in Figure 6. Ten of the 13 infants (77%) had a decline in cortisol at 30 minutes after the initiation of handling, and nine of 13 (69%) infants had a decline in cortisol levels at 60 minutes after the initiation of care. These changes were

Figure 7. Change in Plasma Cortisol with Caregiving (N = 13)
significant when compared by 1 factor (time) (T₁, T₂, T₃) repeated measures analysis of variance (ANOVA), F (1,12) = 16.5, p = 0.002. The effect of gender on change in cortisol was analyzed using a 2-group gender by time repeated measures ANOVA (T₁, T₂, T₃). There was a within subjects effect for time, F (1,12) = 12.1, p = 0.001, and no significant main effect for gender, F (1,12) = 0.97, p = 0.35.

The mean (SD) duration of the infant-nurse interaction was 10 (3.2) minutes. The characteristics of this interaction included: auscultation of heart and breath sounds, changing the infant’s diaper, repositioning the infant, providing gentle oral care and non-nutritive sucking with a pacifier, and placing the infant within nestled boundaries. The duration of the infant-nurse interaction showed no relationship with cortisol reactivity (r (12) = -0.09, p = 0.77).

The results of the bivariate correlations between cortisol reactivity and the infant outcome variables are shown in Table 10. Higher reactivity to handling was associated with older gestational age for establishment of feedings and longer time on ventilation and oxygen. Direct and positive relationships were demonstrated between higher cortisol reactivity and poorer health outcomes.

A hierarchical regression model was used to determine the relative contribution of SNAP, cortisol reactivity, HF, and behavioral distress to infant respiratory health outcomes. SNAP was entered in the first step, followed by change in cortisol (AUC 2) and HF at one month (HF₂) in the second step, and followed by behavioral distress variables (visceral cues) in the final step. The model was not a significant predictor of ventilation duration, F (4,7) = 5.0, p = 0.17.
Table 10. Cortisol Reactivity and Health Outcomes (N = 12)

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Correlation Coefficient (r)</th>
<th>p-value (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{1}$AUC 2 $\rightarrow$ GA at full enteral feedings</td>
<td>.637</td>
<td>.035**</td>
</tr>
<tr>
<td>$^{1}$AUC 2 $\rightarrow$ GA at full oral feedings</td>
<td>.538</td>
<td>.088*</td>
</tr>
<tr>
<td>$^{1}$AUC 2 $\rightarrow$ Vent days</td>
<td>.646</td>
<td>.032**</td>
</tr>
<tr>
<td>$^{1}$AUC 2 $\rightarrow$ O$_2$ days</td>
<td>.586</td>
<td>.058*</td>
</tr>
<tr>
<td>$^{1}$AUC 2 $\rightarrow$ Sepsis</td>
<td>.472</td>
<td>NS</td>
</tr>
<tr>
<td>$^{1}$AUC2 $\rightarrow$ ROP</td>
<td>.367</td>
<td>NS</td>
</tr>
<tr>
<td>$^{1}$AUC2 $\rightarrow$ GA at discharge</td>
<td>.527</td>
<td>.096*</td>
</tr>
<tr>
<td>$^{1}$AUC2 $\rightarrow$ LOS</td>
<td>.498</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^{1}$Pearson Product Moment Correlation  
$^{2}$Spearman Rank Correlation  
$^{2}$Spearman Rank Correlation  
AUC 2= area under curve change from ground to second sample  
ROP = Retinopathy of Prematurity  
GA = Gestational age  
LOS = length of stay

Hypothesis 2b: Heart Rate Variability and Health Outcomes

The hypothesis was that infants with lower heart rate variability at rest would have poorer health outcomes than infants with higher heart rate variability. To measure relationships between HRV and health outcomes, the HF band was chosen as the variable of interest because it reflects the influence of cardiac baroreceptor reflexes and is representative of vagal tone. Results of correlations between HRV and outcomes are depicted in Table 11.
**Table 11. HRV and Health Outcomes (N = 8)**

<table>
<thead>
<tr>
<th>Relationship</th>
<th>r</th>
<th>p-value</th>
<th>Relationship</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>±HF → GA at oral feeds</td>
<td>−.759</td>
<td>.048**</td>
<td>±HF → GA at oral feeds</td>
<td>−.584</td>
<td>.129</td>
</tr>
<tr>
<td>±HF → O₂ days</td>
<td>−.121</td>
<td>.795</td>
<td>±HF → O₂ days</td>
<td>−.639</td>
<td>.088*</td>
</tr>
<tr>
<td>±HF → Vent days</td>
<td>−.287</td>
<td>.533</td>
<td>±HF → Vent days</td>
<td>−.749</td>
<td>.032**</td>
</tr>
<tr>
<td>±HF → Sepsis</td>
<td>−.074</td>
<td>.862</td>
<td>±HF → Sepsis</td>
<td>−.371</td>
<td>.366</td>
</tr>
<tr>
<td>±HF → ROP</td>
<td>−.018</td>
<td>.969</td>
<td>±HF → ROP</td>
<td>−.753</td>
<td>.031**</td>
</tr>
<tr>
<td>±HF → GA at discharge</td>
<td>−.700</td>
<td>.080*</td>
<td>±HF → GA at discharge</td>
<td>−.641</td>
<td>.087*</td>
</tr>
<tr>
<td>±HF → LOS</td>
<td>−.040</td>
<td>.932</td>
<td>±HF → LOS</td>
<td>−.736</td>
<td>.037**</td>
</tr>
</tbody>
</table>

1 Pearson Product Moment Correlation
2 Spearman Rank Correlation

HF spectral output at day 4 - 5 (Ob Rest) was inversely related to gestational age at oral feeding ($r(7) = -0.76, p = 0.048$); infants with higher vagal tone established successful oral feeding at a younger gestational age. HF spectral power at one month ($HF_2$) was highly negatively associated with days on ventilation ($r(7) = -0.75, p = 0.03$), retinopathy of prematurity ($r(7) = -0.75$, $p = 0.03$), and length of hospital stay ($r(7) = -0.74, p = 0.04$), demonstrating that infants with higher vagal tone had lower morbidity. In addition, infants with higher vagal tone were discharged at a younger gestational age ($r(7) = -0.64, p = 0.09$), although this was not statistically significant. A hierarchical multiple regression
procedure was used to determine the relative contributions of SNAP, cortisol, autonomic reactivity and behavioral responses on each of the health outcomes: ventilation days, oxygen days, and length of stay. To test the contribution of each of the variables after controlling for the effects of the prior, variables were entered in three steps; SNAP was entered first, followed by Cort \( T_3 \) and HF\(_2\) in the next step, followed by behavioral distress variables (visceral cues, facial cues) in the final step. If any of the predictor variables were non-significant, they were dropped from subsequent models. The \( R^2 \) for each of the final regression models (and change in \( R^2 \) from the first model to the final model) was: 

- **Ventilation Days** \( R^2 = 0.81 \) (increased from 0.68); 
- **Oxygen Days** \( R^2 = 0.98 \) (increased from 0.85); 
- **Length of Stay** \( R^2 = 0.98 \) (increased from 0.84). 

The betas and \( p \) values for the significant regression models appear in Tables 12, 13, and 14.

HF\(_2\) did not show a unique contribution to the variance predicting ventilation duration or oxygen duration. (Tables 12, and 13). However, in the multiple regression model (Table 14) of length of stay regressed on SNAP, Cort \( T_3 \), HF\(_2\), and Facial Cues, there was a negative correlation between HF\(_2\) (vagal tone at one month of age) and length of stay (LOS), \( F \left( 4,7 \right) = 42, t = -3.7, p = 0.03 \). The results demonstrate that HF\(_2\) showed unique contribution to the variance predicting length of stay and supporting the hypothesis that lower vagal tone may be a useful prognosticator of poor health outcome.
Table 12.
Regression of Ventilation Days on Cortisol, Autonomic Reactivity, and Behavior

<table>
<thead>
<tr>
<th>Final Model</th>
<th>$R^2$</th>
<th>Standardized Beta</th>
<th>SE</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snap</td>
<td>0.81</td>
<td>.605</td>
<td>2.41</td>
<td>1.31</td>
<td>.283</td>
</tr>
<tr>
<td>Cort T3</td>
<td></td>
<td>-.096</td>
<td>1.08</td>
<td>.210</td>
<td>.847</td>
</tr>
<tr>
<td>HF$_2$</td>
<td></td>
<td>-.156</td>
<td>.791</td>
<td>-5.19</td>
<td>.640</td>
</tr>
<tr>
<td>Visceral Cues</td>
<td></td>
<td>.395</td>
<td>14.8</td>
<td>1.27</td>
<td>.293</td>
</tr>
</tbody>
</table>

Snap = Score for Acute Neonatal Physiology
HF$_2$ = High frequency spectral output at 1 month of age

Table 13.
Regression of Oxygen Days on Cortisol, Autonomic Reactivity, and Behavior

<table>
<thead>
<tr>
<th>Final Model</th>
<th>$R^2$</th>
<th>Standardized Beta</th>
<th>SE</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snap</td>
<td>0.98</td>
<td>.308</td>
<td>2.30</td>
<td>1.48</td>
<td>.235</td>
</tr>
<tr>
<td>Cort T3</td>
<td></td>
<td>.151</td>
<td>0.70</td>
<td>1.08</td>
<td>.359</td>
</tr>
<tr>
<td>HF$_2$</td>
<td></td>
<td>-.518</td>
<td>0.97</td>
<td>-2.94</td>
<td>.061*</td>
</tr>
<tr>
<td>Facial Cues</td>
<td></td>
<td>.760</td>
<td>4.10</td>
<td>4.50</td>
<td>.020**</td>
</tr>
</tbody>
</table>

Snap = Score for Acute Neonatal Physiology
HF$_2$ = High frequency spectral output at 1 month of age

Table 14.
Regression of Length of Stay on Cortisol, Autonomic Reactivity, and Behavior

<table>
<thead>
<tr>
<th>Final Model</th>
<th>$R^2$</th>
<th>Standardized Beta</th>
<th>SE</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snap</td>
<td>0.98</td>
<td>-.045</td>
<td>1.86</td>
<td>-.212</td>
<td>.846</td>
</tr>
<tr>
<td>Cort T3</td>
<td></td>
<td>.484</td>
<td>.571</td>
<td>3.37</td>
<td>.044**</td>
</tr>
<tr>
<td>HF$_2$</td>
<td></td>
<td>-.674</td>
<td>.798</td>
<td>-3.71</td>
<td>.034**</td>
</tr>
<tr>
<td>Facial Cues</td>
<td></td>
<td>.767</td>
<td>3.37</td>
<td>4.43</td>
<td>.021**</td>
</tr>
</tbody>
</table>

Snap = Score for Acute Neonatal Physiology
HF$_2$ = High frequency spectral output at 1 month of age

***p<. 01, **p<. 05, *p<. 10
Hypothesis 3 a: Behavioral Responses and Health Outcomes

The hypothesis was that infants with higher behavioral distress would have poorer health outcomes than infants with lower distress. The results of the bivariate correlations between behavioral responses and the health outcome variables are shown in Table 15. Facial distress cues (brow bulge and eye squeeze) demonstrating infant discomfort were positively associated with morbidities including: longer duration of oxygen \( r (11) = 0.57, p = 0.05 \) and a higher incidence of nosocomially acquired infections \( r (11) = 0.58, p = 0.04 \). In contrast, self-consoling behaviors were negatively associated with retinopathy of prematurity \( r (11) = -0.64, p = 0.03 \).

**Table 15. Behavior and Health Outcomes (N = 12)**

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Correlation Coefficient (r)</th>
<th>p-value (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>¹Facial cues → O₂ days</td>
<td>.570</td>
<td>.053*</td>
</tr>
<tr>
<td>¹Visceral cues → Vent days</td>
<td>.532</td>
<td>.075*</td>
</tr>
<tr>
<td>²Facial cues → Sepsis</td>
<td>.575</td>
<td>.040**</td>
</tr>
<tr>
<td>²Self-consoling → ROP</td>
<td>-.641</td>
<td>.025**</td>
</tr>
<tr>
<td>¹Self-consoling → GA at full oral feedings</td>
<td>-.238</td>
<td>NS</td>
</tr>
<tr>
<td>¹Self-consoling → GA at discharge</td>
<td>-.332</td>
<td>NS</td>
</tr>
<tr>
<td>¹Self-consoling → LOS</td>
<td>-.504</td>
<td>.095*</td>
</tr>
</tbody>
</table>

¹Pearson Product Moment Correlation
²Spearman Rank Correlation

ROP = Retinopathy of Prematurity
GA = Gestational age
LOS = Length of stay

*\( p < 0.10 \)
**\( p < 0.05 \)
***\( p < 0.01 \)
Models of hierarchical regression were done to explore the relative contribution of SNAP, cortisol, autonomic reactivity and behavioral responses on health outcomes as previously described. Ventilation duration was regressed on SNAP, Cort T₃, HF₂, and Visceral Cues; oxygen duration was regressed on SNAP, Cort T₃, HF₂, and Facial Cues; and length of stay was regressed on SNAP, Cort T₃, HF₂, and Facial Cues (Tables 12, 13, & 14). The regression models showed no relationship between behavioral distress (visceral cues) and ventilation duration. However, greater facial distress cues during care were associated with longer time on oxygen, F (4, 7) = 44.5, t = 4.5, p = 0.02, and longer length of stays, F (4, 7) = 42.0, t = 4.4, p = 0.02, demonstrating that facial distress cues showed unique contribution in predicting time on oxygen therapy and length of stay and supporting the hypothesis that higher behavior distress may be a useful prognosticator of health outcome.

**Hypothesis 3b: Cortisol Post-Care and Health Outcomes**

The hypothesis was that infants with higher cortisol post-handling (at 60 minutes) would have poorer health outcomes than infants with lower cortisol post-handling. The results of the bivariate correlations between cortisol post-care and the health outcome variables are shown in Table 16. Cortisol levels at T₃, 60 minutes after the initiation of care, were positively associated with poorer respiratory outcomes. The infants with higher cortisol levels at T₃, (one hour post-handling) required longer oxygen therapy (r (11) = 0.66, p = 0.02), longer ventilator support (r (11) = 0.63, p = 0.03), and longer hospital stays (r (11) = 0.75, p = 0.005).
Models of hierarchical regression were done to explore the relative contribution of SNAP, cortisol, autonomic reactivity and behavioral responses on health outcomes as previously described. The regression models (Tables 12, 13) suggest that cortisol at 60 minutes after initiation of care (Cort T₃) did not contribute to the variance in predicting ventilation or oxygen duration. However, in the regression model of length of stay regressed on SNAP, Cort T₃, HF₂, and Facial Cues (Table 14), higher cortisol at recovery was associated with longer length of stays, F (4,7) = 42.0, t = 3.4, p = 0.04. This suggests that Cort T₃ showed a unique contribution to the variance in predicting length of stay and supporting the hypothesis that higher cortisol at recovery may be a useful prognosticator of health outcome.

### Table 16. Cortisol Post-Care and Health Outcomes (N = 12)

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Correlation Coefficient (r)</th>
<th>p-value (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ᵃCortisol T₃ → O₂ days</td>
<td>.663</td>
<td>.019**</td>
</tr>
<tr>
<td>ᵃCortisol T₃ → Vent days</td>
<td>.628</td>
<td>.029**</td>
</tr>
<tr>
<td>ᵃCortisol T₃ → LOS</td>
<td>.746</td>
<td>.005***</td>
</tr>
</tbody>
</table>

³Pearson Product Moment Correlation

**Los = Length of stay**

***p<.01, **p<.05, *p<.10
Hypothesis 4a: Maternal Stress and Sensitivity

Maternal Stress

The hypothesis was that mothers reporting higher stress would be less responsive to their infants than mothers with lower stress. The results of the mean composite scores for the Parent Stress Scale: NICU (PSS: NICU) are shown in Figure 8. Mothers reported their feelings of stress regarding the neonatal intensive care unit environment (sights and sounds), preterm newborn’s behavior, relationship with their newborn, communication with the staff, and overall general stress.

Figure 8. Frequency Distribution of Parent Stress Scale: NICU (N = 13)
Maternal Sensitivity

The results of the Boston City Hospital Assessment of Parental Sensitivity (B-CHAPS) mean scores of mothers’ sensitivity as rated by staff nurses within one week of discharge from the NICU are listed in Table 17.

Table 17. Descriptives of B-CHAPS (N = 12)

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Range</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent appears to modify behavior in response to infant's cues</td>
<td>12</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>3.25</td>
<td>1.22</td>
</tr>
<tr>
<td>Parent modifies behavior in response to nurse’s instructions</td>
<td>11</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>4.00</td>
<td>1.48</td>
</tr>
<tr>
<td>Parent talks to infant</td>
<td>12</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>4.17</td>
<td>1.12</td>
</tr>
<tr>
<td>Parent appropriately tries to bring infant to alert state</td>
<td>12</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>3.42</td>
<td>1.51</td>
</tr>
<tr>
<td>Parent effectively soothes infant</td>
<td>12</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>3.92</td>
<td>1.31</td>
</tr>
<tr>
<td>Parent is attentive to infant</td>
<td>12</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>4.50</td>
<td>1.17</td>
</tr>
<tr>
<td>Parent takes delight in infant</td>
<td>12</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>4.25</td>
<td>1.29</td>
</tr>
<tr>
<td>Parent appears comfortable with touching infant</td>
<td>11</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>4.64</td>
<td>0.67</td>
</tr>
<tr>
<td>Parent appears comfortable with holding infant</td>
<td>9</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>4.33</td>
<td>1.66</td>
</tr>
<tr>
<td>Parent appears comfortable with feeding infant</td>
<td>7</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>3.57</td>
<td>1.90</td>
</tr>
<tr>
<td>Parent appears comfortable with bathing infant</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>2.67</td>
<td>2.52</td>
</tr>
<tr>
<td>BCHAPS average score</td>
<td>12</td>
<td>4.3</td>
<td>.4</td>
<td>4.7</td>
<td>3.98</td>
<td>1.20</td>
</tr>
</tbody>
</table>
The rating scale is as follows: 0 = not observed, 1 - 2 = low sensitivity, 3 = moderate sensitivity, and 4 - 5 = high sensitivity. Nurses' rated mothers as highly sensitive regarding their infant on: modifying behavior in response to instructions, talking softly, attentiveness, taking delight in infant, touching, and holding. In addition, the average sensitivity score for all composites was high.

**Hypothesis 4b: Maternal Parenting Competency**

The results of the mean composite scores for the NICU Parental Belief Scale (PBS) on mothers' self-reports of competency in caring for their preterm infant on: knowledge, interacting with their infants, feelings/emotional needs, and total self-competency are shown in Figure 9.

*Figure 9. Frequency Distribution of NICU Parental Belief Scale (PBS) (N = 13)*
Hypothesis 4 (a) was that mothers reporting higher stress would be less responsive to their infants than mothers with lower stress and (b) mothers that are less responsive to their infants will rate themselves lower in parenting competency than mothers with high responsiveness. There was no relationship between maternal stress PSS: NICU and maternal sensitivity B-CHAPS. Additionally, maternal stress levels did not predict nurse-rated maternal competency of handling at one month of age, thus hypothesis 4 (a) was not supported. However, there was a strong correlation between nurses’ observational ratings of mothers as cue responsive (B-CHAPS) and mothers’ self-ratings of parenting competency (PBS) \( r(11) = 0.65, p = 0.031 \). In addition, there was a strong direct association between B-CHAPS and the premature infant behavior composite of PBS \( r(11) = 0.82, p = 0.002 \). Because of the lack of correlation between predictor and outcomes variables, hierarchical regression was not performed. The correlational analyses demonstrate support of hypothesis 4 (b) regarding nurses’ observational ratings of sensitivity and mothers’ self-reported parenting beliefs.
Secondary Analyses

Analyses were performed to demonstrate the utility of the Score for Neonatal Acute Physiology (SNAP) as a morbidity predictor in the current sample of very low birth weight infants. In addition, the patterns of heart rate and respiratory rate responses to handling are described to delineate a more comprehensive pattern of responses to the handling associated with caregiving.

SNAP and Health Outcomes

The results of the bivariate correlations between SNAP and health outcomes are shown in Table 18. SNAP was highly positively associated with the following morbidity outcomes: time on oxygen ($r (11) = 0.9$, $p = 0.0$),

Table 18. SNAP and Health Outcomes ($N = 12$)

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Correlation Coefficient ($r$)</th>
<th>p-value (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$Snap→ GA at full oral feeds</td>
<td>.642</td>
<td>.024***</td>
</tr>
<tr>
<td>$^1$Snap→ $O_2$ days</td>
<td>.902</td>
<td>.000***</td>
</tr>
<tr>
<td>$^1$Snap→ Vent days</td>
<td>.796</td>
<td>.002***</td>
</tr>
<tr>
<td>$^2$Snap→ Sepsis</td>
<td>.318</td>
<td>.290</td>
</tr>
<tr>
<td>$^2$Snap→ ROP</td>
<td>.684</td>
<td>.014**</td>
</tr>
<tr>
<td>$^1$Snap→ GA at discharge</td>
<td>.673</td>
<td>.016**</td>
</tr>
<tr>
<td>$^1$Snap→ LOS</td>
<td>.909</td>
<td>.000***</td>
</tr>
</tbody>
</table>

Snap = Score for Acute Neonatal Physiology

$^1$ Pearson Product Moment Correlation

$^2$ Spearman Rank Correlation

**p<.01, **p<.05, *p<.10

time on ventilator therapy ($r (11) = 0.8$, $p = 0.002$), length of hospital stay ($r (11) = 0.9$, $p = 0.0$), and retinopathy of prematurity ($r (11) = 0.7$, $p = 0.01$).
SNAP showed a moderate positive association with the following developmental outcomes: the gestational age at full oral feeding \((r (11) = 0.64, p = 0.02)\), and the gestational age at discharge from the NICU to home \((r (11) = 0.67, p = 0.02)\). In contrast, there was no relationship between SNAP and the rate and severity of acquired infection \((r (11) = 0.32, p = 0.29)\).

**Patterns of Response in Relation to Caregiving**

The change in respiratory rate in relation to nursing care is depicted in Figure 10. The mean (SD) respiratory rate at \(T_1\) was 49 (8) breaths per minute (bpm), \(T_2\) was 57 (17) bpm, and at \(T_3\) was 51 (14) bpm. The mean change in respiratory rate was 20 (18) bpm. There was a wide variability of response between subjects, some infants became tachypneic, while others had minimal elevation in breathing rate followed by slowing of breathing at \(T_3\).

*Figure 10. Change in Respiratory Rate with Caregiving (N = 13)*
The change in heart rate in relation to care is depicted in Figure 11. The mean (SD) heart rate was 153 (10) beats per minute (bpm) at T₁, 146 (9) bpm at T₂, and 148 (13) bpm T₃. The mean change in heart rate was 14 (7) bpm. Using a 2 group SNAP categorization (SNAP = 0 - 9, SNAP = 10 - 20) by time repeated measures ANOVA (T₁, T₂, T₃) between and within subject effects were estimated. There was a between subjects effect, F (1,12) = 9.2, p = 0.01, and no within subjects effect, F (1,12) = 1.9, p = 0.18. The higher morbidity SNAP (10 - 20) group infants demonstrated significantly lower mean heart rates pre-handling and during both the 30 and 60 minute post-handling phases than the lower SNAP group infants.

Figure 11. Change in Heart Rate with Caregiving (N = 13)
Chapter 5: Discussion

The findings presented in the previous section were conducted to address the a priori hypotheses based on the review of the literature and the theoretical model. In this section, the interpretation and relevance of the principal findings are discussed. The discussion includes a summary of the results of the correlation and regression analyses. In addition, the hypotheses are discussed based on the findings of the present study and previous research. Strengths and limitations of the study also are offered as well as directions for future research.

Principal Study Findings

Hypothesis 1 (a) was that there would be direct positive relationships between cortisol reactivity and autonomic parameters. There was a positive direct association between change in cortisol from prestimulus (T₁) to 30 minutes post-handling (T₂) and peak change in respiratory rate with handling. Those infants who had a rise in cortisol with handling had a higher increase in respiratory rate (p = .04) associated with caregiving. In addition, the pattern of relationships between the biological variables (higher cortisol and elevated respiratory rate) identified infants with higher physiological instability (tachypnea) to the handling associated with caregiving. In addition, cortisol reactivity was positively associated with sympathovagal balance (LF/HF ratio); higher rise in cortisol with handling was associated with a higher LF/HF ratio suggesting lower
vagal tone. These results support the first hypothesis by demonstrating direct positive relationships between cortisol and autonomic stress measures. This study is one of the few human studies to date on cortisol and autonomic responses to handling in VLBW preterm infants during the first week of life.

In contrast, there was an inverse relationship between cortisol reactivity (AUC 2) and the heart rate at 30 minutes after the initiation of handling. Higher positive cortisol change was associated with lower heart rate at 30 minutes after the initiation of care. This finding is inconsistent with the expected pattern of increased heart rate with cortisol reactivity. A plausible explanation is derived from the Synactive Theory (Als, 1986) suggesting that more vulnerable infants stressed beyond their adaptive capabilities are unable to maintain autonomic stability and will have more significant drops in heart rate, or bradycardia.

Hypothesis 1 (b) was that there would be direct positive relationships between cortisol and behavioral responses. The analyses conducted on the relationships between cortisol and behavioral composites for stress cues demonstrated that higher cortisol reactivity was positively associated with a higher frequency of visceral stress cues (gag, yawn, hiccup), facial distress cues (brow bulge, eye squeeze), and total motor stress cues (startle, finger splay). These results support the hypothesis by demonstrating a direct and positive relationship between biological and behavioral responses. The NIDCAP® observation program purports that visceral reactivity is a normal manifestation of stress response in the preterm infant. Visceral cues represent autonomic instability, suggesting that the infant requires a “time-out” from the handling
stress and should be comforted with supportive positioning and “hands on”
containment to allow for recovery. If stress ensues beyond the infant's innate
ability to adapt and cope with the stress, system instability occurs resulting in an
attenuation of stress cues, loss of muscle tone (flaccidity), and inactivity followed
closely thereafter with bradycardia (drop in heart rate) and or apnea (cessation of
breathing) (Als, 1986). It is likely that sicker, less stable infants would display
lower levels of behavioral responses to handling, as was demonstrated in this
study.

The findings of lower cortisol levels after caregiving in this study are
similar to findings by Acolet and colleagues (1993) who measured plasma
cortisol, adrenaline, and noreadrenaline in a sample of 11 male preterm infants
(mean postnatal age = 20 days) and eight stable controls matched for gestational
and postnatal age. Blood samples were obtained 45 minutes prior to and one
hour after the completion of 20 minutes of gentle massage by an experience
neonatal nurse. The results of the study demonstrated a consistent decline in
cortisol in massage treated infants. The research by Acolet is consistent with
demonstrating statistically significant declines in cortisol after gentle massage in
stable growing preterm infants.

The impact of sound on preterm infant stress system is a well-documented
phenomenon (Bremmer, Byers, & Kiehl, 2003). The hectic morning NICU activity
may have contributed to higher plasma cortisol levels prior to handling.
In addition, by using a morning collection time, the samples may reflect a higher basal arousal than if they had been collected in the afternoon. However, circadian patterns of cortisol have not been clearly demonstrated in preterm infants (de Weerth, 2003). The study infants in this sample demonstrated high levels of arousal and distress in the pre-handling phase, perhaps related to environmental sound and activity. The behavioral observations and hormone samplings for the present study took place during the morning hours when there were higher numbers of personnel in the neonatal intensive care unit (NICU). The high activity levels of staff movement and talking associated with NICU rounds were noted to produce higher infant behavioral distress responses during the pre-handling phase as recorded on the NIDCAP® observation record. Thus, the hypothesis was supported that there were direct positive relationships between cortisol and behavioral variables.

Hypothesis 1 (c) was that there would be a direct positive association between heart rate variability and stress behaviors. The study findings demonstrated that there were strong positive correlations between heart rate variability (HRV) indices and behavioral stress measures. The premature infants with higher HRV expressed a greater number of motor, attentional, facial, and total stress cues as quantified with the NIDCAP® observation method, suggesting a strong relationship between autonomic regulation and stress behaviors. As described by Porges, 1992, higher physiological variability as measured by HRV represents the individual’s neural feedback mechanisms and capacity for neurobehavioral control and regulation. Thus, it would be expected
that the greater the physiological variability, the greater the range of behavioral responses. These findings support the hypothesis by demonstrating direct positive relationships between heart rate variability and behavioral responses.

Hypothesis 2 (a) was that infants with higher cortisol responses to handling would have poorer health outcomes than infants with lower cortisol reactivity. The study findings showed that a rise in cortisol to handling was associated with older gestational ages for establishment of feedings and longer time on ventilation and oxygen. Although the correlational analyses support the hypothesis that higher cortisol reactivity is associated with poorer health outcomes, no significant relationships were demonstrated in the regression models. Thus, cortisol reactivity was not a unique predictor of health outcomes above and beyond other measures.

Of note, the duration of the infant-nurse interaction in the present study had no relationship with change in cortisol from baseline through post-handling phases of care. Using the NIDCAP® approach it was evident, based on the behavioral observation and physiological indices, that the quality of the nurse-infant interaction (cue-responsiveness), rather than the duration of the interaction was important in infant stability and stress reduction. The behavioral observation during caregiving showed that as nurses used cue-based developmentally sensitive care, the infants quieted, displayed fewer stress behaviors, and returned to sleep post-care.

Hypothesis 2 (b) was that infants with lower heart rate variability at rest would have poorer health outcomes than infants with higher heart rate variability.
The spectral density outputs reported in the present study are comparable to those reported by Andriessen, et al., 2003 on a group of 10 stable preterm infants, 27-33 weeks gestational age (GA) who were studied once in the first postnatal week (between day 2-8) of life. Like the research reported by Andriessen and colleagues, in the current study the LF/HF ratio (2.7) was much higher in premature infants than the normative data reported in adults (1 - 1.8). These patterns undoubtedly reflect the immaturity of the parasympathetic nervous system and predominance of the sympathetic system in preterm infants.

In the present study, the second measurement of spectral power obtained at one month of age demonstrated a significant increase in high frequency power (suggesting increased vagal tone) and total spectral power when compared with the initial measurement on postnatal day 4 - 5.

At one-month of age, the HRV measurement in the infants of this study (mean GA of 32 and 3/7 weeks) still showed a predominantly sympathetic system influence. This finding is consistent with studies of vagal tone in preterm and full term infants showing that maturation of the parasympathetic nervous system (as indexed by cardiac vagal tone) typically occurs between 35 - 37 weeks of gestation (Porges, 1992, Doussard- Roosevelt, McClenny, Porges 2001). It would have been ideal in this investigation to have a third measure of HRV at 36 weeks adjusted gestational age. However, the majority of infants were discharged home or transferred back to their referral hospital by that time.

In addition, strong inverse relationships were identified between the HF component of HRV, representative of vagal tone, and health outcomes. Infants
with higher HF spectral power on postnatal day 5 of life established full oral feeding at an earlier GA. In addition, higher HF power at one month of age (32 and 3/7 weeks GA) was associated with younger GA at discharge, suggesting higher regulatory and behavioral competence in these infants. These findings support the second hypothesis by demonstrating a relationship between higher HRV and improved health outcomes. The findings from the present study are consistent with those reported by Doussard-Roosevelt (2001) in a longitudinal study of 20 VLBW infants. Investigators obtained weekly recordings of mean heart rate and cardiac vagal tone at 33, 34, and 35 weeks GA while in the NICU. The neonatal physiological measure of RSA, representative of vagal tone, was correlated with social competence on the behavior child checklist at school age, thus demonstrating a direct relationship between vagal tone and social competence.

The results from the HRV measurement obtained at one-month of age in the present study showed a strong negative correlation between high frequency (HF) spectral output and respiratory morbidity measures. Infants with higher HF at rest had shorter durations of oxygen and ventilation therapies, and had shorter hospital stays in both correlation and regression analyses. These findings support the second hypothesis by demonstrating that lower heart rate variability is associated with poorer health outcomes.

An additional interesting example from the current study that also lends support to this hypothesis is described in the following case. An infant born at 30 and 6/7 weeks GA with a history of mild perinatal depression (SNAP = 6) was
found to have particularly low total spectral output (0.8 u msec$^2$) identified by repeated measurement on day 5 of life. The principal investigator alerted the neonatologist on service caring for the infant of the findings, because of known reports in the literature that have identified low HRV spectral output as a risk factor for sudden infant death syndrome (SIDS) and death (Veerappan, 2000; Verklan, 2004). After the observation of low HRV was noted, this infant remained on cardiorespiratory monitoring and careful nursing observation. On postnatal day 11 of life the infant had a serious apparent life-threatening event (ALTE) also known as “near SIDS” that required a vigorous resuscitation by the NICU team. The infant appeared to have a complete recovery and no sequelae from this event. However, the infant’s hospital course was significant for slow establishment of oral feedings requiring consultation from feeding specialists, and resulted in a prolonged hospital stay. This was highly unusual for an infant who did not have an oral aversion because he never required a breathing tube. In addition, there was no evidence of hemorrhage or other pathology on the cranial ultrasound study to account for the delay in successful oral feeding. This case suggests that HRV may have clinical utility in the early identification of infants who may be at particular risk for feeding and/or other learning difficulties. Spectral power analysis of HRV is a promising, non-invasive technology that may be useful in the identification of at-risk, very low birth weight preterm infants. Through early identification of at-risk infants, early intervention programs can be implemented to potentially enhance neurodevelopmental and cognitive functioning.
Hypothesis 3 (a) was that infants with higher behavioral distress would have poorer health outcomes than infants with lower distress. The infants in the present study that exhibited higher facial cues indicative of distress demonstrated poorer health outcomes including: a higher incidence of nosocomially acquired infections, a longer need for oxygen therapy (in both correlation and regression modeling), and a longer hospital stay (in both correlation and regression modeling). In contrast, more robust infants demonstrated higher self-consoling behaviors that were negatively associated with duration of hospitalization and retinopathy of prematurity. These findings support the hypothesis that infants with higher behavioral distress would have poorer health outcomes than infants with lower distress. In addition, the findings suggest that behavioral indicators of distress (visceral cues and facial cues) are useful in the identification of infants with higher vulnerability to the impact of environmental stimulation.

Hypothesis 3 (b) was that infants with higher cortisol levels (60 min) post-handling would have poorer health outcomes than infants with lower cortisol levels post-handling. The correlation findings were that infants with higher cortisol levels one hour after the initiation of care required more days of ventilation, oxygen therapy, and had longer hospital stays. Both correlation and regression analyses demonstrated that higher cortisol levels post-handling were associated with longer hospital stays, suggesting that limited stress recovery in VLBW infants was an important predictor of poorer health outcome. This study is the first report in the literature to date on the associated finding of high cortisol levels
post-handling during the first week of life as a prognosticator of poorer health outcome in VLBW preterm infants.

Hypothesis 4 (a) was that mothers reporting higher stress will be less responsive to their infants than mothers with lower stress and (b) mothers that are less responsive to their infants will rate themselves lower in parenting competency than mothers with high responsiveness. Mothers of study infants reported both their general stress levels and stress regarding their infants as very high during early hospitalization. The hypothesis that higher levels of maternal stress would result in fewer competencies in maternal sensitivity toward infants and lower parenting competency was not supported. Because there was limited variability in mothers’ self-reports of stress (all mothers reported high levels of stress) determination about the relationship between maternal stress and competency was not possible. Mothers rated by NICU nurses as more competent and sensitive to their infants had higher self-ratings of competency in parenting at one month of age, supporting the final hypothesis.

**Secondary Study Findings**

*Score for Neonatal Acute Physiology (SNAP)*

The score for neonatal acute physiology (SNAP) at 72 hours of age was predictive of long-term morbidity for this sample of mild to moderately ill preterm infants. SNAP was highly positively correlated with not only the developmental outcome variables (GA at full oral feedings, and GA at discharge), but also the health outcome variables (days on oxygen, days on ventilation, retinopathy of prematurity, and length of stay). In the regression modeling, SNAP showed
predicted ventilation duration and oxygen therapy duration. Previous studies have demonstrated the utility of SNAP in predicting outcome in study samples similar to the current investigation. (Richardson, Gray et.al, 1993; Richardson, Phibbs et.al, 1993). In these studies SNAP was an independent predictor of neonatal mortality.

As discussed, the overall principal study findings lend full support to hypotheses 1, and partial support to hypotheses 2, 3, and 4. To qualify the acceptance of these findings and speculation of possible implications, the reader is reminded of the study’s strengths and weakness.

**Strengths**

A major strength of the study was its use of a clinically valid measure to rate preterm infant stress responses (Peters, 1998; White & Porth, 2000). The study design controlled for potentially confounding variables associated with cortisol measurement including: the type of handling stimulus, postnatal age, time of day, type of interaction, and care provider. The cortisol assays were obtained directly from an indwelling catheter so that the collection of samples did not evoke a stress response, thus responses were reflective of the caregiver interaction and NICU environment. An independent lab, without knowledge of the study design or hypotheses, performed the cortisol assays.

Another strength was that a priori hypotheses based on existing literature directed the study design, measurements, and analyses. For consistency, the behavioral observation data were obtained by direct visualization and field notes of two observers, as well as by videotaped recordings. The principal investigator
with demonstrated reliability and certification in the use of the NIDCAP®
naturalistic observation method scored the behavioral data and remained blinded
to the cortisol findings until the coding of behavioral data was completed and
HRV data were analyzed.

In addition, the regression analyses demonstrated that vagal tone and
cortisol post-care remained significant predictors of length of stay above and
beyond other measures. Moreover, the behavioral variable of facial distress
(cues) remained a significant predictor in the models for duration of oxygen
therapy and length of hospital stay, even when other factors were included. This
suggests that facial distress (analogous to pain response) was a unique and
powerful predictor of vulnerability to environmental stress, above and beyond
other measures, and was associated with poorer health outcomes.

Last, the construct of maternal competency was assessed not only by self-
report, but also through nurses’ observational ratings of mothers handling their
preterm infants. Furthermore, all of the questionnaires selected for use in this
investigation have established validity and reliability in the population studied.

Limitations

The present study has acknowledged limitations. First, the sample
consisted of mostly white, very low birth weight (VLBW) preterm infants making it
impossible to generalize to other samples of differing age, condition, or
race/ethnicity. Second, the dimensions of family environment, personal stress,
and parenting competency were measured by self-report of the mothers. Third,
the small sample size may have decreased the power to detect significant
findings and some true relationships may not have been detected because of limited power. In addition, there is a possibility that the small sample size could have produced apparently significant correlations, when in fact, the relationships might be serendipitous. For this reason, it is imperative that the study be replicated to a larger sample.

Furthermore, the large number of analyses in relation to the number of subjects in the study increases the possibility that the study findings may be due to chance. To acknowledge this possibility a Bonferroni correction was done. The standard Bonferroni correction is a multiple-testing adjustment procedure that controls for type I error (the reduction in false discovery rates) on an individual test by adjusting the $p$ value, by dividing the level of significance (alpha) by the number of tests performed (Roback & Askins 2005). For the hypothesis testing in this study, application of the Bonferroni correction decreases the alpha to 0.0125, thus, all of the findings reported as significant in the regression models become nonsignificant. Roback and Askins (2005) argue that although the Bonferroni correction is appropriate in focused studies, the conservative nature of this approach increases the likelihood of type II errors (not detecting an effect when indeed the effect exists) and therefore, should not be employed in initial probing studies, as with this investigation. Roback and Askins (2005) propose that a reasonable approach would be to make the distinction between initial screening studies, where the goal is to identify relationships for later confirmatory testing, in contrast to more focused studies with fewer tests where multiple-testing adjustment procedures, like Bonferroni should be employed. Moreover, in this
study the analyses were guided by existing literature and the findings are biologically plausible, thus it is less likely that the demonstrated significant relationships were spurious.

Finally, the nature of hormone measurement in VLBW infants introduces significant potential for measurement error into the study. Cortisol reactivity has a wide range of variability among subjects and recent evidence suggests that even under ideal laboratory conditions there may be considerable heterogeneity of cortisol secretion, making baseline determinations difficult. Additionally, with reactivity determination there may be variable latency, a lag time of as long as 10 - 30 minutes from the time of stressor stimulus until reactivity (Casey, & MacDonald, 1997). In this study, serial measurements of cortisol were made at 30 and 60 minutes after the initiation of handling. An additional sampling at 120 minutes may have been required to capture the peak reactivity in latent responders. Furthermore, the possibility exists that thirty minutes after the initiation of handling may have been an excessive period of time and the peak response may have been missed.

In addition, it is plausible that the confound of prenatal steroids and/or excessive stress during pregnancy altered normal HPA axis functioning resulting in the inability of the infants to mount a sufficient stress response to handling (Davis, et al., 2004). HPA axis stimulation tests might have been helpful to determine normal adrenal responsivity in the study infants. Because it is standard obstetrical care to administer antenatal steroids to mothers when preterm labor begins prior to 32 weeks gestation to stimulate lung maturity of the fetus, it is an
expected finding that the majority (69%) of the participant mothers in this study received one or more doses of betamethasone antenatally.

Despite the investigator’s best attempts using several different techniques, the difficulties of sampling salivary cortisols in this population could not be solved, thus only plasma samples were collected. Salivary cortisol sampling is ideal because it is non-invasive and reflects the biologically active form. In the opinion of this investigator, the high refusal rate for study participation was primarily caused by the parental view that blood sampling was too invasive for this vulnerable population. In fact, despite making purposeful attempts to allay parental fears, several parents expressed worry about the potential need for a blood transfusion as a direct result of the additional blood sampling necessary for participation in the study, and for that reason they denied participation.

It is also noteworthy that in the present study, mothers of infants with respiratory compromise requiring high-frequency ventilation by day 3 of life were not approached for participation in the study, because of the infants' level of acuity and the emotional distress experienced by mothers. This issue resulted in an unintended selection bias toward recruitment of healthier infants. Nonetheless, this is the first study to test specific hypotheses that combine hormonal, heart rate measures, and behavioral influences within the context of caregiver interaction as predictors of health outcomes on a vulnerable neonatal population.
Conclusions

The findings of this study provide evidence of interrelationships between cortisol and behavioral and heart rate responses to caregiving as predictors of health outcome in very low birth weight infants. All infants exhibited hormonal, behavioral, and heart rate reactivity to handling. The majority (69%) quieted and had lower cortisols and lower mean heart rates at one hour after care. Although, duration of interaction was not directly related to cortisol response, the quality of interaction, use of appropriate “pacing of care” and “hands-on containment” was associated with greater drops in cortisol in the post care period.

This study demonstrated that stress vulnerability as demonstrated by higher cortisol levels at one hour after initiation of handling, was an important predictor of respiratory morbidity. Those infants with persistent tachypnea and elevated cortisol levels one hour post-handling had longer durations of required oxygen therapy and had longer hospital stays. Behavioral distress signals documented using the NIDCAP® behavioral observation system, identified infants who were uniquely vulnerable to the stressors of the environment. Visceral and facial manifestations of distress were associated with morbidity, including higher rates of nosocomial sepsis and longer duration of oxygen. In contrast, the more robust VLBW infants in the study who demonstrated higher motor, attentional, and self-consoling behaviors had greater high frequency HRV and improved developmental and health outcomes.

Mothers of study infants rated their general stress levels as very high during early hospitalization. However, maternal stress levels did not predict self-
ratings or nurse-rated competency ratings of mothers at one month of age. This finding may have been secondary to a lack of variability in stress, as all mothers reported high levels of stress in the early hospitalization phase. Mothers with higher self-ratings of competency in parenting at one-month were rated higher by NICU nurses in competency and sensitivity concerning their infants.

This study adds to the burgeoning evidence that biobehavioral indicators of functioning are useful predictors of health outcomes. Moreover, HRV measurement using spectral analysis is a promising non-invasive technology. In this study there is evidence that vagal tone represents an important homeostatic mechanism that may be an independent measure of stress vulnerability.

Moreover, this is one of the first studies to document the validity of the NIDCAP® behavioral observation measures with biological (cortisol, HRV) measures in the assessment of stress vulnerability as predictive of health outcomes in VLBW preterm infants. Further investigation with a larger sample of newborns is required to confirm the clinical significance of these findings.

Future Directions

The information gleaned from this small-scale investigation will serve as the foundation for future investigations. Future studies on the validity of biobehavioral responses as predictors of outcome in sick newborns should focus on the following areas. First, the reliable and non-invasive technique for sampling saliva in older infants and children (Granger et al., in press; Schwartz, Granger, Susman, Gunnar, & Laird, 1998) has not been successfully adapted for applicability to the very low birth weight preterm infant population. The
exploration of methods for sampling an adequate volume of saliva without causing distress is a critical next step to this program of research. The non-invasive measurement of both HPA and sympatho-adrenomedullary arms of the stress system with salivary collection (Granger, Kivlighan, & Blair, in press) would be an ideal approach for identification of stress and recovery with maternal-infant interactions in stable growing preterm infants after 2 months of age.

Second, application of the techniques of biobehavioral (salivary cortisol, HRV, and behavioral response) measurement to a larger sample of stable preterm infants is an important next step in the author’s program of research. Specifically, the plan is to submit an R03 application to the NICHD branch of the National Institutes of Health to fund a larger scale investigation for more rigorous testing of the hypotheses. There is currently a request for applications by the NICHD on biobehavioral measurement techniques in the study of therapeutic effectiveness of pharmacological and non-pharmacological treatment modalities to relieve discomfort in infants. Plans are to submit an application to this NIH initiative.

Finally, evaluation of strategies for implementing early maternal involvement and understanding of cue-based interventions for ameliorating stress in mothers and infants is warranted. A randomized clinical trial on the application of a parent-centered therapeutic touch/massage program as an intervention strategy to reduce stress, facilitate parent-infant interaction, and promote advocacy and successful parenting is one such program that requires further investigation.
References


during first dose surfactant administration in neonates with RDS. *Pediatric Pulmonology, 17*(4), 246-249.


long-term relations to children’s development. *Nursing Research, 43*(2), 105-110.


Appendix A1
IRB Approval Penn State COM

Milton S. Hershey Medical Center
College of Medicine

DATE: October 28, 2003

TO: Kim Kopenhaver Haider, MSN, CRNP; Pediatrics
FROM: Kevin Gleave, M.D., Executive Chair
Institutional Review Board

RE: IRB Protocol No. 2003-211 – Biobehavioral Patterns of Response in Preterm Infants; Behavioral, Hormonal, and Physiological Markers of Stress

Thank you for your application to the Institutional Review Board (IRB). The above IRB protocol number was assigned for the research and should be included on all future correspondence and documentation. The proposed research met the regulatory criteria for convened board review and was reviewed accordingly. Official approval was granted for this research effective October 28, 2003, and valid until August 18, 2004, at which time IRB reconsideration will be required. This approval includes the following:

- Investigator Written Research Protocol – dated 08/01/2003
- Total entry - Twenty (20) subjects
- Informed Consent - Parental Permission form (dated 09/15/2003)
- Authorization to use protected health information (PHI) – Included in consent form
- Advertisement - None; Questionnaire - Incorporated into protocol
- IRB member exclusions: No investigators for this research serve on the IRB.

Informed consent and Authorization: Only approved investigators may solicit consent for research participation. Subjects or their representatives must receive a copy of the consent form, and for patients include a copy in the medical record along with the protocol abstract. It is the principal investigator’s responsibility to keep original consent forms/authorizations filed in a secure place and to retain them for six years after termination of research that accesses protected health information (PHI), or for two years after termination if no PHI is accessed. Additional requirements apply for FDA and sponsored trials, which the principal investigator should ascertain, if applicable.

Proposing Changes: Federal regulations require prompt reporting to the IRB of any proposed changes in a research activity and prior approval before changes are initiated, except where necessary to eliminate apparent immediate hazards to the subject. (Submit a Modification Request Form to change an existing investigation.)

Adverse Event Reporting: Serious, life-threatening or unexpected adverse events occurring in subjects participating in this research must be reported immediately to the IRB. (Submit the Adverse Event/Safety Report form). Report all other adverse events (i.e., mild or expected reactions) on the Progress Report for renewal.

Investigator Resources: The HSPO/IRB web site at www.hmc.psu.edu/hmc-irb provides many resources, including an Investigator’s Handbook, educational information, forms to report on or modify ongoing research, plus the institution’s federal assurances (FWA), IRB compliance statement and other helpful information.

The Institutional Review Board appreciates your efforts to conduct research in compliance with the institutional policies and federal regulations that have been established to ensure the protection of human subjects. Please feel free to communicate any future questions or concerns regarding this research to the IRB via its administrative arm, the Human Subjects Protection Office.
Appendix A2
IRB Approval University Park Campus

Date: December 3, 2003
From: Mary B. Becker, IRB Administrator
To: Kim Kopenhaver-Haidet
Subject: “Biobehavioral Patterns of Response in Preterm Infants: Behavioral, Hormonal, and Physiological Markers of Stress”

Your above referenced protocol has been reviewed and approved by The Milton S. Hershey Medical Center College of Medicine Institutional Review Board effective October 28, 2003. A dual IRB authorization agreement between Penn State College of Medicine (FWA # 00004251) and The Pennsylvania State University (FWA# 00001534), effective 10/23/03 and limited to this protocol, states that The Pennsylvania State University will rely on the IRB at Penn State College of Medicine to provide review and continuing oversight of this project. Therefore you are cleared by The Pennsylvania State University to proceed with your research and to follow the requirements of the College of Medicine’s IRB. The College of Medicine’s IRB will inform us of any changes to this protocol.

On behalf of the committee and the University, I thank you for your efforts to conduct your research in compliance with the federal regulations that have been established for the protection of human participants.

MBB/mbc
Appendix B

General Clinical Research Center Approval

GENERAL CLINICAL RESEARCH CENTER
COLLEGE OF MEDICINE
THE MILTON S. HERSHEY MEDICAL CENTER
INTER-OFFICE CORRESPONDENCE

Date: December 15, 2003

To: Kim Kopenhaver Haidet Ph.D.(c), MSN, CRNP

From: Gordon L. Kauffman, MD
Chairman, General Clinical Research Center Advisory Committee

Re: 2003-211 "Biobehavioral Patterns of Response in Preterm Infants: Behavioral, Hormonal and Physiological Markers of Stress"

The General Clinical Research Center Advisory Committee reviewed the above referenced protocol submitted for use of the GCRC on November 5, 2003. The Committee approved the protocol pending a response confirming the amount of blood/plasma required for the study and a response on whether the gestational age makes a difference in the cortisol response with a scientific priority score of 1.65. (Scores are used to prioritize visits when requests exceed capacity of either staff or space.) This approval includes sample processing and storage and ancillary support for Plasma Cortisols. Please keep the GCRC aware of any changes in the protocol and consent forms. All changes submitted to the IRB must also be submitted to the GCRC.

Please contact Shirlynn Mottilla, RN, Clinical Head Nurse for the General Clinical Research Center to schedule support. She can be reached at extension 7032 or by e-mail at <shirlynmottilla@hcsh.org>

If you have any questions regarding this review process and/or wish to examine the file of your study, please feel free to contact Rebecca Jenkins, Administrative Manager of the General Clinical Research Center at Extension 5619.

Please note the GCRC uses the IRB number to identify protocols. Please include this number on all correspondence and when scheduling visits.

For purposes of continued support of the Center, documentation of the GCRC contribution in all publications using any of the GCRC resources is required. Manuscripts for publication should include a statement similar to the following: "The nursing care provided by the staff of the Penn State General Clinical Research Center at the M.S. Hershey Medical Center is appreciated," or simply: "The study was supported by NIH Grant M01-RR10732." Copies of all published manuscripts, articles, or abstracts documenting research in the GCRC should be sent to the administrative manager.

The Committee extends best wishes for success of your protocol.
Appendix C

PARENTAL PERMISSION FOR RESEARCH

Penn State College of Medicine
The Milton S. Hershey Medical Center

Title of Project: Biobehavioral Patterns of Response in Preterm Infants:
Behavioral, Hormonal and Physiological Markers of Stress

Principal Investigator: Kim Kopenhaver Haidet Ph.D.(c), MSN, CRNP

Other Investigators: Keith H. Marks, MD, Ph.D., Elizabeth J. Susman, Ph.D.,
Charles Palmer, MB, ChB

Participant’s Printed Name: _____________________________(Mother)
Participant’s Printed Name: _____________________________(Infant)

This is a research study. Research studies include only people who voluntarily
choose to take part. This consent form gives you information about this research, which
will be discussed with you. This consent form may contain words or procedures that you
do not understand. You are urged to ask questions about anything that is unclear to you.
Discuss it with your family and friends and take your time to make your decision. You
will receive a copy of the signed and dated consent form to keep.

1. Purpose of the Research: You and your baby are being offered the opportunity to take
part in this research because your baby was born prematurely (more than 2 months before
the expected date of delivery) and your baby has an existing umbilical line. This research
is being done to learn about how premature babies respond to a standard nursing
procedure, such as: diaper change and change of position. Approximately 20 babies and
their mothers will take part in the research at Hershey Medical Center.

2. Procedures to be Followed: If you agree to participate you will be asked to sign and
date this consent form. You will complete 4 questionnaires. These include questions
concerning you and your baby’s medical information, the teaching you received about
responding to your baby’s cues, how much stress you are experiencing, and your family
support system.

Your baby’s behavior and movement will be videotaped during a 10-minute period of rest
prior to a standard nursing procedure, during the procedure, and 20 minutes after the
procedure is completed. The videotaped behavior will be viewed and scored by a research
nurse.
Twenty minutes before and 30 and 60 minutes after initiation of the nursing procedure, a few drops of your baby’s blood (150 microliters) will be drawn from the existing line placed in the baby’s umbilical cord stump. The blood will be tested to identify the presence of stress hormones. This blood will be taken from a small catheter (flexible tube) already in place for the purpose of routine blood sampling. The infant will not be “stuck” for the purpose of blood sampling.

In addition, information about your baby’s medical condition (results of nursing and medical examinations, laboratory results and chest x-rays) during his/her stay in the neonatal intensive care unit will be recorded for this research and information about your baby’s heart and breathing rates and oxygen levels will be recorded from your baby’s bedside monitor to a laptop computer for this research study. When your infant is one month old, your baby will be videotaped during an interaction with you. The videotaped behavior will be viewed and scored by a research nurse. You will complete a questionnaire on your beliefs about parenting your baby at this time.

3. **Discomfort and Risks:** The sampling of blood from the catheter will not cause discomfort to your baby. With umbilical line blood sampling, there is a slight risk of slippage of the catheter or loosening of a connector causing potential blood loss, but every precaution will be taken to prevent this from happening. Neonatal nurses very experienced in the removal of blood from indwelling catheters will be obtaining the samples. There is a slight risk of loss of confidentiality if you or your baby’s information and videotapes are obtained by someone other than the researchers, but every precaution will be taken to prevent this from happening.

4. a. **Possible Benefits to You:** Neither you nor your baby will benefit from the study.  
   b. **Possible Benefits to Society:** Medical science may gain further understanding of patterns of response in preterm babies. The results of this research may be used to guide further research on caregiving practices to diminish stress in preterm babies, and potentially improving their long-term developmental outcome

5. **Other Options That Could be Used Instead of this Research:** You may decline to participate and you may decline to permit your baby to participate in this study.

6. **Time Duration of the Procedures and Study:** Your baby will be participating in this research while he/she is a patient in the neonatal intensive care unit at Hershey Medical Center. The time of the observation before and after a standard nursing procedure will last between 40-50 minutes. Your baby’s progress will be monitored and recorded for the research throughout her/his hospital stay. The video taping at one month will last 20 to 30 minutes. The questionnaires will take you approximately 20-45 minutes to complete at baseline and 10-20 minutes to complete at one month.

7. **Statement of Confidentiality:**
   a. **Privacy and Confidentiality Measures:** You and your baby’s research records and samples of your baby’s blood that are reviewed, stored and analyzed at The Milton S.
Hershey Medical Center (HMC) and Penn State College of Medicine (PSU) will be labeled with your baby’s initials and a code number. The list that matches your name and your baby’s name with the code number will be kept in a locked file in Ms. Haidet’s office. The research records will be kept in locked file cabinets and in password-protected computer files in the General Clinical Research Center (GCRC) at HMC. The blood samples will be stored in a locked laboratory in the GCRC. The specimens will be destroyed after analysis.

The videotapes of your baby and you interacting with your baby will be labeled with a code number and stored in a locked file cabinet in the principal investigator’s office. Only the researchers and research assistants will have access to these tapes. The tapes will only be stored until the research is completed. At that time they will be destroyed.

For research records shared with researchers in the Department of Biobehavioral Health at Penn State, University Park Campus, you and your baby will not be identified by name, social security number, address, phone number or any other direct personal identifier. The records will be labeled with a code number. The list that matches your name and your baby’s name with the code number will be kept in a locked file in Ms. Haidet’s office. The research records will be stored in locked file cabinets and in password-protected computer files in the Department of Biobehavioral Health. In the event of any publication of presentation resulting from the research, no personally identifiable information will be shared.

b. The Use of Private Health Information

Health information about you and your baby will be collected if you choose to permit your baby to be part of this research study. Health information is protected by law as explained in the HMC Privacy Notice. If you have not received this notice, please request a copy from the researcher. At The Milton S. Hershey Medical Center (HMC) and Penn State College of Medicine (PSU) your child’s information will only be used or shared as explained and authorized in this consent form or when required by law. It is possible that some of the other people/groups who receive your child’s health information may not be required by Federal privacy laws to protect his/her information and may share it without your permission.

For your child to participate in this research you must permit the research team to use your child’s health information. If you do not want us to use your child’s protected health information, your child may not participate in this research. Your permission for the use and sharing of you and your baby’s identifiable health information will continue until completion of the research. At that time the research information not already in your baby’s medical record will be destroyed.

If you choose to participate, you are free to withdraw your permission for the use and sharing of you and your baby’s health information at any time. You must do this in writing as indicated in the HMC Privacy Notice. Write to Ms. Kim Kopenhaver Haidet and let her know that you and your baby are withdrawing from the research study. Her mailing address is Department of Pediatrics H085, The Milton S. Hershey Medical Center, 500 University Drive Hershey, PA 17033.
If you withdraw your permission:

- We will no longer use or share medical information about you and your baby for the reasons covered by your written authorization, except when the law allows us to do so.
- We are unable to take back anything we have already done or shared with your permission.
- We may continue using and sharing the information obtained prior to your withdrawal as necessary to maintain the soundness of the overall research.
- We are required to keep our records of the care that we provided to your baby as long as the law requires.

The research team may use the following sources of health information:

- Videotapes of your baby’s behavior, and you interacting with your baby
- Heart and respiratory rate and oxygen level information,
- Results of nursing or medical examinations,
- Blood hormone levels,
- Results of laboratory tests done to monitor for infections and chest x-rays
- Questionnaire responses
- Medical histories

Representatives of the following people/groups within HMC/PSU are allowed to use and share you and your baby’s health information with other specific groups in connection with this research study:

- The principal investigator, Kim Kopenhaver Haidet,
- The HMC/PSU Institutional Review Board,
- The HMC/PSU Human Subjects Protection Office,
- The other listed investigators and other members of the research team
- Nursing staff and medical staff who work in the Neonatal Intensive Care Unit
- The staff of the General Clinical Research Center (GCRC)
- The Core Endocrinology Laboratory staff

The people or groups listed in the above paragraph may share you and your baby’s health information with the following persons and organizations outside HMC/PSU for their use in connection with this research study:

- The Office of Human Research Protections in the U. S. Department of Health and Human Services
- Researchers in the Department of Biobehavioral Health at Penn State, University Park Campus
- Outside data analysts appointed for this study
- Biostatistics staff at Penn State University, University Park Campus
- The Pediatric Endocrinology Nursing Society, the sponsor of this research study
8. **Costs for Participation**: There is no cost to you for participating in this study. As outlined above, the potential risk for participation in this research study is very low. In the unlikely event that some problem might arise resulting from the research, medical treatment is available and will be provided at the usual charge. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related complications. Costs for such treatment will be charged to your insurance carrier or to you. Some insurance companies may not cover costs associated with research studies. If for any reason these costs are not covered by your insurance, they will be your responsibility. You are not waiving any legal rights you may have by signing this form.

9. **Compensation for Participation**: Neither you or your baby will be paid for participating in the study nor will there be any extra charges for you or your insurer as a result of participation in this research study. Costs for obtaining additional studies that are not part of your baby’s routine hospital care will be covered by research funds or departmental grants.

10. **Research Funding**: The research sponsor, The Pediatric Endocrinology Nursing Society, will reimburse the institution for the heart rate analysis equipment and for the work the research staff does to conduct this research.

11. **Voluntary Participation**: Taking part in this research study is voluntary. If you choose to participate and to allow your baby to take part in this research, your major responsibilities will include: allowing you and your baby’s responses to be videotaped, allowing your baby to have blood samples drawn for stress hormones, and allowing you and your baby’s information to be recorded for this research. You do not have to participate or allow your baby to participate in this research. If you choose to take part, you have the right to stop participation at any time. If you decide not to permit your baby to participate or if you decide to stop his/her participation in the research at a later date there will be no penalty or loss of benefits to which your baby is entitled. In other words, your decision not to allow your baby to participate in this research or to stop taking part in the research will not affect your baby’s access to medical care. During the course of the research you will be informed of any new findings that may affect your willingness to continue allowing you and your baby to participate in this research.

12. **Contact Information for Questions or Concerns**: You have questions or concerns later or if you believe your baby may have developed an injury that is related to this research, you should contact Ms. Haidet at 717-531-8413 or the Newborn Medicine doctor on 24-hour call at 717-531-8941.

If you have questions or concerns regarding you and your baby’s rights as research participants or your privacy and the use of personal health information, you may contact the research protection advocate in the HMC Human Subjects Protection Office at 717-531-5687.
Signature and Permission to Enroll in the Research

Before making the decision regarding enrollment in this research you should have:
- Discussed this study with an investigator
- Reviewed the information in this form and
- Had the opportunity to ask any questions you may have.

Your signature below means that you have received this information, have asked the questions you currently have about the research and those questions have been answered. You will receive a copy of the signed and dated form to keep for future reference.

**Parent/Guardian:** By signing this consent form, you indicate that you give permission for your child to take part in this research study.

_________________________________ ______________ _______ ______________________  
Signature of Parent or Guardian     Date  Time  Printed Name

**Person Explaining the Research:** Your signature below means that you have explained the research to the parent/guardian and have answered any questions he/she has about the research.

_________________________________ ______________ __________ ______________________  
Signature of person who explained this research* Date  Time              Printed Name  
(*Only approved investigators for this research may explain the research and obtain informed consent.)
# Appendix D1

## Timetable

<table>
<thead>
<tr>
<th>PROCEDURES</th>
<th>DUE DATE</th>
<th>PERSONNEL</th>
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<td>Principal investigator held in-services for neonatal intensive care staff nurses, physicians, and management staff on project</td>
<td>Dec 30, 2003</td>
<td>PI, Clinical head nurses, NNPs, Nurses, NICU, CNS, and Outcomes Coordinator</td>
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<tr>
<td>Research Assistant recruitment, and training to establish reliability on coding of videotaped behavior and assessment of sleep/wake cycles, and consistency in data collection methods</td>
<td>Jan 15, 2004</td>
<td>PI, Research Assistants and Nurse Coders</td>
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<tr>
<td>EKG technician and researchers piloted HRV equipment</td>
<td>Jan 30, 2004</td>
<td>PI, Clinical Engineering Technician, and Vascular Lab Consultant</td>
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<td>Began recruitment of infant/mother dyads, with goal of enrolling 1-2 infants per month, with continued longitudinal data collection through one month of age</td>
<td>Feb 5, 2004</td>
<td>PI, Co-Investigators, Research Assistants</td>
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<tr>
<td>Longitudinal data collection and data entry completed</td>
<td>Dec 3, 2004</td>
<td>PI, and Research Assistants</td>
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<td>Submission of poster abstract for presentation at PENS national conference</td>
<td>Dec 10, 2004</td>
<td>PI, Co-Investigators</td>
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<td>Preliminary analysis of Data</td>
<td>Dec 30, 2004</td>
<td>PI, and Data Management Technician</td>
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<tr>
<td>Final analysis of data and completion of Dissertation Thesis</td>
<td>Feb 25, 2005</td>
<td>Principal Investigator</td>
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<tr>
<td>Complete draft of manuscript for submission to peer-reviewed publication</td>
<td>Mar 1, 2005</td>
<td>PI, and Co-Investigators</td>
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<tr>
<td>Prepare poster and PowerPoint presentation of completed study findings and pass oral defense</td>
<td>Mar 14, 2005</td>
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<td>Completion of final report for PENS grant</td>
<td>April, 2005</td>
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<td>Oral &amp; Poster Presentation of study findings at National Conference</td>
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<td>Submission of final manuscript for publication</td>
<td>June, 2005</td>
<td>PI, Co-Investigators</td>
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Appendix D2

Scatter plots of Significant Correlations

Cortisol Reactivity and Autonomic Parameters

change in cortisol from baseline to post-care (T2)

r = -.76

AUC 2 (cortisol area under curve from baseline to sample 2)

r = -69
Cortisol Reactivity and Autonomic Parameters

![Graph showing change in cortisol from baseline to post-care (T2) and peak change in mean respiration rate (bpm).](image)

- Peak change in mean respiration rate (bpm)
  - 60
  - 50
  - 40
  - 30
  - 20
  - 10
  - 0
  - -10
  - r = .59

![Graph showing mean heart rate bpm post stimulus at T2 and AUC 2 (cortisol area under curve from baseline to sample 2).](image)

- Mean heart rate bpm post stimulus at T2
  - 170
  - 160
  - 150
  - 140
  - 130
  - r = -63

AUC 2 (cortisol area under curve from baseline to sample 2)
Cortisol Reactivity and Behavior

AUC 2 (cortisol area under curve from baseline to sample 2)

r = .64

AUC all (Area under curve change from base for all)

r = .60
Heart Rate Variability and Behavioral Responses

Motor cues (twitch, startle, fingersplay, squirm)

attentional cues (fuss, yawn, sneeze, avert)

total power from HRV spectral analysis at obrest

$r = .81$

$r = .72$
Heart Rate Variability and Behavioral Responses

- Total power from HRV spectral analysis at obrest: $r = .87$
- Facial cues (tongue, grimace, eye squeeze): $r = .74$
Cortisol Reactivity and Health Outcomes

AUC 2 (cortisol area under curve from baseline to sample 2)

Gestational Age at Full Enteral Feedings

Days Requiring Mechanical Ventilation

r = .64

r = .65
Cortisol Reactivity and Health Outcomes

AUC 2 (cortisol area under curve from baseline to sample 2)

Vagal tone and Health Outcomes

HF power at 1 month of age

Days on Oxygen

Days Requiring Mechanical Ventilation

r = .59

r = -.75
Vagal tone and Health Outcomes

- Retinopathy of prematurity
  - HF power at 1 month of age

- Length of Hospital Stay (Days)
  - HF power at 1 month of age

Graph showing a negative correlation between HF power at 1 month of age and both retinopathy of prematurity and length of hospital stay. The correlation coefficients are $r = -0.75$ and $r = -0.74$, respectively.
Behavioral Cues and Health Outcomes

Days on Oxygen

r = .57

facial cues (tongue, gape, grimace, eye squeeze)

Documented Sepsis

r = .58

facial cues (tongue, gape, grimace, eye squeeze)
Behavioral Cues and Health Outcomes

self-consoling (suck, hand to mouth, grasp, hand/foot clasp)

Cortisol Post-Care and Health Outcomes

blood cortisol mcg/dl post stimulus at time 60min
Cortisol Post-Care and Health Outcomes

- Days Requiring Mechanical Ventilation
  - Blood cortisol mcg/dl post stimulus at time 60min
  - \( r = 0.63 \)

- Length of Stay (Days)
  - Blood cortisol mcg/dl post stimulus at time 60min
  - \( r = 0.75 \)
SNAP and Health Outcomes

Snap-score for neonatal acute physiology day 4-5

Days Requiring Mechanical Ventilation

Days on Oxygen

$\text{r} = .80$

$\text{r} = .90$
SNAP and Health Outcomes

Snap-score for neonatal acute physiology day 4-5

Length of Stay (Days)

r = .91

Snap-score for neonatal acute physiology day 4-5
## Appendix E1

### NIDCAP® Naturalistic Observation Record

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Appendix E2

Time I __ Time II __  PARENT SURVEY   Today’s Date __________
©Kim K. Haidet, MSN, CNNP and Virginia A. Passero, PhD.,RNC, (rev 7/2003)

Survey completed by:  Mother__  Father___ Both parents___Other_________

Infant’s name __________________ Date of birth _______ Due Date _______
Gestational age ______ NICU admission date ______
Infant Diagnoses______________________________
Parent(s)?names(s)____________________________
_____________________________________________

Mother’s age ______ Married ___  Single/divorced ___  Widowed ___ Father’s age ______
Address_____________________________________
____________________________________________

Phone (_____)__________________

Mother’s highest education: Some high school _ H.S. grad _ Some college _
College grad__GraduateDegree__

Father’s highest education: Some high school _H.S. grad _ Some college _
College grad __ GraduateDegree__

Mother: # pregnancies __ # full term deliveries ___ # preterm deliveries ___
Previous baby in a NICU? Yes No

Pregnancy problems_________________________________________________________

Insurance: Private ___State/government funded ___
Other
**************************************************************************
**********************************************************************
CHECK THE ANSWER THAT BEST APPLIES TO YOU:

1. When were you first actively involved in your baby’s care?
   At admission ___ When I knew he/she would be going home___
   I am not yet actively involved___
   Other ________________________________

2. How did you first become involved in your baby's care?
   I asked to be involved ____ A nurse asked me to be involved ___
   When my baby was stable ____
   When we knew the baby’s discharge date ___
   Other ________________________________

3. What was your favorite way to learn developmental care for your baby?
   Video __ Books and pamphlets ___ Individual demonstration ___
   Hands-on with supervision__ Group__
   Other____________________________________________________

4. Were you taught the following kinds of developmental care for your baby? (check all that apply)
   Gentle touching ___ Talking softly ___ Supportive positioning ___
   Midline positioning___
   Comforting ___ Holding ___ Swaddling ___ Non-nutritive sucking ___
   Kangaroo care ___
   Containment ___ Nesting___ Decreasing noise___ Dimming of
   lights ___Covering the incubator___
   Infant states___
   Infant behavioral cues___ Other_______________________________

5. How involved have you been in your baby's care?
   Very involved ___ Somewhat involved ___ Not involved ___

6. How involved have you been in planning for your baby's care?
   Very involved __ Somewhat involved __ Not involved ___ I would like to
   be more involved ___

7. How competent do you feel to take care of your infant after discharge?
   Very competent ___ Somewhat competent ___ Not competent ___
   Uncertain ___

8. Do you have recommendations or suggestions about the teaching of
   developmental care to parents?
   ______________________________________________________________
Appendix E3

PARENTAL STRESS SCALE: NEONATAL INTENSIVE CARE UNIT

©Margaret S. Miles, RN, PhD 1987  Self Report Format

Nurses and others who work in neonatal intensive care units are interested in how this environment and experience affects parents. The neonatal intensive care unit is the room where your baby is receiving care. Sometimes we call the room the NICU for short. We would like to know about your experience as a parent whose child is presently in the NICU. This questionnaire lists various experiences other parents have reported as stressful when their baby was in the NICU. We would like you to indicate how stressful each item listed below has been for you. If you have not had the experience, we would like for you to indicate this by circling N/A, meaning that you have "not experienced" this aspect of the NICU. By stressful, we mean that the experience has caused you to feel anxious, upset, or tense.

On the questionnaire, circle the single number that best expresses how stressful each experience has been for you. The numbers indicate the following levels of stress:

1 = Not at all stressful  (the experience did not cause you to feel upset, tense, or anxious)
2 = A little stressful
3 = Moderately stressful
4 = Very stressful
5 = Extremely stressful  (the experience upset you and caused a lot of anxiety or tension)

Remember, if you have not experienced the item, please circle NA "not applicable".

Example: Now let's take an example: The bright lights in the NICU. If you feel that the bright lights in the neonatal intensive care unit were extremely stressful to you, you would circle the answer number 5:

NA  1  2  3  4  5

If you feel that the lights were not stressful at all, you would circle the number 1 below:

NA  1  2  3  4  5
If the bright lights were not on when you visited, you would circle NA indicating "Not Applicable":

NA  1  2  3  4  5

Below is a list of the various SIGHTS AND SOUNDS commonly experienced in an NICU. We are interested in knowing about your view of how stressful these SIGHTS AND SOUNDS are for you. Circle the number that best represents your level of stress.

1. The presence of monitors and equipment
   NA  1  2  3  4  5

2. The constant noises of monitors and equipment
   NA  1  2  3  4  5

3. The sudden noises of monitor alarms
   NA  1  2  3  4  5

4. The other sick babies in the room
   NA  1  2  3  4  5

5. The large number of people working in the unit
   NA  1  2  3  4  5

Below is a list of items that might describe the way your BABY LOOKS AND BEHAVES while you are visiting in the NICU as well as some of the TREATMENTS that you have seen done to the baby. Not all babies have these experiences or look this way, so circle the NA, if you have not experienced or seen the listed item. If the item reflects something that you have experienced, then indicate how much the experience was stressful or upsetting to you by circling the appropriate number.

1. Tubes and equipment on or near my baby
   NA  1  2  3  4  5

2. Bruises, cuts or incisions on my baby
   NA  1  2  3  4  5

3. The unusual color of my baby (for example looking pale or yellow jaundiced)
   NA  1  2  3  4  5

4. My baby's unusual or abnormal breathing patterns
   NA  1  2  3  4  5
5. Seeing my baby suddenly change color (for example, becoming pale or blue)  
   NA 1 2 3 4 5

6. Seeing my baby stop breathing  
   NA 1 2 3 4 5

7. The small size of my baby  
   NA 1 2 3 4 5

8. The wrinkled appearance of my baby  
   NA 1 2 3 4 5

9. Having a machine (respirator) breathe for my baby  
   NA 1 2 3 4 5

10. Seeing needles and tubes put in my baby  
    NA 1 2 3 4 5

11. My baby being fed by an intravenous line or tube  
    NA 1 2 3 4 5

12. When my baby seemed to be in pain  
    NA 1 2 3 4 5

13. My baby crying for long periods  
    NA 1 2 3 4 5

14. When my baby looked afraid  
    NA 1 2 3 4 5

15. When my baby looked sad  
    NA 1 2 3 4 5

16. The limp and weak appearance of my baby  
    NA 1 2 3 4 5

17. Jerky or restless movements of my baby  
    NA 1 2 3 4 5

18. My baby not being able to cry like other babies  
    NA 1 2 3 4 5
19. Clapping on baby’s chest for chest drainage
   NA 1 2 3 4 5

We want to ask you how you feel about your own RELATIONSHIP with
The baby and your parental role. If you have experienced the following
situations or feelings, indicate how stressed you have been by them by
circling the appropriate number. Again, circle NA if you did not
experience the item.

1. Being separated from my baby
   NA 1 2 3 4 5

2. Not feeding my baby myself
   NA 1 2 3 4 5

3. Not being able to care for my baby myself (for example, diapering,
bathing)
   NA 1 2 3 4 5

4. Not being able to hold my baby when I want
   NA 1 2 3 4 5

5. Sometimes forgetting what my baby looks like
   NA 1 2 3 4 5

6. Not being able to share my baby with other family members
   NA 1 2 3 4 5

7. Feeling helpless and unable to protect my baby from pain and painful
   procedures
   NA 1 2 3 4 5

8. Being afraid of touching or holding my baby
   NA 1 2 3 4 5

9. Feeling staff is closer to my baby than I am
   NA 1 2 3 4 5

10. Feeling helpless about how to help my baby during this time
    NA 1 2 3 4 5
We are also interested in whether you experienced any stress related STAFF BEHAVIORS and COMMUNICATION. Again, if you experienced the item indicate how stressful it was by circling the appropriate number. If you did not experience the item, circle the NA meaning "Not applicable." Remember, your answers are confidential and will not be shared or discussed with any staff member.

1. Staff explaining things too fast
   NA 1 2 3 4 5
2. Staff using words I don't understand
   NA 1 2 3 4 5
3. Telling me different (conflicting) things about my baby's condition
   NA 1 2 3 4 5
4. Not telling me enough about tests and treatments being done to my baby
   NA 1 2 3 4 5
5. Not talking to me enough
   NA 1 2 3 4 5
6. Too many different people (doctors, nurses, others) talking to me
   NA 1 2 3 4 5
7. Difficulty in getting information or help when I visit or telephone the unit
   NA 1 2 3 4 5
8. Not feeling sure that I will be called about changes in my baby's condition
   NA 1 2 3 4 5
9. Staff looking worried about my baby
   NA 1 2 3 4 5
10. Staff acting as if they did not want parents around
    NA 1 2 3 4 5
11. Staff acting as if they did not understand my baby's behavior or special needs.
    NA 1 2 3 4 5
12. Using the same rating scale, indicate how stressful in general, the experience of having your baby hospitalized in the NICU has been for you.
    NA 1 2 3 4 5
Thank you for your help. Now, was there anything else that was stressful for you during the time that your baby has been in the neonatal intensive care unit?

Please discuss below or on back:

This survey was completed by:  Mother __  Father__  Both Parents__
Appendix E4

The Family Environment Scale

(FES) Form R

©Rudolf H. Moos 1984

There are 40 statements about families on this form. Circle the choice that agrees most closely with how you see your family. Circle neutral only for those choices for which you really cannot decide one way or another.

a = Strongly Agree  b = Agree  c = Neutral  d = Disagree  e = Strongly Disagree

1. Family members really help and support one another… . a b c d e

2. We fight a lot in our family …………………………… a b c d e

3. We feel it is important to be the best at whatever we do…a b c d e

4. We often talk about political and social problems…….. a b c d e

5. Family members rarely become openly angry………….. a b c d e

6. Getting ahead in life is very important in our family…… a b c d e

7. We rarely go to lectures, plays, or concerts……………… a b c d e
8. Friends often come over for dinner or to visit ............ a b c d e

9. We are generally very neat and orderly .................. a b c d e

10. There are very few rules to follow in our family......... a b c d e

11. It's hard to “blow off steam” at home without upsetting somebody........................................a b c d e

12. Nobody in our family is active in sports, Little League, bowling, etc......................... a b c d e

13. There is a feeling of togetherness in our family....... a b c d e

14. We tell each other about our personal problems....... a b c d e

15. Family members hardly ever lose their tempers....... a b c d e

16. We are not that interested in cultural activities ........ a b c d e

17. We often go to movies, sports events, camping, etc… a b c d e

18. Being on time is very important in our family.......... a b c d e

19. There are set ways of doing things at home.......... a b c d e
20. Family members often criticize each other............. a b c d e

21. We always strive to do things just a little better the
    better the next time.................................. a b c d e

22. We rarely have intellectual discussions................. a b c d e

22. There is a strong emphasis on following rules in our
    family.............................................. a b c d e

24. We fight a lot in our family ............................. a b c d e

25. Family members rarely worry about job promotions,
    school grades, etc.................................a b c d e

26. Family members are not very involved in recreational
    activities outside work or school................. a b c d e

27. Family members make sure their rooms are neat...... a b c d e

28. There is very little group spirit in our family......... a b c d e

29. Money and paying bills is openly talked about in our
    family ................................................a b c d e
30. In our family, we don't try that hard to succeed...
31. Each person's duties are clearly defined in our family
32. We really get along well with each other
33. Family members often try to one-up or outdo each other
34. Family members go out a lot
35. Rules are pretty flexible in our household
36. There is plenty of time and attention for everyone in our family
37. There are a lot of spontaneous discussions in our family
38. Family members really like music, art, and literature
39. Dishes are usually done immediately after eating...
40. You can't get away with much in our family...
Appendix E5

B-CHAPS
Boston City Hospital Assessment of Parental Sensitivity
Jean Gardner Cole, MS

Name of Infant: __________________________________________________
Date of visit: _____________________________________________________
Length of visit: ___________________________________________________
Completed by: _____________________________________________________

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<th>Superior</th>
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<tr>
<td>2. Parent modifies behavior in response to nurse’s instructions</td>
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<tr>
<td>3. Parent talks to infant</td>
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<td>4. Parent appropriately tries to bring infant to alert state.</td>
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<td>5. Parent effectively soothes infant</td>
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<td>6. Parent is attentive to infant</td>
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<td>7. Parent takes delight in infant</td>
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<td>8. Parent appears comfortable with:</td>
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<td>b. holding</td>
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<td>c. feeding</td>
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<td>d. bathing</td>
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Additional comments:
________________________________________________________________
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________________________________________________________________
________________________________________________________________
The Boston City Hospital Assessment of Parental Sensitivity (B-CHAPS)  
Jean Gardner Cole, MS

Description of B-CHAPS items:

1. This item evaluates the sensitivity of the parent's responses to her/his infant's behavior. It implies that she/he seeks interaction when the infant is appropriately alert, that she/he demonstrates understanding of the infant's signs of overstimulation, such as state change, change in tone of body and color change, that she/he “reads” her/his infant well and responds accordingly, providing “time out” if indicated.

2. The parent shows an interest in and is not resistant to teaching and advice from the staff. She/He incorporates some suggestions into her/his caregiving style and appears to be open to learning about what the staff have observed about the infant’s behavioral style.

3. Parent talks, coos or uses some form of verbal interaction with the infant. She/He treats the infant as if verbal interaction is an appropriate and positive form of communication.

4. The parent recognizes when it is a good time to try to bring the infant to an alert state and has effective techniques for doing so, e.g.; gentle talking or gentle rocking up and down. She/He does not attempt to engage the infant’s attention at inappropriate times such as when the infant is over stimulated or in quiet sleep.

5. The parent employs appropriate and effective soothing techniques for the infant. She/He modifies these maneuvers in response to the infant’s cues, e.g.; stops talking if this is too stimulating; stops rocking for the same reason and just holds the infant quietly.

6. While in the NICU, the parent directs her/his attention to her/his infant for significant periods of time. Her/His primary interest is in her/his infant and she/he is less concerned with the goings on around her/him.

7. Parent appears to feel positive about her/his infant as evidenced by smiles at the infant’s behavior, positive statements about the infant, and pride in the infant’s progress.

8. Parent demonstrates confidence and competency in these tasks with appropriate preparations, efficiency of time, and sensitivity to behavioral cues as evidenced by comments and “time outs”.

NICU Parental Beliefs Scale

Bernadette Mazurek Melnyk, PhD, RN-CS, PNP

Copyright, 1997

(Please do not duplicate or use this instrument without written permission from the author).

Below are 18 statements that relate to you and your baby’s hospitalization. Hospital experiences differ for every parent. There are some parents who are not so sure about their baby’s needs and how they can best meet them while they are in the neonatal intensive care unit (NICU), while other parents are more sure about how to help their baby through this experience. There are no right or wrong answers to the following statements. Please circle the number that best describes your agreement or disagreement with each statement.

1. I know what characteristics and behaviors are common in premature babies hospitalized in the NICU.
   1 Strongly disagree  2 Disagree  3 Neither agree or disagree  4 Agree  5 Strongly agree

2. I am sure that what I do for my baby will be what is best to help him/her deal with being in the NICU.
   1 Strongly disagree  2 Disagree  3 Neither agree or disagree  4 Agree  5 Strongly agree

3. I feel comfortable in caring for my baby in the NICU.
   1 Strongly disagree  2 Disagree  3 Neither agree or disagree  4 Agree  5 Strongly agree

4. I know what characteristics and behaviors to expect in my baby while he/she is in the NICU.
   1 Strongly disagree  2 Disagree  3 Neither agree or disagree  4 Agree  5 Strongly agree
5. I am sure about what things I can do to best help my baby get through the NICU experience.

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<td>Disagree</td>
<td>Neither agree</td>
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6. I am sure that I can meet my baby’s emotional needs while he/she is in the NICU.

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7. I know why my baby has the characteristics and behaviors that he/she does in the NICU.

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8. I feel confident in telling the nurses and doctors about what will best help my baby while he/she is in the NICU.

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9. I am clear about how to help take care of my baby in the NICU.

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10. I know how my baby will probably respond to me while he/she is in the NICU.

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11. I am sure about how my emotions will affect my baby while he/she is in the hospital.

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<td>Neither agree</td>
<td>Agree</td>
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12. I am clear about how my baby will react when he or she is getting too much stimulation in the NICU.

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<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strongly disagree</td>
<td>Disagree</td>
<td>Neither agree</td>
<td>Agree</td>
<td>Strongly agree or disagree</td>
</tr>
</tbody>
</table>
13. I am sure about the things that I can do to make my baby feel most secure while he/she is in the NICU.

<table>
<thead>
<tr>
<th>Strongly disagree</th>
<th>Disagree</th>
<th>Neither agree or disagree</th>
<th>Agree</th>
<th>Strongly agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
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</table>

14. I know how my baby’s appearance and behaviors are different from a full-term baby’s appearance and behaviors.

<table>
<thead>
<tr>
<th>Strongly disagree</th>
<th>Disagree</th>
<th>Neither agree or disagree</th>
<th>Agree</th>
<th>Strongly agree</th>
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</table>

15. I know the best times to communicate with or interact with my baby.

<table>
<thead>
<tr>
<th>Strongly disagree</th>
<th>Disagree</th>
<th>Neither agree or disagree</th>
<th>Agree</th>
<th>Strongly agree</th>
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<tbody>
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<td>1</td>
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</table>

16. I am confident in asking the doctors and nurses questions about my baby’s medical condition.

<table>
<thead>
<tr>
<th>Strongly disagree</th>
<th>Disagree</th>
<th>Neither agree or disagree</th>
<th>Agree</th>
<th>Strongly agree</th>
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<td>2</td>
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</table>

17. I know what my baby will do when he or she is stressed.

<table>
<thead>
<tr>
<th>Strongly disagree</th>
<th>Disagree</th>
<th>Neither agree or disagree</th>
<th>Agree</th>
<th>Strongly agree</th>
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</table>

18. I am clear about what my baby will look or act like when he or she is ready to communicate with me.

<table>
<thead>
<tr>
<th>Strongly disagree</th>
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<th>Neither agree or disagree</th>
<th>Agree</th>
<th>Strongly agree</th>
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</tbody>
</table>
Appendix E7

Chart Audit Record
©Kim K. Haidet, MSN, CNNP (rev 7/2003)

Date of Audit by

INFANT INFORMATION:
HMC Hospital Number __________ Name _____________________________
Subject #________
Date of Birth ______ Date HMC NICU adm _____ Transfer Date _____
To __________
D/C Home_____ Rehospitalization date_____
Location __________ Cause __________
Gender female male
Gestational Age (Attending’s Admit note) ___________ Birth Weight _______

Growth Parameters% Wt_____L_____HC_____ 

Admission Diagnoses 1 Prematurity 2 RDS 3 Sepsis/Infection 4 Intrauterine
Growth Restriction/Small for Gest.5 Other________

Documented Sepsis Age_____ CBC, BCX, Urine, CSF  Documented Sepsis
Age_____ CBC, BCX, Urine, CSF

Documented Sepsis Age_____ CBC, BCX, Urine, CSF  Documented Sepsis
Age_____ CBC, BCX, Urine, CSF

Days on Vent______ Days on O2______ Age at Full Enteral_____

One Month of Age Diagnoses (D/C narrative) 1 Prematurity 2 Resp Insuff 3
Sepsis/Infection 4 Patent ductus 5 GI Reflex 6 Hyperbili 7 IVH GR 8 Apnea 9
Anemia 10 Chronic Lung Disease 11 Hernias 12 Other ________________

Growth Parameters% Wt_____L_____HC_____
MATERNA L INFORMATION:
Prenatal Care: Yes/No Gravida ___ Para ___ Term ___ Preterm ___ Ab ___ LC ___
Significant History ____________________________________________
Siblings (ages) ______________________________________________
Pregnancy Problems: 1 Preterm labor 2 Hypertension/HELLP 3 Diabetes 4 Infection
5 Bleeding/Previa/ITP 6 PROM 7 Incomp cervix/uterine abn 8 Substance abuse
9 Other

Delivery Type: 1 Vaginal 2 Cesarean (indication) _____ 3 Assisted:
forceps/vacuum
Pitocin Anesthesia _____ Other Pharmacologic agents
________________________________________________________

Delivery Events: Apgars: ______
Resuscitation_________________________________________

Marital Status: 1 Married 2 Single 3 Widowed 4 Divorced
Domestic Violence: Y N
Race: 1 Caucasian 2 Black 3 Hispanic 4 Other

Maternal Employment: 1 Homemaker 2 Factory 3 Clinical/Office 4 Cashier 5 Stylist 6 RN 7 Admin/Teaching 8 Sales 9 Other

Father’s Employment: 1 Unknown 2 Unskilled Labor/Trucker 3 Skilled labor/Factory 4 Teacher 5 Researcher 6 EMT/Supervisor 7 Other__________

PATIENT EDUCATION RECORD NEONATAL: (Developmental teaching by Nurses):
  Date _______ Strategy V W D Demo _____ Evaluation C R
  Date _______ Strategy V W D Demo _____ Evaluation C R
  Date _______ Strategy V W D Demo _____ Evaluation C R
Vita

Kim Kopenhaver Haidet

Education:  **Doctor of Philosophy (Ph.D.)** Department of Biobehavioral Health, College of Health and Human Development, The Pennsylvania State University, May 2005

**Master of Science in Nursing (M.S.N.)** Perinatal Nursing, School of Nursing, The University of Pennsylvania, August 1989 (With Highest Distinction)

**Bachelor of Science (B.S.)** Nursing, College of Health and Human Development, The Pennsylvania State University, May 1986 (With Distinction)

Grant/Research Experience:


Selected Publications:

Haidet, K.K., Susman, E.J., West, S.G., Marks, K.H. (Accepted 2005)  