

Oxytocin: Biomarker of Affiliation and Neurodevelopment in Premature Infants

DISSERTATION

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By

Ashley Marie Weber, MS, RN

Graduate Program in Nursing

The Ohio State University

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Dissertation Committee:

Professor Deborah Steward, Chair and Academic Advisor

Professor Tondi Harrison, Dissertation Advisor

Professor Abigail Shoben, NRSA Co-Investigator

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Abstract

Extremely premature infants, born at 28 weeks gestation or less, are at greatest risk for poor neurodevelopmental outcomes. While survival of these infants has improved in the past decade, neurodevelopmental outcomes have not. Because early life experiences affect brain structure and function, the quality of these experiences is one of the most important factors affecting optimal development. Reliable markers of neurobiological processes underlying development are necessary so that research can accurately monitor mediators of neurodevelopmental outcomes. Oxytocin (OT) has the potential to be a neurobiological marker of social processes that offer neuroprotection for the infant. OT acts as a buffer for the stress response system and provides protection to the brain during inflammation, ischemia, or injury. OT has been strongly linked to neurodevelopmental outcomes in animal models, particularly those outcomes related to social cognition and emotion regulation. No studies measuring OT have been conducted in premature infants, nor has the association of oxytocin levels and neurodevelopment for these infants been investigated.

The purpose of this study is to 1) describe OT levels in plasma, urine, and saliva in premature infants through 34 weeks gestation and 2) determine if OT levels vary with maternal-infant interaction, neurobehavioral organization, and infant stress exposure.

Thirty-seven premature infants, born gestational ages 25-28 6/7 weeks, were

longitudinally followed until 36 weeks gestation. Plasma and urine samples were collected at 14 days of life, then weekly until 34 weeks. Data on infant and environmental variables were abstracted from the electronic medical record. Infant social engagement behaviors was measured by the Parent-Child Early Relational Assessment, during a videotaped feeding when the infant was at one-quarter full oral feeds. Infant stress exposure was measured weekly by the Neonatal Infant Stressor Scale. Neurobehavioral organization was measured by the NICU Network Neurobehavioral Scale at 36 weeks gestation.

Plasma OT levels significantly decreased with age, at a rate of 15% per week. Urine OT levels did not significantly change with age. However, more research is needed before concluding that urine is not an acceptable noninvasive measurement in this population. Both plasma and urine exhibited wide variability across age, but values were significantly stable within infants. Plasma and urinary OT levels were not correlated, both within and between infants. Moreover, OT levels were not related to infant social engagement behaviors or infant neurobehavioral organization. We hypothesize that the stressful nature of the NICU environment may contribute to decreasing OT in premature infants. Future research must replicate these results, as well as determine how stress and the NICU environment impact OT levels in premature infants. We also hypothesize that before the emergence of coordinated movements and behaviors, premature infants primarily socially interact with their caregivers through their physiology. Future research should investigate associations among the physiologic coregulation of a dyad, coregulation of dyadic behaviors, and infant neurodevelopment. OT may serve as an

important biomarker when investigating the window of development that encompasses the infant's transition from the biologic to the social world.

Dedication

This document is dedicated to my Buckeye family and friends. I would be nothing without you. I also would like to dedicate my work to NICU families, and I hope this dissertation reflects the courage, strength, and determination shown by your babies each and everyday.

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Vita

June 2006Saint Joseph Academy
2010.....B.S.N., Nursing, Ohio State University
2012.....M.S. Nursing, Ohio State University
2010 to presentDoctoral Candidate, College of Nursing,
The Ohio State University

Publications

Weber, A.M. & Harrison, T.M. The effect of maternal-infant interaction on physiological outcomes during feeding in premature and term infants over the first year of life. *Research in Nursing and in Health*, 37(6), p. 478. DOI: 10.1002/nur.21618

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Weber, A.M., & Ahijevych, K.L. (2010). Determining Bitter Taste Prevalence in a Sample of Undergraduate Students at The Ohio State University. *Journal of Undergraduate Research (JUROS)*, 2.

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Table of Contents

Abstract	ii
Dedication	v
Acknowledgments.....	vi
Vita.....	vii
List of Tables	xii
List of Figures	xiii
Chapter 1: Introduction	1
Chapter 2: Maternal–Infant Interaction in the NICU as a Mechanism for Reducing the Effects of Allostatic Load on Neurodevelopment in Premature Infants	5
Schorre’s Regulation Theory.....	7
Impact of Maternal Behavior on Healthy Infant Neurobiology	7
Impact of Neurobiology on Dyadic Affect Synchrony	14
Application of Schorre’s Regulation Theory to the Premature Infant.....	15
Neurological Development.....	16
Quality of Maternal–Infant Interaction.....	20
Suboptimal Interactions and Infant Neurobiology	22

Clinical Implications	24
Implications for Research.....	26
Conclusion.....	27
Chapter 3: Maternal-Infant Interaction and Oxytocin-based Processes underlying	
Infant Neurodevelopment	32
The Genetic Level: Prenatal Effects with and without Oxytocin.....	34
The Epigenetic Level: Maternal Behavior and Postnatal Effects	35
Priming: Maternal Stimuli for the Infant’s Developing OT System	37
The Molecular Level: G-Proteins, Receptors, and OT.....	39
The Cellular Level: Membrane Depolarization and Development of Autoregulation..	39
The Neural Circuit Level: Neurotrophins and Establishment of Circuits.....	41
The Multi-Circuit / Organ-System Level: Established Circuits and the OT System	44
The Dopaminergic Reward Pathways	44
Hypothalamic-Pituitary-Adrenal Axis (HPA) and the Sympatho-Adreno-Medullary (SAM) Response System.....	46
Parasympathetic nervous system regulation.....	48
Peripheral Targets.....	50
The Behavioral Level: Emerging Social Interactions	52
Discussion: Application to the High-Risk Human Infant	53

Stressors and High-Risk Infant Neurobiology.....	54
Future Directions in Research	57
Conclusion.....	58
Chapter 4: Oxytocin Trajectories in Extremely Premature Infants during NICU	
Hospitalization	66
Theoretical Framework and Background.....	68
Methods.....	69
Sample	69
Procedure	70
Measures	70
Data Analysis.....	71
Results.....	73
Discussion	74
Conclusion.....	81
Chapter 5: Oxytocin Trajectories, Infant Social Engagement, and Neurobehavior in	
Extremely Premature Infants	89
Theoretical Framework and Background.....	90
Methods.....	93
Sample	93

Procedure	94
Measures	96
Potential Confounders	98
Data Analysis.....	101
Results	103
Discussion	105
Conclusion.....	115
References	125
Appendix A: Appendix	176

List of Tables

Table 1 Recruitment and Enrollment for Study	82
Table 2 Maternal Demographics for Sample	83
Table 3 Infant Demographics for Sample	84
Table 4 Plasma OT Samples	85
Table 5 Urinary OT Samples	86
Table 6 Linear Mixed Model of Logged Oxytocin Trajectories.....	87
Table 7 The Covariance Matrix of the Bivariate Random Intercept Model	88
Table 8. Summary for Main Outcomes and Cofounders in the DAG Model	121
Table 9 Models for Determining Associations with Infant Social Engagement	122
Table 10. Models for Determining Associations with Infant Neurobehavior	123
Table 11 Directed Acyclic Graph (DAG) Models	124

List of Figures

Figure 1. Pathways associated with infant neurobiological changes in response to positive maternal interaction.....	29
Figure 2. Impact of the neonatal intensive care unit (NICU) environment on stress neurobiology and development in premature infants.....	31
Figure 3. Nested hierarchies of effects of oxytocin.	60
Figure 4. Positive feedback loop of oxytocin, maternal-infant interaction, and infant neurodevelopment.....	61
Figure 5. Communication processes and communicators involved in establishing neural circuits.....	62
Figure 6. Oxytocin Pathways in the Brain and Body.....	64
Figure 7. Directed Acyclic Graph (DAG) Model: Part II.....	117
Figure 8. Directed Acyclic Graph (DAG) Model: Part II.....	119

Chapter 1: Introduction

Although the prematurity rate in the United States has decreased steadily over the past decade (Reedy, 2007), prematurity is still the largest contributor to morbidity and mortality in perinatal health care. Extremely premature infants, those born at 28 weeks gestation or less, have the highest morbidity (50%) and mortality (50%) of those infants born prematurely (Glass et al., 2015). Of those extremely premature infants who survive, as many as 50% will have moderate to severe disability (Patel, 2016), due to this population's high risk for experiencing brain injury and/or altered brain development (Jarjour, 2015).

The advent of a premature birth marks a stark interruption in brain development and the start of distressing early life experiences within the highly technological Neonatal Intensive Care Unit (NICU). Neurological structures designed to mature during fetal life must now mature in the NICU and process complex, multisensory, and often painful stimuli (Tannous Elias, dos Santos, & Guinsburg, 2014). These stimuli are a source of infant distress; i.e., the infant perceives the stimulus/event as negative and responds in a maladaptive way, either physiologically, psychologically, and/or emotionally (Hatfield & Polomano, 2012). Extremely premature infants can be exposed to as many as 16 distressing events a day (Carbajal et al., 2008). Examples of these events include, but are not limited to, clinical procedures (e.g. suctioning, intravenous catheter placement, lab

draws), handling, and mechanical ventilation. Infant responses to distressing events are detrimental to brain development (Anand, 2000a, 2000b). These distressing events contribute to additional neuronal cell death, reduced brain volumes (Bhutta & Anand, 2001; Peterson et al., 2000), impairments in brain structure and function (Bhutta, Cleves, Casey, Craddock, & Anand, 2002; G. C. Smith et al., 2011), and ultimately, deficits in neurodevelopment.

Neurobiological processes that counter maladaptive consequences of distressful early life experience and promote healthy function have received little attention in the research literature (Pickler et al., 2010). This dissertation expands the research literature surrounding adaptive neurobiological processes by proposing that oxytocin (OT) has the potential to serve as a biomarker of neuroprotective processes which are shaped through early infant social experience.

Moreover, the dissertation provides preliminary evidence to support OT as a reliable, sensitive measure of the effects of early experiences in the NICU on neurodevelopment in premature infants. This research incorporates the novel investigation of hormonal levels of OT in premature infants. This dissertation also examines relationships between OT levels and conditions of infant experience that have been linked to neurodevelopmental outcomes in the extremely preterm population. The specific aims of this dissertation are:

Primary Aims:

Aim 1) Describe the developmental trajectory of plasma OT levels (i.e., how age affects OT levels over time) from 27-34 weeks corrected gestational age.

Aim 2) Compare OT levels in plasma and urine

Aim 3) Examine relationships between plasma OT levels and conditions of early

infant experience in the NICU associated with neurodevelopmental outcomes. Our hypothesis are:

H3a) Higher plasma OT levels will be associated with more positive maternal-infant interaction.

H3b) Higher plasma OT levels will be associated with greater infant neurobehavioral organization.

To address these aims, we recruited thirty-seven premature infants, born gestational ages 25-28 6/7 weeks, and longitudinally followed them until 36 weeks corrected gestational age (CGA). Appendix A describes the protocol for timing of procedures and measure administration. Plasma and urine were collected at 14 days of life, then weekly until 34 weeks CGA. Infant social engagement behaviors were measured by the Parent-Child Early Relational Assessment during a videotaped feeding interaction with the mother. Neurobehavioral organization was measured by the NICU Network Neurobehavioral Scale at 36 weeks CGA. Potential confounders were abstracted from the infant's medical record.

The following Chapters of this Manuscript-based Dissertation will address these aims in detail. Chapter 2 describes the guiding framework for the doctoral candidate's study, Schore's Regulation Theory. Schore's model contends that early life experiences result in a cascade of neurobiological processes which are critical for adequate neuronal network formation, structural brain growth, and brain function (Schore, 1996, 2001). Through developmentally-supportive early life experiences, the infant is able to develop adaptive stress response systems, socioemotional competencies, and self-regulation (Schore, 2005). The candidate published her synthesis of Regulation Theory, and its application to premature infants, in the peer-reviewed journal *Biological Research for*

Nursing (Weber, Harrison, & Steward, 2012).

Inspired by Regulation Theory, the candidate chose to further study the prosocial hormone OT, due to its neuroprotective and experience-dependent characteristics (Vargas-Martínez, Uvnäs-Moberg, Petersson, Olausson, & Jiménez-Estrada, 2014). Based on extensions of Regulation Theory, a review of OT and its impact on neurodevelopment has been submitted for publication to *Biological Research for Nursing* and is currently under review. This review of the neurodevelopmental importance of OT, as well as its application to high-risk infants (e.g. extremely premature infants), comprises Chapter 3 of this Dissertation. Chapter 4 details the methods and results of Aims 1 and 2, describing the developmental trajectory of OT in a sample of extremely premature infants. To the candidate's knowledge, this is the first documentation of OT levels in living premature infants. Finally, Chapter 5 discusses the methods and results of Aim 3, and reports the associations between OT levels in extremely premature infants and early indicators of neurodevelopment in the NICU. This is the first study of its kind, and the candidate will build on her dissertation findings to understand early and developmentally supportive caregiver-infant interactions strengthen neural pathways and neurobiological processes involving OT.

Chapter 2: Maternal–Infant Interaction in the NICU as a Mechanism for Reducing the Effects of Allostatic Load on Neurodevelopment in Premature Infants

Approximately 500,000 infants are born prematurely each year in the United States, accounting for about 12% of all live births (Mathews, Minino, Osterman, Strobino, & Guyer, 2011). Prematurity is the largest contributor to morbidity and mortality in perinatal health care (Reedy, 2007). Neurologic deficits are one of the most significant morbidities. Up to 15% of premature infants will be diagnosed with cerebral palsy, and approximately 50% will display cognitive, motor, or behavioral problems later in childhood (Bhutta, Cleves, Casey, Cradock, & Anand, 2002). In addition, children who were born prematurely have significantly poorer academic performance and a higher incidence of learning disabilities when they reach school age than those born at term (Brevaut-Malaty et al., 2010).

The premature infant is exposed to multiple stressors inherent in the highly technological neonatal intensive care unit (NICU), enduring 10–16 painful procedures a day as well as excessive noise, light, and handling (Carbajal et al., 2008; Lester et al., 2011). It is possible that the neurodevelopmental outcomes observed in premature infants are caused, in part, by alterations in the developing brain that result from repeated exposure to stressors and the infants' subsequent physiologic responses. The concepts of allostasis and allostatic load lend credence to this hypothesis. Allostasis refers to the

process of maintaining homeostasis or stability through change in integrated physiologic systems (e.g., nervous, endocrine, and immune systems) that mediates short-term adaptation to environmental challenges or stressors (McEwen & Gianaros, 2011). Allostatic processes are healthy and necessary for adequate short-term adaptation. However, when allostatic processes are repeatedly induced, prolonged, not rapidly terminated, or inadequate they no longer mediate a healthy adaptation to stress. These dysregulated allostatic processes produce changes in physiologic mediators and contribute to allostatic load, defined as prolonged dysregulation of the allostatic systems resulting in detrimental health consequences. Allostatic load has not been well defined for premature infants, and many of the mechanisms, relationships, and measures described in the adult allostatic load model may need adaptation to apply to developing infants and children (Johnson, Bruce, Tarullo, & Gunnar, 2011). We propose that suboptimal health outcomes in premature infants, such as neurodevelopmental delays, socioemotional problems, and poor self-regulation could be considered indicators of allostatic load.

The quality of the relationship between the mother and infant affects the health and neurodevelopment of the infant. Admission of their infant to the NICU is a difficult experience for mothers as they confront their own set of multiple stressors. The developing relationship between the mother and infant can be significantly altered by the repetitive stress exposures that both experience. Neurobehavioral processes that counter or prevent maladaptive consequences of exposure to stressors and that support adaptation and healthy function have received little attention in the research literature. Schore's regulation theory suggests that positive maternal–infant interactions shape development

of adaptive, healthy stress response systems through changes in infant neurobiological processes and brain structure and function (Schorre, 1996, 2001). The purposes of this article are to explain the regulation of infant neurobiological processes during interactions between mothers and healthy full-term infants in the context of Schorre's regulation theory, to identify threats to these processes for premature infants, and to propose principles of clinical practice and areas of research necessary to establish a supportive physical, social, and emotional environment in order to prevent or reduce allostatic load in these vulnerable infants.

Schorre's Regulation Theory

Regulation theory identifies the crucial biological, neurological, and psychological principles involved in maternal–infant interaction and subsequent self-regulation and attachment security (see Figure 1; Schorre, 1996, 2001). These principles include the following: (a) Right hemispheric development and function is central to regulation in a social context; (b) The processes that operate in right hemispheric development involve the orbitofrontal cortex, the mesocortical and mesolimbic dopaminergic pathways, the hypothalamic–pituitary–adrenal (HPA) axis and sympathetic-adrenal-medullary (SAM) stress response system, parasympathetic nervous system regulation, and genetic and epigenetic changes; (c) The structure, functions, and development of the right hemisphere depend on the infant's experience, primarily with the mother; and (d) Repeated or unrelieved stress may permanently alter the structure and function of these neurobiologic processes.

Impact of Maternal Behavior on Healthy Infant Neurobiology

The orbitofrontal cortex and dopaminergic pathways. Interactions between mothers and young infants occur most commonly during holding. The mother's holding environment (Feldman, 2007) provides the infant with numerous sources of sensory stimulation. By holding her infant close to her body, the mother allows the infant to experience her unique scent. The mother looks at her infant with an animated and joyful facial expression and attempts to meet the infant in a mutual gaze. She may talk to her infant in a soothing and loving tone. This holding interaction will often include feeding, and the infant tastes milk. With this interaction, the infant experiences olfactory, auditory, gustatory, visual, and tactile stimuli. The orbitofrontal cortex is essential in processing these important signals (see Figure 1).

Activated by these maternal stimuli, the infant's right orbitofrontal cortex (Carlsson, Lagercrantz, Olson, Printz, & Bartocci, 2008) transmits this sensory information through the mesocortical pathway to the midbrain's ventral tegmental area (VTA), the primary area for production of dopamine in the brain (Schoore, 1996). The mesocortical pathway connects the sensory, motor, and orbitofrontal areas of the cortex with the VTA (Wise & Bozarth, 1984). When neurons of the VTA are activated, dopamine is released and transmitted through the neurons of the mesolimbic pathway. The mesolimbic pathway connects the cortex with the limbic areas of the brain including the hippocampus, amygdala, and nucleus accumbens (NAC), areas of the brain known as reward centers as they are responsible for feelings of pleasure, emotion, mood, and reward (Gianoulakis, 2009). Through a separate dopaminergic pathway, the orbitofrontal

cortex also communicates with the hypothalamus (Schore, 1996), the critical initiator of the HPA stress response.

The release of dopamine within the infant's developing brain has substantial effects on neurobiological processes. Through the mesolimbic pathway, dopamine is released into the NAC and other reward centers such as the amygdala and hippocampus. Dopamine acts on these reward centers to produce a calming effect on the infant accompanied by intense pleasure, interest, and motivation (Weller & Feldman, 2003). Dopamine also acts on the hypothalamus to increase production and release of corticotropin-releasing hormone (CRH), which stimulates the pituitary to release endogenous opiates. These opiates stimulate the reward centers as well as the VTA, creating pleasure in the infant and exciting dopamine neurons, causing more dopamine to be released (Gianoulakis, 2009). Additionally, dopamine increases gene transcription of the precursors of endogenous opiates (Schore, 1996). The result is a positive-feedback loop involving bursts of dopamine and opiates during maternal–infant interaction in which the infant experiences intense emotions which motivate bonding and attachment behaviors (Depue & Morrone-Strupinsky, 2005).

HPA axis and the SAM stress response systems. Dopamine's action on the hypothalamus to release CRH activates the HPA axis. Hypothalamic CRH initiates production of adrenocorticotrophic hormone (ACTH). Release of ACTH stimulates the adrenal cortex to release cortisol and the adrenal medulla to release norepinephrine and epinephrine, thus activating the sympathetic-adrenal-medullary (SAM) stress response system (see Figure 1). Cortisol, norepinephrine, and epinephrine are the primary effector

hormones and neurotransmitters of the stress system, exerting influence on cardiac, respiratory, metabolic, skeletal, and gastrointestinal systems (Chrousos, 2009).

Norepinephrine and epinephrine work in the periphery to increase heart rate, respiratory rate, blood pressure, metabolism, and blood flow to skeletal muscles (Charmandari, Tsigos, & Chrousos, 2005). Cortisol inhibits secretion of CRH through a negative feedback loop (Stansbury & Gunnar, 1994), which serves to downregulate the stress response and prevent hyperreactivity of the HPA axis.

The stress response is also activated through dopaminergic stimulation of the amygdala. The amygdala releases CRH, which signals the locus coeruleus in the brain stem to release norepinephrine and epinephrine, thus increasing arousal, alertness, and attention during an interaction. In turn, norepinephrine and epinephrine released from the locus coeruleus stimulate the hypothalamus to release CRH, activating the HPA axis (Gunnar & Vazquez, 2006).

Release of these hormones and neurotransmitters in the brain triggers central nervous system (CNS) responses, which enhance attachment and provide the infant experience with adaptive responses to stress. Cortisol enhances memory, learning, and positive emotions (Haley, Weinberg, & Grunau, 2006; Stansbury & Gunnar, 1994) as well as the processing of sensory information to facilitate emotion regulation and behavioral responses to stress (Stansbury & Gunnar, 1994). Norepinephrine and epinephrine facilitate arousal, alertness, attention, oxygenation, and cognition in the brain (Chrousos, 2009). Thus, by setting in motion the release of dopamine and activation of the HPA and SAM systems during maternal–infant interaction, the right orbitofrontal

cortex controls both central and peripheral autonomic, physiologic, and stress responses of the infant (Schore, 1996).

Parasympathetic nervous system regulation. Maternal–infant interactions result in enhanced sympathetic activity through stimulation of the SAM system both through the locus coeruleus and the adrenal medulla. In addition, the simultaneous withdrawal of parasympathetic activity enhances sympathetic function (Schore, 1996, 2001). Regulation of the parasympathetic nervous system occurs via the right hemisphere of the brain. The nucleus ambiguus is controlled by the amygdala and cortex and is the source of the right vagus nerve (see Figure 1). The vagus, which has both afferent (sensory) and efferent (motor) fibers, allows rapid communication between peripheral and CNS through projections terminating in the heart, soft palate, pharynx, larynx, esophagus, lungs, and gastrointestinal tracts (Porges & Furman, 2011). When the infant’s parasympathetic pathway is activated by sensory stimuli from the mother, the infant’s right vagus reduces stimulation of the pharynx, larynx, facial muscles, and sino-atrial (SA) node of the heart (vagal withdrawal). This change in vagal regulation will result in the vocal intonations, facial expressions, and heart rate changes associated with emotions during maternal–infant interaction. Moreover, an increasing heart rate will support the enhanced metabolic requirements of infant arousal.

With continued arousing stimuli from the mother, the infant’s vagus nerve, interdependent with other neuroendocrine and neurophysiologic systems, eventually senses changes outside the bounds of homeostasis in visceral organs and higher cortical areas and responds by increasing vagal stimulation of the heart. Enhanced vagal activity

slows the heart rate, reduces sympathetic effects (Charmandari et al., 2005; Porges & Furman, 2011), and enhances parasympathetic activity, resulting in conditions supportive of rest and growth (Porges & Furman, 2011). Behaviorally, the infant averts gaze, its expression changes from active alert to quiet disinterest, and vocalizations cease. The attuned mother, sensitive to the infant's facial, vocal, motor, and emotional cues, will respond by decreasing maternal stimuli. The mother will break mutual gaze, cease vocalizations, and allow the infant to rest. Reductions in maternal stimuli inhibit the dopaminergic neurons of the mesocorticolimbic pathways, thereby reducing the emotional response resulting from endogenous opiates and reducing the sympathetic response from the HPA and SAM stress response systems (Charmandari et al., 2005). Through these changes in the provision of maternal stimuli during interaction, the mother serves as an external regulator of the infant's physiologic and stress response (Posner & Rothbart, 2009; Schore, 2001).

Repetition of sensitive maternal–infant interactions, beginning with birth, causes the release of neurohormones and neurotransmitters and the continued use of the neural pathways that process maternal stimuli, leading to substantial neuronal and synaptic growth in the infant (Kalsbeek et al., 1987). In addition, Schore posits that maternal stimuli initiate release of brain-derived neurotrophic factor (BDNF) from the infant's hippocampus and cerebral cortex. BDNF supports differentiation of synapses and promotes growth of dopamine neurons (Fish et al., 2004; Schore, 2001). This early enhanced growth of the corticolimbic areas of the brain is associated with optimal development of the skills observed within the first few months of life, including the

ability to see, vocalize, turn the head to sound, smile, exhibit joint attention, and finally interpret and predict others' intentions (Schore, 2001). Continued use of these pathways through maternal–infant interaction strengthens and enhances the efficiency of these neurobiological processes, thus developing within the infant adaptive and effective systems that can efficiently respond to stressful challenges.

Genetic and epigenetic changes. Regulation theory also addresses genetic and epigenetic influences on right-brain development, attachment, and self-regulation. Given the extensive production of nuclear and mitochondrial genetic material in the neonatal brain during the first 2 years of life, Schore postulates that the socioemotional environment provided by the mother programs genetic code. This programming develops neonatal brain connections and structures (Schore, 2001). One way in which this occurs is through the mother's ability to regulate the infant's production of neurohormones, particularly dopamine (Schore, 1996). Dopamine is a critical neuromodulator in pathways associated with regulation of behavior; thus, genes that influence CNS dopamine levels have considerable influence on self-regulatory function (Posner & Rothbart, 2009). Research supports Schore's proposed genetic and epigenetic mechanisms for maternal effects on neurodevelopment. Children with genotypes associated with higher levels of dopamine have improved behavioral regulation, executive attention, and affect (Posner & Rothbart, 2009) and less impulsivity and risk taking (Sheese, Voelker, Rothbart, & Posner, 2007). Importantly, parenting quality interacts with these genes to influence behavior in young children (Posner & Rothbart, 2009; Sheese et al., 2007). Researchers have shown that, in animal models, quality of maternal care regulates gene expression

(Fish et al., 2004). Epigenetic changes associated with low quality of maternal care produce alterations in the infant's cellular activity that are directly related to behavioral abnormalities, including diminished exploratory behaviors, increased anxiety, hyperactive locomotion, and cognitive deficits (Burton, Lovic, & Fleming, 2006; Coplan et al., 1996; Jackowski et al., 2011). Another series of studies in rat pups has demonstrated that higher quality maternal care is associated with reduced CRH mRNA expression in the hypothalamus and the amygdala (Meaney, 2001). These epigenetic changes were associated with reduced stress reactivity in the pups. These human- and animal-based studies provide significant support for Schore's regulation theory, which identifies genetic modification as a key mechanism for maternal effects on infant behavior (Fish et al., 2004; Meaney, 2001; Sheese et al., 2007).

Impact of Neurobiology on Dyadic Affect Synchrony

The ability of the mother to provide suitable stimuli for and induce neurobiological changes in the infant allows for development of emotional regulation within the infant and affect synchrony within the dyad. According to Schore, affect synchrony is the mutual adjustment of stimulation, arousal, and attention in order to match affective states between mother and infant (Schore, 2001). In essence, the mother assists the infant in processing stimuli during face-to-face interactions by adjusting her behavior to the infant's abilities, creating similar inner psychological states. The mother's and infant's right brains are each responsible for processing the facial and emotional expressions emitted from the other (Schore, 2001; Strathearn, Li, Fonagy, & Montague, 2008). Together, the right brains of the dyad form a communication system, grounded in

biology, in which the dyad's right limbic systems subconsciously exchange emotional information and mirror themselves during the interaction (Schore, 2001). This psychobiological mirroring allows the mother and infant to experience the same emotions during maternal–infant interaction. Moreover, repeated, synchronized, and positive interactions organize the neonatal right brain by increasing the utility and connectivity between cortical (prefrontal and orbitofrontal cortex) and subcortical (amygdala, hippocampus, hypothalamus, NAC, VTA) brain regions. Ultimately, affect synchrony and the psychobiological communication system it generates support the development of infant self-regulation and attachment (Feldman, 2007).

Application of Schore's Regulation Theory to the Premature Infant

Schore's regulation theory is valuable for understanding the complexities that surround neurodevelopmental outcomes in premature infants. While maternal behavior during interaction provides a large proportion of the environmental stimuli responsible for neurodevelopmental outcomes in healthy infants, the physical and social characteristics of the NICU environment have a critical influence on neurodevelopmental outcomes in premature infants. Maternal stimuli during repeated interactions in the NICU may either counteract the negative effects of environmental stimuli or serve as an additional stressor if the interactions are not supportive. A model depicting proposed relationships is presented in Figure 2.

The neurodevelopmental outcomes of premature infants are a unique case because development of the immature brain and CNS must occur in the extrauterine environment. In addition, the beginning stages of the maternal–infant relationship, critical for fostering

neurodevelopment, occur within the environmental context of the NICU. Importantly, the effects of early maternal–infant interactions can impact the quality of the attachment that evolves over time. Mehler and colleagues (2011) found that mothers who interacted with their premature infant in the period immediately following delivery were more likely to have infants who exhibited secure attachment behaviors. Other researchers have demonstrated that premature infants who are insecurely attached have an increased number of neurodevelopmental delays when compared to premature infants who are securely attached (Brisch et al., 2005). Therefore, in considering long-term neurodevelopmental outcomes, it is important to understand both the maturational trajectory of the brain and CNS and the qualitative nature of the maternal–infant interaction occurring within the NICU.

Neurological Development

Neurological maturation. Development of the brain and CNS follow an intricately complex plan. Maturation of the limbic circuit (amygdala, hippocampus, dopamine pathways) occurs early in gestation. Thus, these structures are most likely adequate in the premature infant (Arnold & Trojanowski, 1996; Machado & Bachevalier, 2003). In addition, the major neurotransmitters, including dopamine and norepinephrine, are present in early gestation (de Graaf-Peters & Hadders-Algra, 2006), while cortisol is not produced until after 24 weeks' gestation (Nykanen, Heinonen, Riepe, Sippell, & Voutilainen, 2010). During the second and third trimesters, the brain undergoes significant cell migration, neurogenesis, neuronal differentiation, synapse formation and elaboration, axonal ingrowth, glial cell proliferation, and myelination (de Graaf-Peters &

HaddersAlgra, 2006; Lubsen et al., 2011). During the last trimester, pruning and plasticity of the infant brain is at its highest, with almost 50% of neurons in the developing brain experiencing programmed cell death (Volpe, 2000). Animal models have shown that connections between the orbitofrontal cortex and the prefrontal cortex develop during the last trimester and that dopamine innervation of the orbitofrontal cortex matures near birth (Machado & Bachevalier, 2003). Therefore, it is highly likely that in premature infants the orbitofrontal cortex and its connections with the mesocortical and mesolimbic pathways are extremely immature.

Premature birth interrupts neurodevelopment at a critical stage. Depending on gestational age, many of the neurological structures are still actively differentiating and organizing at birth, and these maturational processes now must occur within the context of the NICU. In this highly technological environment, premature infants are exposed to numerous physiologic as well as environmental stressors that have the potential to permanently alter the developing brain and CNS (Pickler et al., 2010). These stressors include the presence of noxious stimuli such as excessive noise, light, handling, and painful procedures (Carbajal et al., 2008) as well as the absence of important regulating interventions such as holding, breastfeeding, and comforting touch (Hanley, 2008; Lester et al., 2011). Physiologic stressors include infection and other health disruptions related to the infant's physiologic immaturity (Bhutta & Anand, 2002; Volpe, 2009). Through exposures to these multiple stressors, premature infants face high rates of additional neuronal cell death. Excessive cell death and, ultimately, reduced brain volume result in impaired development of brain structure and function (Volpe, 2009). This vulnerability to

neuronal cell death early in life increases risk of damage to the orbitofrontal cortex and its connecting pathways. In animal models, damage to these areas causes devastating effects on socioemotional development (Machado & Bachevalier, 2006).

Effects of stressors on premature infant neurobiology. Repetitive exposure to stressors in the NICU environment may cause abnormal levels of neurotransmitters and neurohormones and has the potential to prevent and disorder neuronal network formation and the functional capabilities that develop as a result of structural brain growth (Grunau, Holsti, & Peters, 2006; Grunau et al., 2009). Alterations in these neurobiological processes during this critical period of brain maturation may significantly contribute to allostatic load in premature infants. These infants begin life acutely ill and facing a large number of environmental stressors, resulting in immediate activation of allostatic processes meant to protect homeostasis. These allostatic processes result in changes in behavior, heart rate, blood pressure, oxygen saturation, stress hormone secretion, and intracranial pressure (Anand, 1998). Repeated exposure to stressors overwhelms allostatic systems and results in chronic dysregulation of allostatic processes. Pathophysiologic alterations commonly seen in premature infants, including hypotension, hyperglycemia, insulin resistance, apnea and bradycardia, hypoxemic episodes, and respiratory acidosis, could contribute to allostatic load in this population.

Another concern for premature infants is that the physiologic systems associated with allostasis are developmentally immature and may not be capable of responding appropriately. For example, impaired autonomic nervous system function (Longin, Gerstner, Schaible, Lenz, & Konig, 2006) and relative adrenal insufficiency (Fernandez

& Watterberg, 2009) may limit the ability of these systems to adequately respond to stressors. The premature infant could experience multiple sources of allostatic load, including (1) repeated hits from multiple different stressors, (2) failure to habituate to the same stressor, (3) prolonged response to a stressor, and (4) inadequate response to stressors (McEwen & Gianaros, 2011). The interaction between developmental immaturity and these different sources of allostatic load may impact the course of structural brain development and subsequent neurodevelopmental outcomes (Fernandez & Watterberg, 2009; Longin et al., 2006).

Because the brain is the central mediator of allostatic processes and promotes adaptation to stress, it is also the primary organ impacted by allostatic load (McEwen & Gianaros, 2011). It is well documented that, compared to term infants, premature infants have smaller brain volumes in the cortex (sensorimotor, premotor, midtemporal, parieto-occipital, and subgenual areas) and subcortex (the corpus callosum, amygdala, hippocampus, and basal ganglia) and display altered structural and functional connections in the brain (Lubs et al., 2011). Importantly, Smith and colleagues (2011) demonstrated a relationship between increased exposure to stressors and decreased brain size and altered neural network development in premature infants. These anatomical differences are correlated with poorer neurodevelopmental outcomes including motor delays (Gadin et al., 2012) and deficits in social–emotional behavior (Rogers et al., 2012) and cognitive performance (Myers et al., 2010).

Effects of altered neurobiology on function. Schore describes the functional capabilities that can be impaired as a result of alterations in neuronal network formation,

including attention, arousal, responsiveness, physiologic maturation, emotional regulation, learning, and memory formation (2001). Premature infants are known to have developmental delays or deficits in all of these areas (Atkinson & Braddick, 2007; Foster-Cohen, Friesen, Champion, & Woodward, 2010; O'Shea et al., 2008). The brain regions responsible for these functional capabilities (amygdala, hippocampus, hypothalamus) also bear the brunt of disease burden attributed to allostatic load (Ganzel, Morris, & Wethington, 2010). We hypothesize that the developmental alterations premature infants experience are developmentally appropriate indicators of allostatic load (McEwen & Gianaros, 2011).

Quality of Maternal–Infant Interaction

Consistent high-quality maternal–infant interactions are critical for maximizing neurodevelopment in premature infants. For these vulnerable dyads, the foundation for this relationship begins in the NICU environment. Numerous factors can impact maternal behavior in interacting with her infant, including physical separation between infant and mother (Wigert, Johansson, Berg, & Hellstrom, 2006). Animal models have demonstrated the critical importance of maternal physical presence on infant physiology, including regulation of autonomic, neuroendocrine, behavioral, and electrophysiological processes (Hofer, 1994). Mother's close physical presence through skin-to-skin contact (kangaroo care) is associated with enhanced autonomic regulation, state regulation, and cognitive development (Feldman & Eidelman, 2003) as well as accelerated brain development (Scher et al., 2009). Premature infants and their mothers often miss crucial interactions during the first few days and weeks of life due to the physiologic instability and

immaturity of the infant with resultant limitation on interaction through holding, feeding, and touching (Silberstein et al., 2009; Thoyre, Shaker, & Pridham, 2005). This absence of early maternal–infant interaction may contribute to impairments in the premature infant’s later capacities for emotional regulation and cognition (Silberstein et al., 2009).

In addition, physiologic dysregulation in response to multiple stressors in the NICU environment may inhibit the infant from organizing behavior during interactions (Anand, 1998). Absence of coordinated behaviors and clear cues serve as a source of frustration for mothers, as it is difficult to understand or respond to their infants’ needs. Clarity of infant cues thus affects maternal behavior, yet premature infants may not have the capacity to overcome physiologic dysregulation and appropriately contribute to the success of an interaction. As a consequence, developmentally supportive experiences with the mother are likely to be limited or prevented.

Another factor with the potential to impact the developing relationship is the numerous stressors mothers experience within the unfamiliar environment of the NICU. These stressors include not only physical attributes of the NICU such as intimidating life-support equipment, monitors, intravenous and nasogastric tubes, oxygen cannulas, isolettes, and alarms (Miles, Funk, & Kasper, 1991) but also psychosocial characteristics of the NICU experience such as uncertainty of the infant’s survival, loss of a healthy birth, grief over their infant’s health status, and powerlessness in the care of their infant (Obeidat, Bond, & Callister, 2009). The short-term effects of these NICU stressors include emotional reactions of sadness, guilt, disappointment, sense of failure, alienation, and loss of self-esteem (Dudek-Shriber, 2004). The mother must be able to overcome her

reactions to the stressful nature of the NICU in order to forge a relationship with her infant.

Maternal stress has significant long-term implications for maternal mental health, including psychiatric illnesses such as anxiety, depression, and stress disorders (Bellini, 2009; Shaw et al., 2009). In one study, approximately 35% of NICU mothers experienced acute stress disorder, 15% suffered from posttraumatic stress disorder (PTSD), and 39% met the criteria for postpartum depression (Lefkowitz, Baxt, & Evans, 2010). Mothers with postpartum depression are more likely than those without to perceive themselves as incompetent caregivers and have lower parental satisfaction (Ngai, Wai-Chi Chan, & Ip, 2010). Maternal psychiatric illness contributes to negative parenting behaviors and poor health outcomes in their children (Wan & Green, 2009). Given that mental illness is known to adversely affect attachment outcomes (Wan & Green, 2009), and that mothers already report problems with bonding in the NICU (Obeidat et al., 2009), mothers of premature infants are at high risk of strained relationships and poor attachment with their vulnerable infants (Forcada-Guex, Borghini, Pierrehumbert, Ansermet, & Muller-Nix, 2011). The consequences of postpartum stress exposure in mothers are likely to affect neurodevelopmental outcomes for premature infants through effects on the quality of their interaction with their infant.

Suboptimal Interactions and Infant Neurobiology

A positive interaction can serve as a protective factor in decreasing the risk for long-term neurodevelopmental problems in preterm infants (Nicolaou, Rosewell, Marlow, & Glazebrook, 2009). Negative interactions, on the other hand, can prevent the infant

from receiving neuroprotective maternal stimulation and increase risk of neurodevelopmental problems. Premature infants who are insecurely attached have more impairments in neurodevelopment than those with more secure attachments (Brisch et al., 2005; Forcada-Guex, Pierrehumbert, Borghini, Moessinger, & Muller-Nix, 2006). Researchers have characterized mothers of premature infants who are insecurely attached as being less sensitive, more controlling and intrusive, and inconsistent in their interactions (Forcada-Guex et al., 2006; Poehlmann et al., 2011). These mothers are not able to provide the stimulation, sensitivity, and supporting behaviors required for the development of critical neurobiological processes.

There is little research on the neurobiologic effects of suboptimal interactions in human infants. Research with animal models demonstrates associations of inadequate maternal care with low levels of hippocampal glucocorticoid receptors (Ladd, Huot, Thirvikraman, Nemeroff, & Plotsky, 2004). Fewer glucocorticoid receptors result in inefficient regulation of the negative feedback loop responsible for stopping the stress response. Researchers have also seen hypersensitivity in dopamine, opioid, norepinephrine, and serotonin receptors, which is associated with anxious and depressive behaviors (Lewis, Gluck, Beauchamp, Keresztury, & Mailman, 1990; Martin, Spicer, Lewis, Gluck, & Cork, 1991; Rosenblum et al., 1994). These changes in receptors result in hyper-reactive or inadequate responses to stressful stimuli, significantly increasing the organism's allostatic load. Intermittent maternal separation in rats leads to HPA-axis hyperactivity and high levels of CRH, ACTH, and norepinephrine (Coplan et al., 1996). These abnormally high levels of glucocorticoids and catecholamines are associated with

apoptosis, decreased brain volume, and impaired circuitry of the mesocortical and mesolimbic pathways (Fuchs, Czeh, Kole, Michaelis, & Lucassen, 2004; van der Beek et al., 2004). Long-term behavioral consequences of this toxic neurochemistry and subsequent alterations in brain structure result in affective disorders such as depression, anxiety, PTSD, and chronic fear (Fuchs et al., 2004; Gunnar & Quevedo, 2008). These findings in animal research have important implications for human infants, suggesting that, while a sensitive caregiver is a significant buffer to hyper-reactivity of the stress response, an insensitive caregiver can be a significant source of stress.

Given that quality of caregiving is related to structural and functional brain development in infants, the effects of the characteristic NICU environment on the quality of maternal–infant interaction may be an important factor in infant neurodevelopment. Nurses can help mitigate the risk of the stressful NICU environment and support neurodevelopment in these vulnerable infants by facilitating high quality maternal–infant interaction.

Clinical Implications

Understanding the neurobiological pathways through which maternal behavior impacts neurodevelopment and attachment outcomes helps clinicians in caring for vulnerable premature infants and their mothers. Using regulation theory as a framework, nurses can assess infants and mothers and implement interventions that enhance maternal–infant interaction and promote attachment.

According to regulation theory, high-quality maternal care may be neuroprotective, suggesting several areas of potential intervention: First, all mother-infant

dyads need to be assessed for maternal mental health, maternal responsiveness, and quality of the dyadic interaction. Assessment should include the level and impact of chronic stressors occurring in the NICU that are risk factors for poor neurodevelopment and insecure attachment. These stressors include prolonged maternal separation, lengthy hospital stays, high severity of infant illness, and presence of maternal mental illness (Bellini, 2009; DudekShriber, 2004). Infants and mothers with these risk factors require additional attention, assessment, and intervention. Interventions could include provision of educational resources, such as teaching mothers about premature infant behaviors, how to interpret cues, and how to best support development; referrals for mental health or developmental services (e.g., counseling, social work, and family therapy); and referrals to community, social, and financial support resources, such as NICU parent support groups (Fowles & Horowitz, 2006). Second, interventions need to be directed at supporting infant physiologic responses and enhancing maternal responsiveness and sensitivity through maintaining proximity of mother and infant and enhancing contact between members of the dyad (Feldman, Weller, Sirota, & Eidelman, 2003). Contact can be maximized through kangaroo care, breastfeeding, rooming in, and nurturing touch or massage. Third, minimize the adverse environmental stimuli inherent in the NICU through effective pain control, clustered cares, noise reduction, and cycled lighting. Research has shown that these interventions reduce infant physiologic stress and improve neurodevelopmental outcomes (McAnulty et al., 2009). Finally, and most importantly, place mothers at the center of NICU nursing care by ensuring that they are respected collaborators in their infants' care, thus empowering them to be advocates for themselves

and their infants. Such active participation enables mothers to claim a more active role in parenting, significantly reduces maternal stress levels, and promotes attachment (Browne & Talmi, 2005; Melnyk et al., 2006).

Implications for Research

Schore's regulation theory provides a rich framework for examining psychological, social, biological, and behavioral factors associated with infant neurodevelopment that can incorporate infant, maternal, and dyadic health (Schore, 2001). To date, research examining maternal–infant interactions in the premature infant population has primarily focused on relationships between maternal–infant interactions during feeding and infant feeding skill in the first year of life (Pridham, Steward, Thoyre, Brown, & Brown, 2007), infant physiological responses during feeding (Brown, 2007; Thoyre & Brown, 2004), weight gain and motor development (Pridham, Brown, Sondel, Clark, & Green, 2001), and behavioral measures of neurodevelopment (Feldman & Eidelman, 2006). Research is urgently needed to investigate effects of maternal–infant interaction within the context of the NICU environment on structural brain development, stress reactivity, and other biological measures of neurodevelopmental outcomes. Additionally, the concept of allostasis in the context of an immature infant developing in an intensive care environment needs to be more clearly characterized and defined. Allostatic systems and allostatic load, when applied to a developing infant or young child, reflect early experience and immature development of the mediating systems rather than the acute responses of mature systems and the cumulative wear and tear associated with allostasis and allostatic load in adults (Johnson et al., 2011). Research is needed to

examine the effects of this early and repeated stimulation of immature nervous, endocrine, and immune systems and the potential moderating effects of maternal caregiving on the infant's adaptive allostatic responses and future health.

Although effects of the NICU experience on maternal mental health have been studied, we know little about relationships among maternal neurobiology, parenting skills, and infant neurobiology. Research at the dyadic level could examine biobehavioral influences of the NICU environment on infant and maternal affect synchrony, resonance, and mutual physiologic regulation. Longitudinal analyses of biobehavioral outcomes of stress in the dyad are critical for determining the effects of early experience on parenting quality, maternal health, infant neurodevelopment, and dyadic attachment over time. Research addressing the broader effects of the physical, socioemotional, and psychological characteristics of the NICU environment on infant neurodevelopment, allostatic load, and attachment outcomes is critically needed. While investigators have examined individual environmental factors, future research must analyze these factors concurrently and cumulatively. Sophisticated analytic methods and multisite studies allowing for large sample sizes will be needed to parse out effects associated with infant health condition, maternal caregiving, and physical environment.

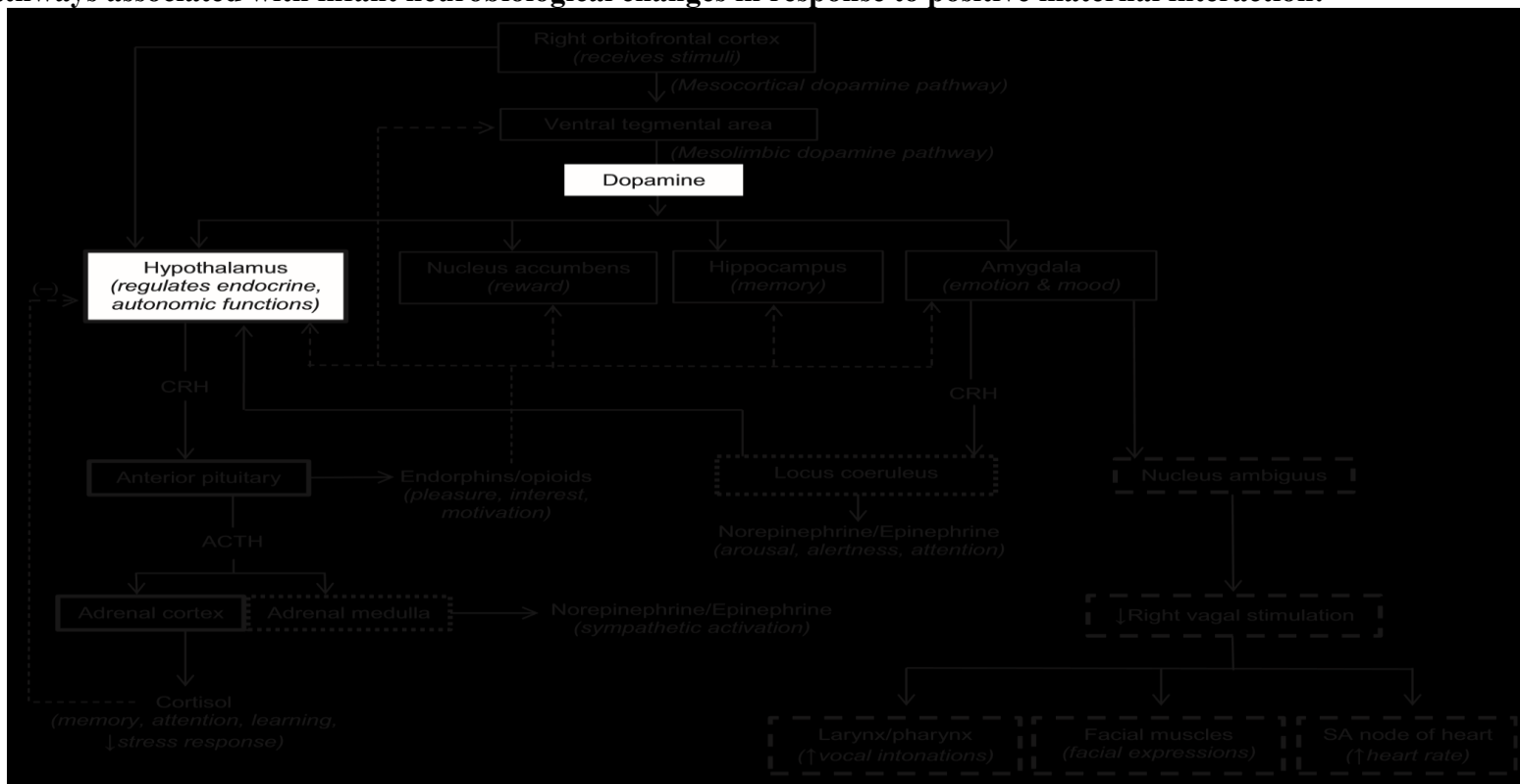
Conclusion

Schore's regulation theory provides a useful framework for understanding the NICU experience of premature infants and their mothers as well as for providing nursing care to these vulnerable families. During this sensitive period, repeated exposure to stressors has the potential to alter brain structure and function through chronic

dysregulation of allostatic processes. Premature infants are at high risk of significant allostatic load and associated physical, emotional, and behavioral problems. Enhancing neuroprotective maternal–infant interactions may moderate the adverse effects of the stressful NICU environment, reduce allostatic load, and improve infant neurobehavioral and attachment outcomes. Research is critically needed to develop and test nursing interventions directed at assisting mothers in supporting optimal neurodevelopment in their premature infant.

Figure 1. Pathways associated with infant neurobiological changes in response to positive maternal interaction.

29



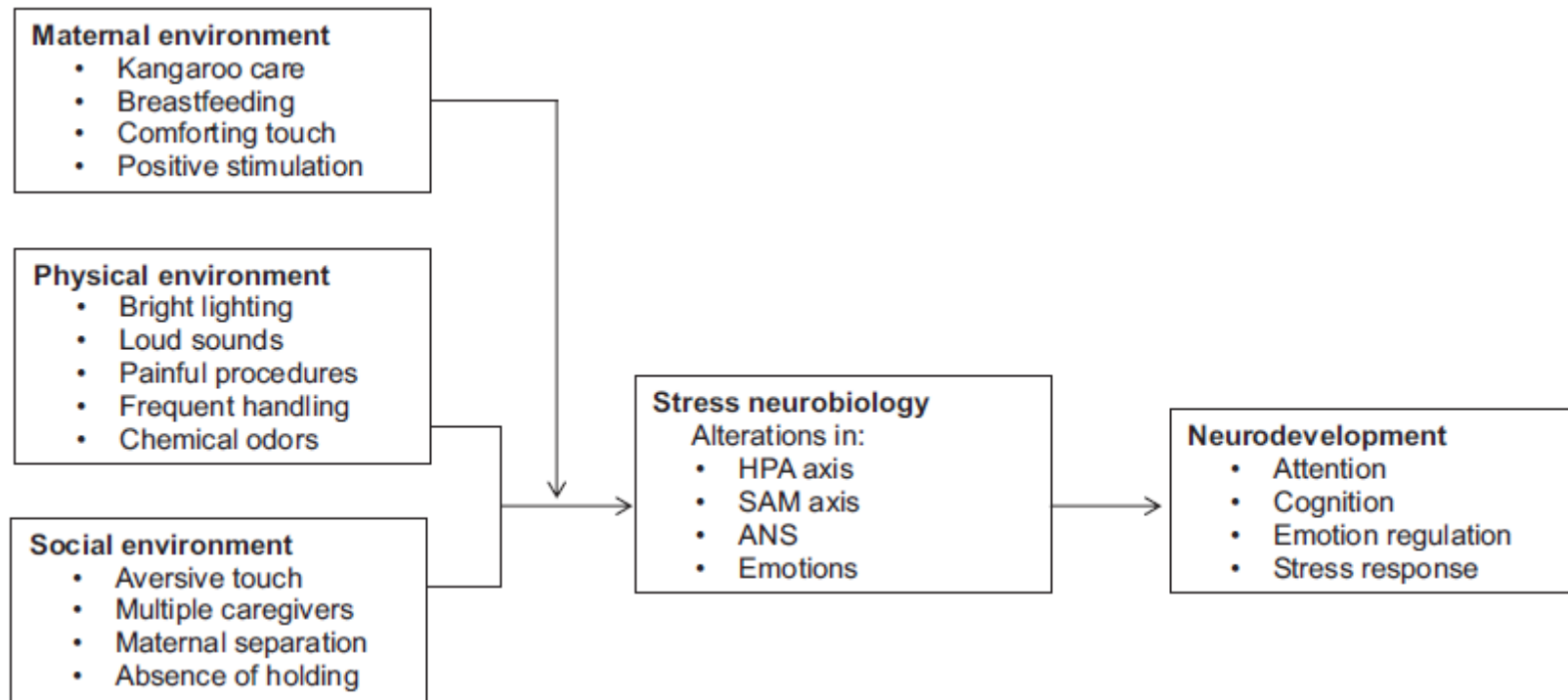
ACTH = adrenocortiotropic hormone; CRH = corticotrophin-releasing hormone; SA = sinoatrial. Thick black-bordered boxes indicate hypothalamic–pituitary–adrenal (HPA) axis.

(Continued)

(Figure 1 continued)

Dotted boxes indicate sympathetic-adrenal-medullary (SAM) axis. Dashed boxes indicate regulation of parasympathetic nervous system. Dashed lines indicate feedback loops. Stimuli received by the orbitofrontal cortex are relayed to the ventral tegmental area (VTA) through the mesocortical dopamine pathway. Through the mesolimbic dopamine pathway, the VTA releases dopamine into the NAC, hippocampus, and amygdala. Dopamine is also released into the hypothalamus, which initiates the HPA axis. Progression of the HPA axis results in endogenous opioid release and ACTH from the pituitary, cortisol release from the adrenal cortex, and norepinephrine (NE) and epinephrine (Epi) release from the adrenal medulla. Opioids enhance pleasure, interest, and motivation and augment dopamine release from the VTA in a positive feedback loop. Cortisol increases attention, memory, and learning and inhibits CRH secretion from the hypothalamus in a negative feedback loop. NE and Epi in the periphery increase cardiovascular responses and sympathetic activity. Dopamine also activates the amygdala to release CRH, signaling the locus coeruleus to release NE and Epi. NE and Epi act in the brain to increase arousal, attention, alertness, and sympathetic activity. NE and Epi in the brain also stimulate the hypothalamus to release CRH which also activates the HPA axis. Parasympathetic activity is regulated through dopaminergic activation of the amygdala. The amygdala regulates the nucleus ambiguus, source of the right vagus nerve. Parasympathetic activity is regulated through dopaminergic activation of the amygdala. The amygdala regulates the nucleus ambiguus, source of the right vagus nerve. In a positive, stimulatory interaction, the vagus withdraws parasympathetic activity to the larynx, pharynx, facial muscles, and sinoatrial node of the heart. Change in vagal regulation causes changes in vocal intonations, facial expressions, and heart rate during the interaction.

Figure 2. Impact of the neonatal intensive care unit (NICU) environment on stress neurobiology and development in premature infants.



31

The physical and social environments of the NICU result in stress neurobiology that is suboptimal for development. However, the environment provided by sensitive and responsive maternal caregiving can moderate this risk by providing positive stimulation and supporting neurobiological processes that are conducive to optimal development. ANS = autonomic nervous system; HPA = hypothalamic–pituitary–adrenal; SAM = sympatho-adrenal medullary.

Chapter 3: Maternal-Infant Interaction and Oxytocin-based Processes underlying Infant Neurodevelopment

Oxytocin (OT), an affiliation hormone released during supportive social interactions, provides an exemplar of how social environments are reflected in our neurobiology from the beginning of life. Over the past several decades, a growing body of OT research has uncovered previously unknown functions of OT, including modulation of parenting behaviors, neuroprotection, affiliation, and bonding. Nurses are in a unique position to use innovative discoveries made by the biologic sciences to inform nursing practice and to advocate for health care interventions that improve patient outcomes. Improvement of outcomes depends on a deeper understanding of neurobiological processes that foster resilience and adaptation in the context of high-risk social environments.

Regulation Theory provides a strong framework for describing how a mother's social interactions with her infant affect neurodevelopment through a symphony of molecules that form the neurobiological milieu of the developing infant brain (Schoore, 2001). Researchers must examine not only how each molecule contributes to the milieu, but also how each contributes to brain structure, function, and outcomes. In a previous paper detailing the basic tenets of Regulation Theory in the context of infancy (Weber et al., 2012), we reviewed how social stressors negatively influence infant brain biology,

producing aberrant changes in brain structure and function and contributing to neurodevelopmental impairments. In the current paper, we discuss how OT-based social processes contribute to infant neurobiology.

We propose that early, consistent, and developmentally-supportive social interactions positively influence brain biology—not only by protecting the developing structure of the infant brain during times of stress, but also by augmenting the maturation of the developing brain in a way that contributes to optimal neurodevelopment. OT is necessary for a variety of neural processes related to neurodevelopment, an umbrella term that refers to the generation, shaping, maturation, reshaping, and regeneration of the nervous system throughout life. OT's critical role in infant neurodevelopment has been described in scientific discoveries within the last decade (Vargas-Martínez et al., 2014).

The purpose of this paper is to describe the unique role that OT plays in facilitating affiliation, bonding, and infant neurodevelopment. At the time Schore developed Regulation Theory, OT research was its early stages. This paper offers an extension to Regulation Theory by adding OT's unique contribution as a player in the brain's neurobiological symphony underlying infant development. In the following sections, we argue that OT is a necessary and critical component for optimal neurodevelopment of all infants. We structure our discussion of the socially-based, neurobiological processes of OT through its effects in the nested hierarches of genetic, epigenetic, molecular, cellular, neural circuit, multi-organ, and behavioral levels (Figure 3). Our discussion is also presented chronologically, as OT works through numerous positive feedback loops during infant neurodevelopment, beginning prenatally and

continuing through life (Figure 4). While we present our argument in terms of positive relationships, we acknowledge that *regulation* of OT levels within the ideal range, for each brain region and context of the stimulus, is essential for optimal neurodevelopment.

The Genetic Level: Prenatal Effects with and without Oxytocin

Numerous knockout models have been used to determine the developmental and functional importance of OT, including models that disrupt or delete (1) OT genes, (2) neurogenesis of OT neurons, (3) compounds necessary for OT synthesis (e.g. the CD38 gene), (4) OT-based signaling pathways, (5) OT receptors (OTR), or (6) OT uptake from the cell. These transgenic animals suffer from numerous social and physiological impairments, including profound social amnesia, neglectful parenting, aggression, depression, anxiety, hyperactivity, social ineptness, dysregulation of energy balance, and many other functional deficits (Higashida et al., 2010). These models, along with data from human OT-based genetic syndromes, provide strong evidence of the significant developmental effects of dysregulation within the OT system (Francis et al., 2014). Syndromes in humans associated with OT dysregulation, such as Prader-Willi, Fragile X, and Williams Syndrome, produce profound deficits in socioemotional regulation and cognition, evidenced by the common symptoms of anxiety, emotional lability, hyperactivity, impulsivity, decreased “theory of mind”, lack of empathy, and developmental delays (Francis et al., 2014). Many of these social deficits are also seen in high-risk infants with greater prevalence than their healthy term peers (Msall & Park, 2008).

Genetic priming of the dyad’s OT systems begins during pregnancy (Figure 4,

Stages 1-2). Estrogen, heightened during pregnancy, parturition, and post-partum, is a powerful augmenter of maternal OT and OTR (Broad, Kendrick, Sirinathsinghji, & Keverne, 1993). The expression and distribution of maternal OTR noticeably increase throughout pregnancy and lactation, especially in regions containing the dopaminergic reward pathways (Baskerville & Douglas, 2010). Moreover, vagino-cervical stimulation during labor produces a surge of OT (Figure 4, Stage 3), which results in the initiation of supportive maternal care after birth (Figure 4, Stage 4) and the promotion of bonding (Keverne & Kendrick, 1994). In turn, post-partum supportive maternal behaviors reduce methylation within the estrogen ER α gene, causing up-regulation of the ER α gene promoter, and increased estrogen sensitivity (Sharma & Meaney, 2001). This methylation further increases OTR expression in both mother and infant (Francis, Champagne, & Meaney, 2000), and continues the positive feedback loop of the estrogen/OT cycle after birth (Figure 4, Stages 5-6). This positive feedback loop was recently demonstrated in human generational studies, in which maternal variants of the OTR and/or CD38 genes were shown to be associated with infant genes, both maternal and infant peripheral OT levels, and the infant's socioemotional health (Apter-Levy, Feldman, Vakart, Ebstein, & Feldman, 2013; Feldman, Monakhov, Pratt, & Ebstein, 2015). An infant's genetic profile will ultimately affect socioemotional health and neurodevelopment—however, it is through modifiable epigenetics that nurses will be able to provide supportive social interventions and promote best outcomes.

The Epigenetic Level: Maternal Behavior and Postnatal Effects

The previous paragraphs describe genetic factors that prenatally program the

infant's OT system. After birth, supportive maternal behaviors program development of the infant's OT system through epigenetic changes (Sharma & Meaney, 2001; Szyf, Weaver, & Meaney, 2007). Using animal models, Meaney and colleagues brilliantly demonstrated effects of maternal behavior on infant's OT system (Meaney, 2001). High licking and grooming (HLG) dams (i.e. female rats) display supportive maternal behaviors, such as the nurturing touch provided by licking, grooming, and arched backed nursing. These HLG dams and their pups both express high levels of OT and OTR (D. D. Francis et al., 2000). However, low-licking and grooming (LLG) pups reared in HLG environments also express increased levels of OT and OTR (Sharma & Meaney, 2001). In sum, all pups reared in HLG environments had greater OTR binding and increased brain activity in regions associated with social behaviors (Champagne, 2009). The pups also displayed decreased anxiety, increased exploration, and greater response to novelty (Szyf et al., 2007). These revolutionary studies demonstrated that supportive maternal behaviors modify the epigenetics of infant OT/OTR expression. Importantly, maternally-induced epigenetic changes in the pups translated into changes in infant social behaviors.

Translational research in humans is beginning to echo the important findings of animal models. In human mothers, variants of the OTR and/or CD38 genes are associated with higher peripheral OT levels, and these mothers display more supportive parenting behaviors, such as maternal warmth, touch, speech, gaze, contingent responses, and high quality of affect (Feldman et al., 2015). OT levels across pregnancy and postpartum are not only associated with frequency of supportive maternal behaviors, but also with optimal coordination of interaction with the neonate's alert state (Feldman, Gordon,

Schneiderman, Weisman, & Zagoory-Sharon, 2010; R. Feldman, Gordon, & Zagoory-Sharon, 2011). Parents with higher OT levels also provide more emotionally rich descriptions of their neonates (Feldman, Weller, Zagoory-Sharon, & Levine, 2007). Additionally, parents who display more affectionate touch show significantly greater increase in their own OT levels after interaction (Feldman et al., 2010).

Like the HLG dummies, human mothers demonstrating highly supportive behaviors activate and enhance development of OT systems in their infants through epigenetic changes. These mothers raise children who show higher levels of OT, as well as higher levels of empathy, engagement, social reciprocity, and psychological wellness (Apter-Levy et al., 2013). These infants have increased social cognition at 18 months, as well as increased affect recognition, non-verbal skills, and pragmatic language skills as children (Wade, Hoffmann, Wigg, & Jenkins, 2014). Importantly, infants who inherit low-risk OT alleles and experience developmentally-supportive maternal care become children with more highly regulated OT systems and the best neurodevelopmental outcomes (Apter-Levy et al., 2013; Ruth Feldman, 2015c, 2015d).

Priming: Maternal Stimuli for the Infant's Developing OT System

According to Regulation Theory, a supportive mother provides developmentally-appropriate sensory stimuli during interactions that contribute to the development of the growing infant brain (Schoore, 2001). These sensory stimuli include a mother's holding environment, soothing voice, loving gaze, animated facial expressions, comforting scent, and nourishing breastmilk. New research is uncovering the processes through which OT facilitates the sensory processing of tactile, auditory, visual, olfactory, and gustatory

stimuli. Recent research using animal models demonstrate that OT “mediates early experience–dependent cross-modal plasticity in the sensory cortices” (Zheng et al., 2014, p. 391). For example, OT underlies such sensory experiences as odor recognition (Wacker & Ludwig, 2012), attentiveness to facial expressions (Luo et al., 2015), and neuromodulation of the gustatory circuit (Beets, Temmerman, Janssen, & Schoofs, 2013). OT works within the prefrontal cortex to process signals from the olfactory bulb, auditory cortex, visual cortex, and gustatory circuit to facilitate high-order cognitive functions such as socially-based learning, motivation, and dominance hierarchy (Bicks, Koike, Akbarian, & Morishita, 2015). OT enhances the neural processing of facial stimuli by the orbitofrontal and visual cortices to attract the dyad to each other’s social cues (Luo et al., 2015).

These biological discoveries are of critical importance to human infants, showing that sensory experience controls the production and release of OT (Freeman, Inoue, Smith, Goodman, & Young, 2014). Subsequently, OT in the brain and body enact a variety of critical cognitive-based social functions, such as “modulating visual attention, processing auditory and multimodal sensory stimuli, and controlling orienting responses to visual stimuli,” skills that are critical to the development of an infant’s socioemotional health (Freeman et al., 2014, p. 128). In sum, Zheng and colleagues conclude, “Sensory experience is critical to development and plasticity of neural circuits” (Zheng et al., 2014, p. 391). In the following sections, we will review how the OT system, in coordination with other key neurobiological systems, uses sensory experiences from supportive social interactions to shape the developing infant brain.

The Molecular Level: G-Proteins, Receptors, and OT

The breadth and depth of OT's ability to shape the developing infant brain is based in gene transcription and cell-signaling pathways required by neurons to synthesize proteins necessary for all of the key neural processes involved in neurodevelopment (Figure 5).

OT is a nine-amino acid peptide whose receptor is part of the G-Protein-Coupled Receptor (GPCR) family, the body's largest class of membrane receptors. Once activated, GPCRs open ion channels on the cell membrane to influence enzymes and second messenger molecules that travel and communicate within the cell (Gimpl & Fahrenholz, 2001; Koehbach, Stockner, Bergmayr, Muttenthaler, & Gruber, 2013). These second messenger molecules can also open ion channels (e.g. calcium channels) inside the cell to further affect membrane charge and polarity. Through manipulation of intracellular and extracellular ion stores, OT influences the biochemical activity of many subcellular components, including enzymes, ion channels, the cytoskeleton, and ion pumps. Moreover, OT's influence on ion stores produces positive charge in the cell (i.e. depolarization), as well as voltage changes on the cell membrane (an electric potential).

The Cellular Level: Membrane Depolarization and Development of Autoregulation

The intracellular signaling properties of OT have cellular and extracellular implications for the OT neuron. Depolarizations and electric potentials provide the basis of neuron survival and function, as they allow the neuron to receive, process, and respond to stimuli. Electric potentials are also the primary mode of neuron-to-neuron communication (Vargas-Martínez et al., 2014). OT-induced depolarizations travel along

the cell membrane to create potentials which release OT-filled vesicles into the extracellular space. In this way, the OT neuron also uses autocrine effects to *autoregulate* (Philippe Richard, 1998). Autoregulation is a unique autocrine property of OT neurons—it allows OT neurons to control their own firing activity locally, with their own receptors and peptides. For the developing infant brain, autoregulation means that a single neuronal stimulus can “prime” OT neurons, allowing them to release OT for long periods in response to a previous stimulus and in anticipation of future ones. OT neurons, on extended-release autoregulation, seep OT locally to affect surrounding groups of OT neurons (Lemos et al., 2012; Philippe Richard, 1998). In this way, for example, a kangaroo care nursing intervention immediately after birth can prime and autoregulate OT neurons for extended periods of time.

Through seepage, OT can diffuse into immediate brain regions (e.g. lateral septum, amygdala, and hippocampus) at such high concentrations from the hypothalamus that OT can regulate key neural circuits within the brain (Vargas-Martínez et al., 2014). Seepage of OT into surrounding brain areas categorizes OT as a neuromodulator, or regulator of neuron groups. In addition to seepage from the soma and dendrites, axonal release of OT adds localized, time-sensitive control of distant neural circuits, which produce the complex social behaviors required during maternal-infant interaction (Vargas-Martínez et al., 2014). Through these combinations of dendritic, somatic, and axonal release of OT, the OT neuron can clearly communicate with other neural circuits by a peptide-induced “Morse Code.” Like Morse code, the OT neuron’s peptide message is interpreted through the frequency, duration, timing, strength, and overall pattern of OT

release (Vargas-Martínez et al., 2014).

These diverse release mechanisms of OT, as well as the distribution and number of OTR in specific brain regions, provides OT with pinpoint accuracy in communication and collaboration with other neural circuits (Viero et al., 2010). This pinpoint accuracy is informed within the context of experience-dependent neuromodulation of OTR expression and OT release within the brain (Carter, 2014). In the next section, we will describe how the OT system establishes itself and other key neural circuits through neuronal communication.

The Neural Circuit Level: Neurotrophins and Establishment of Circuits

Before neural circuits are established (Figure 5), OT must interact with many neuromodulators, all hallmarked by their central role in development: neurotrophins, cholecystokinin (CCK), γ -amino-butyric acid (GABA), and glutamate, among others. OT significantly increases the levels of neurotrophins, a group of proteins which act as growth factors to stimulate and control neurogenesis in the developing infant brain (see Figure 5). Neurotrophins can also prevent excessive apoptosis and induce differentiation of progenitor cells into neurons (Askvig, Leiphon, & Watt, 2012). Specifically, research has shown that OT increases levels of brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), ciliary neurotrophic factor, (CNTF), and CCK (Askvig et al., 2012; Bakos, Lestanova, Strbak, Havranek, & Bacova, 2014; Havranek et al., 2015).

Hypothalamic OT neurons co-express CCK, and release CCK in response to neural activity (Verbalis, McCann, McHale, & Stricker, 1986). Like estrogen, CCK is a powerful augmenter of OT and OTR. More importantly, CCK can induce OT release

even in the absence of neural activity (Hashimoto et al., 2005; Iwasaki et al., 2015). CCK stimulates the synthesis of neurotransmitters, peptides, and trophic factors necessary for proteins and enzymes that form circadian rhythms (Weller & Feldman, 2003), critical for optimal neurodevelopment. CCK, along with BDNF, NGF, and CNTF, also stimulates the synthesis of neurotransmitters, formation of neuronal spines and synapses, and elongation of axons. By participating in these key neural processes, neurotrophins regulate neuron excitability, differentiation, connectivity, and survival (Bakos et al., 2014; Hill, Warren, & Roth, 2014).

OT communicates not only with neurotrophins, but also with GABA, to augment the aforementioned neural processes. Indeed, an immature neuron's excitability, differentiation, connectivity, and *phenotype* (i.e., whether it is excitatory, inhibitory, or both) determine its integration pattern within organized and efficient neural circuits (Ben-Ari, 2014). During early infant brain development, OT facilitates the shift of GABA's actions from excitatory to inhibitory (Ben-Ari, 2014), and therefore assists in determining the *phenotype* of a neuron.

GABA, like OT, uses electrical activity of the cell membrane as a "Morse code", through the duration, timing, strength, and pattern of neuronal firing. Based on previous messages, GABA can induce either a strong hyperpolarizing or strong depolarizing action on the immature neuron (Cellot & Cherubini, 2013). These differentiating and distinctive electric signals tell the neuron when to "switch" potential (i.e. hyperpolarize or depolarize), and also act as a conductor for integrating a neuron into its rightful circuit. This developmental shift of GABA is essential for the establishment and integration of

the most fundamental neural circuits in the human brain (Cellot & Cherubini, 2013). Thus GABA, like OT, influences neural plasticity through the activity of controlled, precise, and experience-dependent electronic potentials within neural circuits (Cellot & Cherubini, 2013).

OT-GABA interactions produce giant depolarizing potentials (GDPs) within the brain (Cherubini, Griguoli, Safiulina, & Lagostena, 2011). A GDP is the synchronous depolarization, (i.e. simultaneous activation), of a large group of neurons. GDPs are essential to forming synaptic connections that create functioning brain networks (Mohajerani & Cherubini, 2006). When groups of neurons are consistently and simultaneously depolarized, the number and strength of their synaptic connections increase in a structurally profound way, and lead to the formation of a neural circuit (Egorov & Draguhn, 2013). In essence, the brain requires consistent and synchronized activity to establish its circuits. If the appropriate developmental sequence of neuronal activity is interrupted or changed, then neurons and their functional circuits are pathologically altered in a way that contributes to developmental disorders of the brain (Dehorter, Vinay, Hammond, & Ben-Ari, 2012).

In summary, as a GPCR, OTR participate in an extraordinary range of physiologic functions (Koehbach et al., 2013) that explain OT's actions on a vast array of the brain's activities, such as reward, mood, social recognition, memory, bonding, and social engagement. OT, through combinations of somatic, dendritic, and axonal release, communicates with other neurons through its peptide messaging. OT can also integrate these neurons into developing or existing neural circuits (Ferri & Flanagan-Cato, 2012).

Finally, OT/GABA-induced GDPs create massive synchronization and organization of neuronal groups, leading to establishment and maturation of infant brain circuits. In this way, OT-based intracellular, cellular, and intercellular pathways mediate the link between early infant experience and neurodevelopment. The next section will discuss the main neural circuits of OT influence.

The Multi-Circuit / Organ-System Level: Established Circuits and the OT System

The primary site of OT synthesis is the supraoptic nuclei (SON) and the paraventricular nuclei (PVN) of the hypothalamus (Ferri & Flanagan-Cato, 2012). The hypothalamus is a key neurohormonal center, receiving numerous endocrine, metabolic, and neural signals from the body, processing these signals, and activating effector pathways to produce hormonal, autonomic, and behavioral responses (Calabrò et al., 2012) (see Figure 6).

A critical area of the brain for infant behavior and development, the hypothalamus regulates many physiologic functions, including thermoregulation, blood pressure, sleep cycles, and digestion. These functions generate infant behaviors of thirst, sleep, arousal, and hunger. Regulation of physiologic functions is the cornerstone for synchronized, supportive interactions and mother-infant attachment—social functions regulated by the OT system.

The Dopaminergic Reward Pathways

Projections of OT neurons in the central nervous system (CNS) are extensive (see Figure 6), including hypothalamic projections to the nucleus accumbens, hippocampus, amygdala, and ventral tegmental area (VTA), crucial components of the dopaminergic

reward system (Strathearn, 2011). The mesocorticolimbic dopamine pathways control responses to natural rewards, such as social interaction (Weber et al., 2012). Both dopamine and OT facilitate social behaviors, affiliation, bonding, and attachment (Ruth Feldman, 2015d).

Dopamine and OT closely coexist in many brain regions (e.g. VTA, nucleus accumbens, hippocampus, amygdala, prefrontal cortex), with parallel receptor binding sites and neuronal fibers (Insel, 2003). Numerous studies have shown that OT stimulates mesocorticolimbic dopamine neurons to release dopamine (Young, Lim, Gingrich, & Insel, 2001). Conversely, dopamine stimulates OT neurons, which co-express dopamine receptors, to release OT (Numan & Young, 2015). Co-regulation of brain areas such as the hippocampus and amygdala allow for OT/dopamine interactions to heighten the brain's assignment of importance to social cues during maternal-infant interaction (Love, 2014).

Relevant brain functions affected by OT/dopamine interactions include social recognition, preference, memory, desire for close proximity, and approach/engagement behaviors (Coria-Avila et al., 2014). In healthy dyads, these brain functions are recruited to support behaviors that define sensitive and nurturing maternal-infant interaction. For mothers with high levels of OT and maternal-infant synchrony, greater brain activations occur in the nucleus accumbens and dopaminergic reward pathways when viewing their infants. These mothers also display increased functional connectivity among reward pathways, emotion circuits, and empathy networks (Atzil, Hendler, & Feldman, 2014).

Hypothalamic-Pituitary-Adrenal Axis (HPA) and the Sympatho-Adreno-Medullary (SAM) Response System

Regulation Theory describes how supportive maternal stimuli provides the infant with the experience of adaptive responses to stress, through dopaminergic activation of the HPA axis and SAM stress response systems (Schoore, 2001). Healthy responses to stress bathe the infant brain with OT and other neurotransmitters and neurohormones, such as corticotrophin-releasing factor (CRH), adrenocorticotrophic hormone (ACTH), cortisol, endorphins, norepinephrine, and epinephrine (see Figure 6). OT is released during eustress and distress, but has differential effects based on the severity, duration, and type of stressor (Harari-Dahan & Bernstein, 2014).

For example, supportive maternal behaviors provide eustress experiences for the infant. Eustress releases OT from the PVN to stimulate ACTH secretion, and improves regulation of the HPA axis and SAM stress response systems (Jovanovic et al., 2014; J. A. Smith et al., 2015; Omri Weisman, Zagoory-Sharon, & Feldman, 2013). As a known anxiolytic (MacDonald & Feifel, 2014), OT decreases social anxiety and, thus, increases a mother's willingness to engage and interact with her infant. In this way, OT's direct effect on anxiety has an indirect effect on a mother's social engagement, which will also effect an infant's social engagement during interaction (See Figure 4). OT's ability to decrease social anxiety in the dyad produces substantial improvements in socially-based behaviors and emotions during maternal-infant interaction (Harari-Dahan & Bernstein, 2014; Neumann & Slattery, 2015).

During unsupportive maternal behaviors and infant distress, OT can suppress

physiologic and psychologic reactivity to stress by inhibiting CRH neurons, thus diminishing CRH secretion and decreasing cortisol (Kormos & Gaszner, 2013). OT and CRH are reciprocally expressed in the PVN, and this anatomical connection allows OT to alter the firing of CRH neurons through paracrine cell signaling. If OT signaling is blocked, social buffering of the stress response does not occur (Hostinar & Gunnar, 2015).

The divergent effect of OT in the stress response systems (i.e. eustress vs. distress effects) is based on context and nature of the stressor (Neumann & Slattery, 2015). This divergence may explain why OT is so powerful as an anxiolytic—it can produce approach/ engagement or avoidance/aggressive behaviors in accordance with appropriate, adaptive anxiety responses to a stressor (Harari-Dahan & Bernstein, 2014; Maroun & Wagner, 2015). These contextual and stress-specific interactions are a result of coordinated OT communication with either excitatory (e.g. glutamate, norepinephrine) or inhibitory (e.g. GABA, serotonin) neurotransmitters. OT directly modifies expression of the monoamine neurotransmitters, including GABA, glutamate, norepinephrine, and serotonin (Fuxe et al., 2010).

OT also modifies activity of sympathetic nerves, especially in the upper thoracic region of the spinal cord (Norman et al., 2011). Descending OT nerves projecting into the spine and the peripheral nervous system assist with the pain- and motor-related functions of the fight-flight response (Moreno-López, Martínez-Lorenzana, Condés-Lara, & Rojas-Piloni, 2013) (see Figure 6). The sympathetic effects of OT release may explain why holding and breastfeeding are such powerful analgesics for the infant (Carbajal, Gall, &

Annequin, 2004). OT-based regulation of the infant's stress, anxiety, and mood assists with the emergence of emotion regulation during the first year of life (Maroun & Wagner, 2015) and the development of distinct and context-dependent social behaviors and emotions, such as trust, engagement, empathy, and cooperation (Bosch, 2011; Olf et al., 2013).

Parasympathetic nervous system regulation

The OT system is capable of facilitating hormonal-autonomic interactions in the body, including autonomic responses to social stimuli (Kemp et al., 2012). OT neurons project into the amygdala and the brain stem nuclei, areas critical for autonomic and cardiovascular control (see Figure 6). Connections with the amygdala enable OT to assist in establishing associations among infant experience of a maternal stimulus, its rewarding/aversive nature, and subsequent experientially-based emotion. The amygdala connects with the nucleus ambiguus, which is the primary source of the vagus nerve, a crucial parasympathetic regulator of the cardiovascular and social engagement system (Porges, 2003). OTR are located along pathways regulating the vagus, and OT is associated with vagal regulation in human adults and infants (Grippe, Trahanas, Zimmerman, Porges, & Carter, 2009).

Parasympathetic Nervous System Regulation of the Heart. OT is released in the human brain and pituitary after vagal stimulation (Grippe et al., 2009; Gutkowska, Jankowski, & Antunes-Rodrigues, 2014). When circulating OT reaches the atria of the heart, OTR respond by releasing atrial natriuretic peptide (ANP), nitric oxide, and more OT to promote cardiovascular homeostasis. Ground-breaking research has recently

discovered that the atria are able to release OT *with or without* central regulation. This means that an independent OT system exists in the heart. In fact, central OT concentrations found in the hypothalamus are comparable to those in the atria. Atrial OT release not only regulates electrical activity within the heart, but also regulates natriuresis, glucose metabolism, endothelial cell growth, cell motility and migration, apoptosis, and fibrosis throughout the body. Regulation of these key cellular functions by OT results in important morphogenetic, angiogenic, neuromodulator, antioxidant, and cardioprotective properties (Gutkowska et al., 2014).

OT also has significant vascular effects, including reducing blood pressure, heart rate, inflammation, and metabolic rates. These protective brain effects are produced by stimulating ANP and nitric oxide release, generating new cells, and promoting healing after infant brain insults such as infarction, hemorrhage, and traumatic injury during birth (Jankowski, Gonzalez-Reyes, Noiseux, & Gutkowska, 2012; G. J. Norman et al., 2010). The OT-based physiologic regulation of these autonomic and neurovascular parameters are the foundation of infant self-regulation. Autonomic regulation contributes to the physiologic regulation of emotions and other socially-based oscillators, such as infant arousal, attention, orientation, and engagement during parent-infant interaction (R. Feldman, 2006, 2009; Ruth Feldman, Magori-Cohen, Galili, Singer, & Louzoun, 2011).

Parasympathetic regulation of the social engagement system. The social engagement system is responsible for physiologic states associated with supportive social interactions and engagement behaviors that encourage dyadic attachment. The system consists of the vagus, motor neurons in the cortex, and the muscles of the head, face,

larynx, pharynx, and neck (See Figure 6). Because this social system includes neural control of these muscles, the system can regulate social expressions and infant engagement cues during a social experience, such as eyelid opening, eye contact, facial expressions, vocalizations, and head-turning towards auditory stimuli (Porges, 2003). Importantly, OT heavily coordinates with the social engagement system to produce the emotions and infant engagement cues displayed during parent-infant interaction (Nagasawa, Okabe, Mogi, & Kikusui, 2012).

Developmentally-supportive maternal stimuli have the potential to create visceral signals in the periphery that can be sensed by OTR on afferent vagal nerve fibers (see Figure 6), transmitted to the infant brain, registered as emotional signals, relayed back to the efferent vagus and motor neurons, and transmitted into infant engagement cues (Iwasaki et al., 2015; Porges, 2003). In this way, OT's coordination with the vagal nerve is critically important for the foundation of infant socioemotional regulation. Increases in human parenting behaviors produce a parallel increase in their infant's OT levels, vagal response, social engagement, gaze, exploration, and social reciprocity (O. Weisman, Zagoory-Sharon, & Feldman, 2012). Thus, synchronized loops of parent-infant interaction, based in synchronized vagal feedback, allow for coordinated release of OT in the dyad. In sum, the vagus not only provides a communication pathway between central and peripheral OT systems, but also creates a biobehavioral pathway for autonomic regulation and affect synchrony in the dyad (Feldman, 2006).

Peripheral Targets

OT is unique in that it has the properties and functionality of a neurotransmitter,

neuromodulator, and a neurohormone (Knobloch & Grinevich, 2014). In other words, OT can be locally released from presynaptic OT neurons to adjacent postsynaptic neurons (Wang & Hatton, 2007), regionally from somatodendritic diffusion into groups of neurons, or distally through transport into the lymph, CSF, or blood (Carter, 2014). OT release from dendrites of the PVN, the center of the OT system, diffuse into the third ventricle, and thus, the CSF (Knobloch & Grinevich, 2014). The release of OT into the bloodstream by the pituitary is the primary contribution to plasma OT levels during crucial times of life, including birth, lactation, and suckling (Brown, Bains, Ludwig, & Stern, 2013).

Other mechanisms have been studied examining brain-body communication within the OT system, including OT's paracrine functions with cytokines, macrophages, T lymphocytes, antigen-presenting cells, and protein kinases (Pittman, 2011; Vargas-Martínez et al., 2014). A burgeoning area of research is studying how OT, as stimulated by maternal social support, promotes optimal coordination between the infant's developing immune and nervous systems, and how those psychoneuroimmunology conditions contribute to the maturation of the infant brain (Pittman, 2011). Finally, of critical importance is OT neuronal projections, which are abundant in the periphery and connect to the heart, kidneys, adrenal glands, pancreas, gastrointestinal tract, and reproductive organs. Many of these organs (e.g. the heart) can release OT independently and interdependently of OT signaling within the brain. Organ-based OT signaling provides another critical communication pathway for maternal stimuli to be received by the infant's peripheral OT system and relayed to the central OT system of the infant brain

(Gutkowska et al., 2014). In light of the discussed evidence from the neural circuits of OT, we conclude that parents shape not only their infant's OT system, but also the OT-based processes that significantly shape brain development.

The Behavioral Level: Emerging Social Interactions

An interesting phenomenon, based in bidirectional and positive feedback mechanisms, is the relationships among interaction behaviors, OT, and neurodevelopment (see Figure 4). Higher levels of maternal OT elicit more supportive maternal behaviors (Ruth Feldman, 2015d), which enhance infant neurodevelopment through both OT-based (see Figure 4, Stage 7) and non-OT based pathways. Conversely, supportive maternal behaviors increase maternal and infant OT (Ruth Feldman, Gordon, Influx, Gutbir, & Ebstein, 2013), which itself optimizes infant neurodevelopment (Figure 4, Stages 5-7). Moreover, neurotypical, socially-adept infants are more likely to elicit supportive maternal behaviors during interaction (Ruth Feldman, 2015a)—completing the positive, bidirectional loop of the relationship between supportive maternal behaviors and infant development (Figure 4, Stage 7).

In human infants, positive relationships exist among healthy term infants' salivary OT levels, the social environment provided by parents, and infants' socioemotional competencies (Ruth Feldman, 2015b). Increases in parental OT levels produce parallel increases of OT in their infants and increase infants' social engagement during parent-infant interaction (O. Weisman et al., 2012). Furthermore, infants with OTR gene alleles categorized as "low-risk" have increased social cognition at 18 months, as well as increased reciprocity, engagement, and empathy (Wade et al., 2014). These data suggest

OT mediates the relationship between early, supportive social experiences and social cognition, a key facet of early infant neurodevelopment. In the following sections, we apply the scientific findings of OT's role in neurodevelopment to development of high-risk infants and families. In this paper, we define "high-risk infants" as those neonates exposed to stressful environments or chronic medical conditions, and thus are vulnerable to the debilitating effects of stress on the developing brain. Stressful environments can be defined by a variety of situations, including experiences such as hospitalization in the Neonatal Intensive Care Unit (NICU), poverty, prematurity, abuse, exposure to addictive substances, parental anxiety, postpartum depression, chronic illness, and developmental delays.

Discussion: Application to the High-Risk Human Infant

OT is present in human fetal brain tissues as early as 14 weeks gestation and significantly increases throughout gestation in brain regions such as the pituitary and the hypothalamus (Khan-Dawood & Dawood, 1984). The number of OT neurons in the fetus is equal to that of an adult by 25 weeks gestation (Wierda et al., 1991). Furthermore, preliminary results from our lab confirm that OT is present and measurable in the blood, urine, and saliva of premature infants as young as 25 weeks gestation. However, the nuclear volume, dendritic branching, morphology, and electrophysiology properties of the infant's OT neurons are still extremely immature at term birth, and rapidly develop during the first few weeks of life (Vargas-Martínez et al., 2014).

During the first two weeks of a rat's life (roughly equivalent to the first year of human life in terms of brain development), the characteristics and activity of OT neurons

change dramatically. The membrane potentials of OT neurons stabilize, neuronal activity becomes organized, glutamatergic activity emerges, and autoregulation of OT neurons begins in the hypothalamus. Autoregulation creates a feverish, yet short-lived, sprouting of dendritic branches, transforming the morphology of OT neurons into “mature” cells of the nervous system. GDPs begin massive remodeling of the infant brain into organized, interactive neural circuits (Vargas-Martínez et al., 2014).

Because the OT system undergoes massive amounts of change and restructuring during infancy, the first year of life constitutes both a critical and sensitive period for the OT system (Ruth Feldman, 2015b). Accordingly, early social environments and maternal behavior can have enormous influence on neurobiological processes that involve OT and shape neurodevelopment. Early infant experience is a crucial mediator between the infant’s biological risk and resulting neurodevelopmental outcomes.

Stressors and High-Risk Infant Neurobiology

The interaction between developmental immaturity and exposure to stressful social environments jeopardizes optimal neurodevelopment for high-risk infants (Carbajal et al., 2008). Immature neurons are exceptionally vulnerable to neuronal excitotoxicity, apoptosis, oxidative stress, and inflammation—conditions known to increase with stress exposure (Griesmaier & Keller, 2012; Gudiño-Cabrera, Ureña-Guerrero, Rivera-Cervantes, Feria-Velasco, & Beas-Zárate, 2014). Importantly, high levels of excitotoxic amino acids, free radicals, and pro-inflammatory cytokines are key factors in the pathophysiology of many brain pathologies, including periventricular leukomalacia (PVL). PVL is the most common type of brain injury in premature infants and a major

contributor to neurodevelopmental deficits (Griesmaier & Keller, 2012; Van Steenwinckel et al., 2014). Excitotoxicity, oxidative stress, and inflammation are also linked with a variety of other neonatal disease pathologies underlying developmental disorders of the infant brain, including hypoxic-ischemic encephalopathy, intraventricular hemorrhage, and cerebral palsy (Andrikopoulou et al., 2014). High levels of OT may reduce risk of brain injury through reducing oxygen reactive species, inflammatory markers, and microglial activity (Karelina et al., 2011).

Supportive maternal behaviors have the potential to prevent or reverse the detrimental effects of stress on the developing infant brain, while unsupportive behaviors can be a significant source of social stress for the infant (Weber et al., 2012). Pathologic stress conditions, such as insensitive caregiving, can produce complex changes in the genetic expression, concentrations, and interactions of OT and other neuromodulators (Olf et al., 2013). Pathologic stress decreases OT levels and OT/OTR expression (Kormos & Gaszner, 2013). Glucocorticoids released during times of intense stress and depression inhibit OT neurons, decreasing the anxiolytic effects of OT. Along with others, we hypothesize that unsupportive maternal behaviors, such as those stemming from maternal neglect, intrusiveness, abuse, anxiety, and depression, cause dysregulation of the infant's OT system, and predispose high-risk infants to impaired structural, connective, and functional brain development (De Bellis & Zisk, 2014).

Recent OT literature supports our hypothesis. Children with histories of trauma, neglect, severe poverty, and abuse have chronically altered levels of OT, cortisol, dopamine, and serotonin, leading to hyperactive HPA and SAM systems. These children

also often have significant developmental, social, psychological, and general behavioral deficits (De Bellis & Zisk, 2014). Suboptimal maternal care is known to be correlated with high-risk OT gene variants in the dyad, dopamine dysfunction, anxiety and depression disorders, cardiovascular disease, and obesity (Ruth Feldman, 2015c, 2015d; Omri Weisman et al., 2015). While supportive parenting may be biologically rooted in OT and reward networks, developmentally unsupportive parenting incorporates stress hormones and fear pathways (Atzil et al., 2014). Intrusive mothers show greater brain activation in the amygdala, premotor cortex, and orbitofrontal cortex when viewing pictures of their infants. These mothers display increased functional connectivity in stress pathways, fear circuits, and networks associated with insufficient behavioral inhibition (Atzil et al., 2014; Strathearn, 2011).

These studies inform the biological underpinnings of the difficult parenting situations seen with high-risk infants. Stress alters parenting behaviors, creates suboptimal interactions that are not developmentally supportive, and strains the parent-infant relationship. Parents of high-risk infants often report difficulties in assuming the parent role and bonding with their infant (Obeidat, Bond, & Callister, 2009). Insensitive caregiving diminishes infant OT production, which can contribute to devastating neurodevelopmental deficits in communication, emotion regulation, social interaction, memory, and behavior (Bales & Perkeybile, 2012). Given the importance of maternal behavior in promoting healthy neurocognitive development, OT provides a biological basis for interventions that decrease parent-infant stress, increase developmentally supportive parenting behaviors, and improve neurodevelopmental outcomes for high-risk

infants.

Future Directions in Research

OT has great potential as a distinctive biomarker of social interactions that provide neuroprotection and promote neuromaturation in the vulnerable infant population. Study of variation in OT levels could provide researchers and clinicians with a greater understanding of how interventions and the context of the stressful social environments shape the trajectory of infant neurodevelopment. Few studies integrate measures of stressor exposure, the neurobiological milieu of the developing brain, structural neural circuit growth, and functional infant outcomes. The addition of OT to existing measures of neurobiological processes underlying development represents progress towards a more comprehensive, scientific investigation of human development. Currently, there is an enormous gap in knowledge of how the human neurobiology of parenting and infant behaviors are linked during the neonatal period. Most studies linking dyadic neurobiology and synchronous behaviors study offspring as children, adolescents, or most commonly, adults. Moreover, the explosion of OT research in the basic sciences during the past decade warrants new and innovative applications to high-risk infants and their families. Scientists must move beyond the current children targeted for OT research, e.g. autism and ADHD, to reach additional vulnerable populations.

Successful implementation of foundational, descriptive studies of normative values for measures of OT will pave the way for interventional research. A diverse array of plausible clinical interventions exist that could improve infant outcomes through OT pathways, including programs to increase mother-infant contact, maternal knowledge of

infant development, infant physiologic regulation, and dyadic resiliency to stress. In addition, given that touch is a potent stimulator for OT release (Dunbar, 2010), effects of interventions that increase physical contact (e.g. kangaroo care, massage, hand containment, scent exchange) are critically needed.

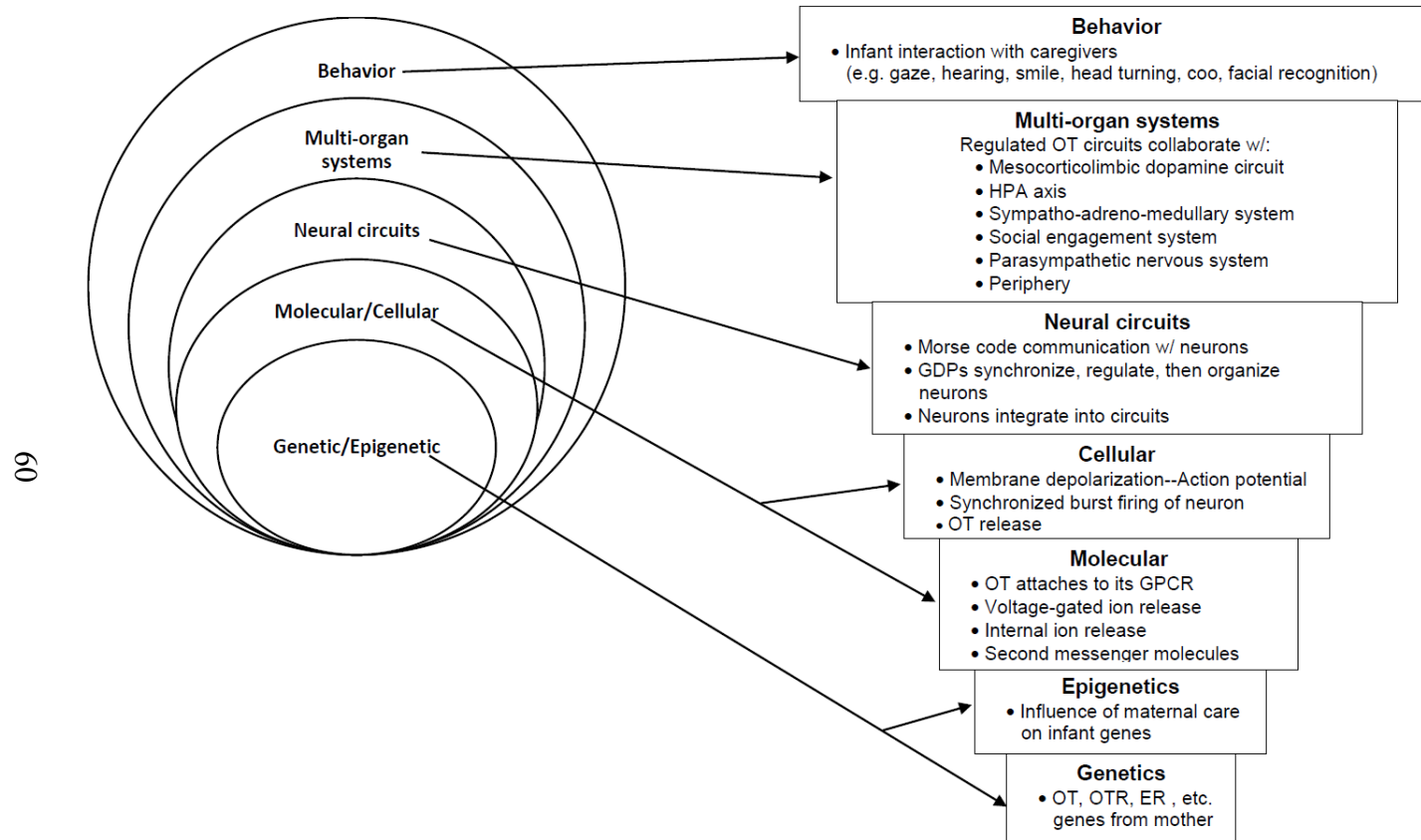
Measuring relationships of OT with other types of interventions include those directed at optimizing parents' roles through helping parents to reduce stress, depression, and anxiety, while increasing their knowledge about supporting their infant's development (Melnyk, Crean, Feinstein, & Fairbanks, 2008). This strategy has been successful for multiple interventions for high-risk infants, such as those affected by prematurity, neonatal abstinence syndrome, and congenital anomalies (Milgrom et al., 2010). However, the relationships of positive outcomes with OT levels in these populations is unknown.

Conclusion

Interactions between infants and their parents serve as key events through which infant neurobiological systems are shaped by the OT system. As we use the growing body of OT literature to understand the biological underpinnings of neurodevelopment throughout life, the clinical potential of monitoring OT function is clear. Nurses have a unique opportunity to use the emerging OT literature to inform practice by applying these basic science concepts to vulnerable patient populations. The discipline of nursing has consistently promoted the use of social support, therapeutic relationships, and community resources to facilitate healing and growth. As we look for nursing's place in the newly developing paradigm of healthcare delivery, OT is a unique hormone that biologically

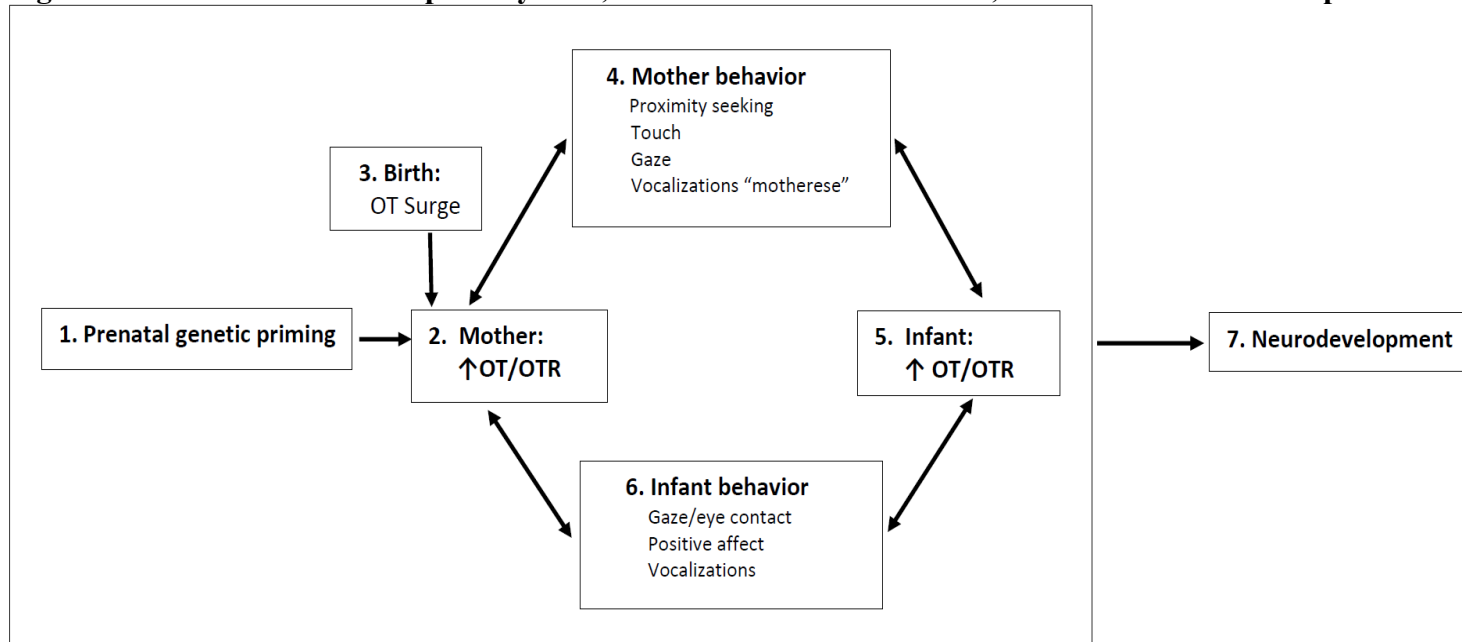
tells us where nursing should be—present and socially supportive of our patients and their families when they need us the most.

Figure 3. Nested hierarchies of effects of oxytocin.



OT = oxytocin, OTR = oxytocin receptors, ER = estrogen receptors, GPCR = G-protein-coupled receptor, GDP = giant depolarizing potentials, HPA = hypothalamic-pituitary-adrenal axis

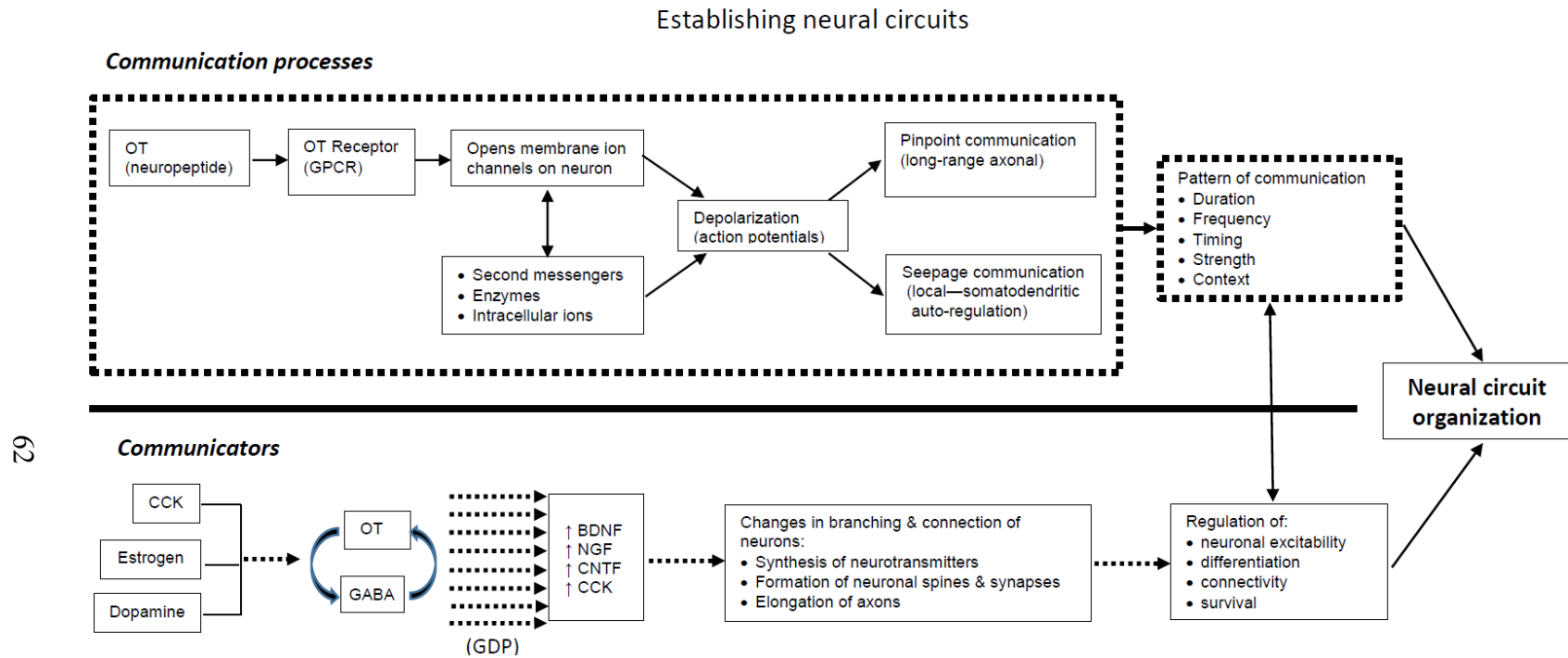
Figure 4. Positive feedback loop of oxytocin, maternal-infant interaction, and infant neurodevelopment.



61

OT = oxytocin; OTR = oxytocin receptors. A mother's genetics affect her OT trajectory during pregnancy and after birth. Birth leads to a surge of OT release in the mother and subsequent initiation of supportive maternal behaviors toward her infant. Thus, the newborn infant is immediately exposed to supportive maternal care, spurring infant OT system development. The infant's response to the mother continues a positive feedback loop of OT activities/functioning, ultimately enhancing infant neurodevelopment.

Figure 5. Communication processes and communicators involved in establishing neural circuits.



62

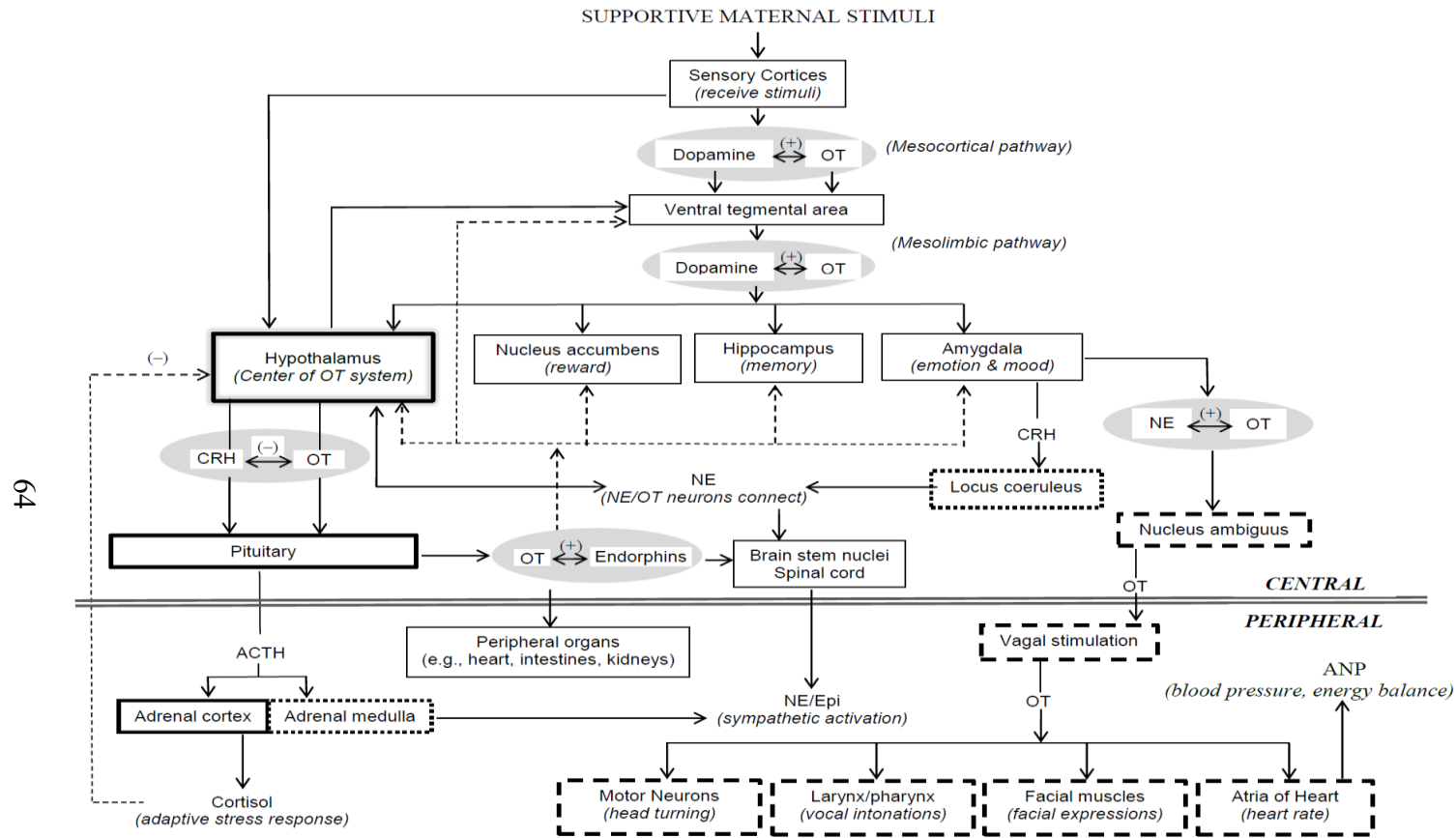
OTR = oxytocin receptors, GPCR = G-protein-coupled receptor; CCK = cholecystikinin; GABA = γ -amino-butyrac acid; GDP = giant depolarizing potentials; BDNF = brain-derived neurotrophic factor; NGF = nerve growth factor; CNTF = ciliary neurotrophic factor. Boxes below the thick black line depict the neurotrophins involved in communication for development of neural circuits; boxes above the thick black line depict the molecular/cellular communication processes.

(Continued)

(Figure 5 continued)

These communication processes occur in the positions indicated by the dashed arrows. Communication processes: The OT neuron communicates when its neuropeptide attaches to the OTR, a GPCR. Once activated, the OTR opens the neuron's membrane ion channels to stimulate second messengers, enzymes, and intracellular ion stores. These molecules promote membrane depolarization and initiation of an action potential. Through action potentials, OT neurons engage in somatodendritic and axonic release of OT. The neuropeptide OT acts on the OT neuron (autoregulation), priming the OT neuron to seep. Through patterns of pinpoint and seepage communication, the OT neuron uses the duration, frequency, timing, and strength of OT release as a "Morse Code" communication. Communicators: A symphony of hormones act as communicators in organizing the developing infant's neural circuits. CCK, estrogen, and dopamine augment OTR and OT levels. Multiple depolarizations (action potentials) are created by OT/GABA interactions, producing GDPs, which are electrical communications on a massive, synchronized scale (depicted by multiple stacked dotted lines). The GDPs regulate levels of BDNF, NGF, CNTF, and CCK, critical neurotrophins that act on the brain's neurons to promote the growth and branching of neurons through the regulation of neurotransmitter synthesis, neuronal spine and synapse formation, and axon elongation. Maturation changes in neuronal branching lead to the regulation of a neuron's excitability, differentiation, connectivity, and survival. Through these hormonal and electrical communications, neural circuits form, ultimately impacting infant neurodevelopment.

Figure 6. Oxytocin Pathways in the Brain and Body.



OT = oxytocin, CRH = corticotrophin-releasing hormone, NE = norepinephrine, Epi = epinephrine, ACTH = adenocorticotrophic hormone, ANP = atrial natriuretic peptide.

(Continued)

(Figure 6 continued)

Thick black-bordered boxes indicate hypothalamic-pituitary adrenal (HPA) axis. Dotted boxes indicate sympathetic-adrenal-medullary (SAM) axis. Dashed boxes indicate regulation of parasympathetic nervous system. Dashed lines indicate feedback loops. Oval areas indicate interactions between OT and other hormones of key neurobiological systems. Thick double line delineates OT central and peripheral activity. Stimuli received by the infant's sensory cortices are relayed to the ventral tegmental area (VTA) through the mesocortical dopamine pathway. Through the mesolimbic dopamine pathway, the VTA releases dopamine into the nucleus accumbens, hippocampus, and amygdala. OT and dopamine neurons coexist and interact within the mesocorticolimbic pathways. OT is released from the periventricular and supraoptic nucleus of the hypothalamus, which is the center of the OT system. OT inhibits actions of the HPA axis, resulting in lowered (1) ACTH release from the pituitary, (2) cortisol release from the adrenal cortex, and (3) norepinephrine (NE) and epinephrine (Epi) release from the adrenal medulla. OT also increases endogenous opioid release to enhance pleasure, interest, and motivation. OT augments dopamine release from the VTA, forming a positive feedback loop. OT, along with dopamine, activates the amygdala to release CRH, signaling the locus coeruleus to release NE and Epi. The amygdala regulates the nucleus ambiguus, source of the right vagal nerve. OT stimulates the vagus, a key component of the parasympathetic and social engagement systems. The vagus withdraws parasympathetic activity to control the larynx, pharynx, facial and neck muscles, and atria of the heart. Change in vagal regulation causes changes in vocal intonations, facial expressions, heart rate, and blood pressure, as well as energy balance through ANP release.

Chapter 4: Oxytocin Trajectories in Extremely Premature Infants during NICU Hospitalization

Prematurity is the largest contributor to morbidity and mortality in perinatal health care (Matthews, MacDorman, & Thoma, 2015), with neurologic deficits being one of the most significant morbidities. Extremely premature infants, those born at 28 weeks gestation or less, are the most vulnerable population for experiencing brain injury and/or altered brain development that results in a range of neurodevelopmental deficits (Ancel et al., 2015; Stoll et al., 2015). For example, up to 70% of these infants display white matter abnormalities on Magnetic Resonance Imaging (MRI), and 75% displaying atypical neurodevelopment at 5 years of age (Jarjour, 2015; Volpe, 2009). Moreover, improvement in the rates of neurologic morbidities have not kept pace with improvements in mortality (Hintz et al., 2011). These morbidities place considerable burden on families, the health care system, and society (Korvenranta et al., 2010; Soilly, Lejeune, Quantin, Bejean, & Gouyon, 2014).

For infants born prematurely, development of the immature brain and nervous system must occur in the extrauterine environment of the Neonatal Intensive Care Unit (NICU). During the last trimester of pregnancy, the rate of growth, pruning and plasticity of the infant brain is at its highest, with almost 50% of neurons in the developing brain experiencing programmed cell death (Volpe, 2009). In the highly technological NICU

environment, infants are exposed to distressing physiologic as well as environmental events that have the potential to permanently alter the developing brain (Pickler et al., 2010). These distressing events contribute to additional neuronal cell death (Adnan T. Bhutta & Anand, 2002; A. T. Bhutta & Anand, 2001), reduced brain volumes (Lind et al., 2011), impairments in brain structure and function (G. C. Smith et al., 2011), and ultimately, deficits in neurodevelopment (Rogers et al., 2012).

Socially-based interventions (e.g. skin-to-skin contact between mother and baby) that reduce distressing early life experiences can improve structural and functional brain development (R. Feldman & Eidelman, 2003; Milgrom et al., 2010; Montirosso et al., 2012). For example, Milgrom used weekly sensitivity training to teach parents how to monitor and reduce stress cues in their premature infants. Parent training was significantly and positively related to maturation and connectivity of the white matter tracts in premature infants. However, the biological mediators of these relationships and the mechanisms driving positive outcomes are not fully understood. Research to counteract or prevent the maladaptive consequences of distressing early life experience depends on a deeper understanding of biological processes that foster resilience and adaptation in the context of the NICU environment. The development of a reliable biomarker of these processes is sorely needed for evaluation of interventions which promote developmentally supportive experiences (Pickler et al., 2010). Reliable markers of early social experiences that impact development are necessary so that researchers can accurately monitor important mediators of neurodevelopmental outcomes.

Oxytocin (OT) has the potential to be a neurobiological marker of social

processes that offer neuroprotection for the infant. OT, an affiliation hormone released during supportive social interactions, contributes to a range of neurologic functions, including modulation of infant social behaviors, neuroprotection, affiliation, and bonding. Moreover, measurement of OT has not been reported in the literature for premature infants.

Theoretical Framework and Background

Schore's Regulation Theory was chosen as a guiding framework for this study (Schore, 2001). Schore's model contends that early life experiences result in a cascade of neurobiological processes which are critical for adequate neuronal network formation, structural brain growth, and brain function. The OT system undergoes significant maturation during early fetal life and infancy. OT is present in human fetal brain tissues as early as 14 weeks gestation and significantly increases throughout the course of gestation in areas of the brain such as the pituitary and the hypothalamus (Burford & Robinson, 1982; Kuwabara, Takeda, Mizuno, & Sakamoto, 1987). In term human infants, OT is measurable in plasma (Kuwabara et al., 1987), urine (White-Traut, Powlesland, Gelhar, Chatterton, & Morris, 1998), saliva (R. Feldman, Gordon, & Zagoory-Sharon, 2010) and cerebral spinal fluid (Clark et al., 2013). OT has not been longitudinally measured in human infants, including premature infants, therefore stability during infancy is unknown. OT is very stable in children and adults (Ruth Feldman, Gordon, Influx, Gutbir, & Ebstein, 2013; Levine, Zagoory-Sharon, Feldman, & Weller, 2007).

The purpose of this study is to measure OT in the extremely premature population and describe its longitudinal developmental trajectory within the complex and

technological NICU environment. This study compares OT levels in plasma and urine to demonstrate that urine is a valid specimen for measuring OT noninvasively in premature infants. For our first aim, we hypothesize that OT trajectories will be relatively stable in premature infants, similar to results found in children and adults. For our second aim, we hypothesize that OT levels in urine and plasma will increase with age, and that urine and plasma levels will be correlated. Finally, recommendations for future sample sizes will be estimated based on the effects and estimates found with this novel study design.

Methods

Sample

To address the purpose of this study, premature infants were recruited from three Midwestern Level III NICUs. Inclusion criteria included English-speaking mothers who gave birth to premature infants ranging from 25-28^{6/7} weeks gestation. Exclusion criteria were chosen due to their influence on infant brain development and/or neurobiological processes: history of maternal drug abuse (Ornoy, 2003; Thompson, Levitt, & Stanwood, 2009), presence of major congenital or chromosomal abnormality, grade 3 or 4 intraventricular hemorrhage (Bolisetty et al., 2013), hypoxic ischemic encephalopathy (Logitharajah, Rutherford, & Cowan, 2009), metabolic disorders involving the adrenal system (Khulan & Drake, 2012) and necrotizing enterocolitis requiring surgical intervention (Ta et al., 2011).

Power analysis was conducted using a standard formula (Laird, Donnelly, & Ware, 1992), in which we inferred conservative estimates from cross-sectional studies with term infants (R. Feldman et al., 2010; Weisman, Zagoory-Sharon, & Feldman,

2012). We assumed a variance of 1000, a linear change of 9 pg/ml per week, and a correlation of 0.2. With these levels and, on average, five measures per subject, a sample of 50 infants will provide at least 80% power to detect a positive rate of change in OT level.

Procedure

Infants were recruited during the first two weeks of life by directly approaching the mother while visiting in the NICU. All participating mothers signed a written consent form approved by the institutional review boards of the academic institution and participating clinical sites. Sample collection started approximately on Day of Life 14, then continued weekly until the infant achieved 34 weeks corrected gestational age (CGA). Sample collections occurred between the hours of 2300 and 0200 to control for the diurnal variation of OT. Urine was collected by cotton ball, placed in the diaper during the previous diaper change prior to data collection. Blood collection occurred by heelstick unless the infant had an arterial line, and was concurrent with blood draws required for clinical treatment. Blood was collected into chilled EDTA (1mg/ml) BD microtainer tubes (Becton, Dickson, & Company, USA) containing Aprotinin (10 μ L/mL of blood). All samples were collected before feeding as OT levels are reported to be influenced by the act of suckling (Lupoli, Johansson, Uvnäs-Moberg, & Svennersten-Sjaunja, 2001) and digestion (Verbalis, Blackburn, Hoffman, & Stricker, 1995; Verbalis, McCann, McHale, & Stricker, 1986). After collection, samples were immediately placed on ice, processed, and transferred to a locked -80°C freezer.

Measures

Plasma and urinary OT were measured using a commercially available EIA kit (Peninsula Laboratories International, Inc., San Carlos, CA, KIT S-1355.0001). Samples were processed in accordance with successful protocols previously described in the literature (R. Feldman et al., 2010; R. Feldman, Gordon, & Zagoory-Sharon, 2011). Samples were immediately be placed on ice and transported by the PI to the Heart Center Laboratories, then centrifuged at 4°C at 2000g for 10 min. Supernatants were collected and stored at –80°C until assayed. Samples were visually examined for adequate volume and gross contamination by hemolysis, stool, sediment, or diaper cream. One plasma sample was excluded from analysis due to insufficient volume, and 9 urine samples were excluded due to visible contamination.

Samples from the same infant were assayed simultaneously to avoid inter-assay variation, and all measurements were performed in duplicate. Samples were assayed in batches to minimize the time spent in storage. Inter-assay and intra-assay coefficients of variation were 15% and 7%, as reported by the manufacturer. Urinary OT levels were normalized to total creatinine to control for dilution of the urine, and creatinine concentrations were measured using a commercially available Creatinine Colorimetric Assay kit (Cayman Chemical Co.). Data were analyzed using GraphPad Prism software.

Data Analysis

An overall alpha level of 0.05 was chosen for analysis. OT levels were naturally logged, due to the skewed distribution of the data. Linear mixed models (LMMs) described the mean trajectory of OT levels, as well as infant-level trajectories over the course of gestation. CGA (Age) was modeled as a continuous variable, centered at 27

weeks CGA.

The model form is: $Y_{it} = (b_o + \mu_{oi}) + (b_l + \mu_{li}) * (\text{Age}_{it} - 27) + \epsilon_{it}$.

In the model, i indexes the infant, t the corrected gestational age. The two b terms (b_o , b_l) are fixed effects providing the mean intercept and slope coefficients. The two μ terms (μ_{oi} , μ_{li}) are random effects, which adjust the mean parameters for infant-specific variation in OT trajectory. Infant-specific trajectories provide estimates of the between-infant variation in overall OT level (random intercepts) and its rate of change over time (random slopes). Fluctuation of OT levels about an infant's trajectory were estimated by the variation in ϵ , which estimates scatter of infant i 's data about his/her linear fit.

We used a bivariate random intercepts model to estimate correlations between plasma and urinary OT levels over time, both between and within infants. The model form is:

$$Y_{it} = (b_o^Y + \mu_{oi}^Y) + b_l^Y * (\text{Age}_{it} - 27) + \epsilon_{it}^Y$$

$$W_{it} = (b_o^W + \mu_{oi}^W) + b_l^W * (\text{Age}_{it} - 27) + \epsilon_{it}^W$$

The model form adds to the Aim 1 model, where Y denotes the sample source as plasma, W the sample source as denotes urine (Weiss, 2005). The model permits an unstructured covariance structure for the ϵ terms, from which the correlation estimates between plasma and urine within an infant will be derived. The covariance of the random effects will permit estimates of the correlation between plasma and urine in the overall sample.

Power analysis for future studies was conducted using the method recommended by Stroup (Stroup, n.d.). We will use the estimated parameters from the results of Aim 1

to determine the number of infants needed to detect OT levels decreasing at a rate of 10 pg/ml per week in plasma.

Results

Results for the recruitment of subjects are presented in Table 1. Demographic and descriptive statistics for the 37 mothers and premature infants who were successfully enrolled in the study are presented in Tables 2 and 3. Summary statistics for values of plasma and urinary OT are presented in Tables 4 and 5. As seen in these tables, the standard deviations of both plasma and urinary OT levels were quite large, indicating significant variability in the OT levels found in extremely premature infants. When considering all the possible timepoints that were available to collect our plasma and urinary data (n=220), intermittent missingness was 55% and 37% for plasma and urinary OT levels, respectively. Intermittent missingness is common in longitudinal research designs, and especially in research with vulnerable populations. Moreover, because the incidence of infants born 25-26 weeks gestation is lower than infants born at 27-28 weeks, we recruited less infants born 25-26 weeks and captured less OT data from 27-29 weeks gestation. Hence, the majority of our OT data encompasses the 30-34 week range.

The results of our first aim, detailing the trajectory of plasma and urinary OT levels, are presented in Table 6. Plasma OT levels decreased with age, with a 14% decrease in the geometric mean per week ($b = -0.15$ $p = 0.006$). There was no evidence that urine OT levels changed with age ($b = 0.04$ $p = 0.579$). Both plasma and urine exhibited wide variability across infants, but values were relatively stable within infants. Furthermore, the intraclass correlation coefficient (ICC) of plasma was 0.75, while urine

was 0.62, indicating strong stability of OT levels within infants.

The hypothesis of our second aim, that plasma and urinary OT levels would be correlated, was not supported by our data. Neither the between-infants' estimates ($r = 0.19$ $p = 0.54$) of correlation between urine and plasma levels, nor within-infants' estimates ($r = 0.31$ $p = 0.14$) of correlation in the residuals of urine and plasma levels were statistically significant. The covariance matrix of the Bivariate Random Intercept Model, from which we derived the estimates of the correlations, is presented in Table 7 (Weiss, 2005).

After recruiting 37 premature infants over a period of two years, we conducted an interim statistical analysis to determine if additional recruitment would be necessary. In comparison to our original power analysis parameters, we detected a variance in plasma OT levels of 2500, average observations per subject of 3, and a correlation of 0.3. However, we detected a much larger effect in the change of OT levels per week (~14% decrease in the geometric mean per week). Thus, results showed that we achieved enough statistical power to detect clinically significant results, if they existed, in our sample. We decided to close recruitment for our study at that time.

Discussion

The purpose of our study was to measure OT in the extremely premature population and describe its longitudinal developmental trajectory. This study also compared OT levels in plasma and urine to demonstrate that urine is a valid specimen for measuring OT noninvasively in premature infants. The results of our first aim describe the developmental trajectory of OT in the plasma of extremely premature infants. As

demonstrated in Table 6, plasma OT levels significantly decreased with age, and also demonstrated a clinically meaningful effect of a 14% decrease in the geometric mean per week. To our knowledge, this is the first assessment of the trajectory of OT levels in living premature infants.

We had hoped that OT levels would increase, indicating that infants are maturing and able to engage in more advanced social interactions with their parents. OT is released in response to social interaction, and acts as a neuroprotective buffer against the detrimental effects of stress on the infant brain. However, if stress is overwhelming, then OT neurons are inhibited by stress hormones, and OT levels decrease in a maladaptive way. It is possible that the cumulative stressors present in the NICU environment have longitudinal, and detrimental, effects on plasma OT levels. Previous research with premature infants has supported this hypothesis, as studies have shown significant, negative associations between cumulative stress exposure and various measures of infant brain biology and/or maturation, including cortisol trajectories (R. E. Grunau, Weinberg, & Whitfield, 2004; Ruth E. Grunau et al., 2007), heart rate variability (Mehler et al., 2015), brain width, altered diffusion, and functional connectivity (G. C. Smith et al., 2011). In addition, significant, negative associations exist between stress exposure and a wide variety of measurements related to the OT system, including OT/OTR gene expression (Ruth Feldman, Monakhov, Pratt, & Ebstein, 2015), OTR binding and OT immunoreactivity (Bales & Perkeybile, 2012; Bales, Boone, Epperson, Hoffman, & Carter, 2011), and OT levels (Smith et al., 2015). However, it is important to note that studies investigating relationships between OT and stress used either adult human

subjects or animal models.

Interestingly, in the 1980s some researchers concluded that OT levels significantly increased over time from 11-42 weeks gestation, particularly in the fetal hypothalamus and pituitary (Burford & Robinson, 1982; Khan-Dawood & Dawood, 1984). Our study was not consistent with this conclusion. However, several factors could have accounted for these differing results. First, these studies were conducted in the 1980s, with different methods (tissue-based radioimmunoassay) and small sample sizes (e.g., $n=8$). Second, infants in these studies had expired, thus tissue samples were not collected *in vivo*. Third, tissue samples were collected using a cross-sectional design, instead of longitudinally. The nature of cross-sectional designs, conducted with fetuses of various gestational ages with severe medical conditions that resulted in death, could have created the illusion of an increased longitudinal effect over time. More research is needed to determine if the OT trajectories of extremely premature infants, who have incredibly stressful hospitalizations and long length of stays, differ from the trajectories of very preterm, late preterm, and full-term infants.

Urine and plasma OT levels were not correlated over time. While we did find a significant decrease in OT levels over time within plasma, urinary OT levels did not follow this same trend. Several reasons could provide explanation for this result which pertain to the strengths and limitations of the research procedure and measures. First, there was significantly more variability in urinary OT levels that we attribute to measurement error. Infants often stooled or had large amounts of barrier cream added to their diaper. Both could have contaminated our specimens and impacted obtained OT

levels. We did not restrict the use of diaper cream in our protocol due to the high risk of skin breakdown in this population (Oranges, Dini, & Romanelli, 2015). Second, it is unknown whether urine interacts with cotton, which is the method we used to collect specimens (Polito, Goldstein, Sanchez, Cool, & Morris, 2006). Future research studies may need to use urine bags as a collection method to provide clean urine samples for analysis. We did not chose the urine bag collection method in order to minimize stressful procedures required for the protocol and avoid the risk of skin breakdown from the bag, which was a significant strength of the study procedures.

Third, because we did not catheterize infants during the previous care, so as to minimize stressful procedures (Nagasawa, Kikusui, Onaka, & Ohta, 2009), the urine collected could have been unprocessed for up to five hours, increasing the risk for OT degradation in the specimen (Reyes et al., 2014). Researchers may consider collecting urine as soon as the infant voids, to avoid excessive pooling of urine samples. The instantaneous measure of plasma is much different than the long-term, average measure that urine levels provide, due to the time required to fill the bladder and void (Reyes et al., 2014). Future research needs to determine how unstable OT samples are to degradation and variability in sampling procedure. Previous research has claimed that acidity of the urine acts as a preserving agent and prevents OT from degrading prior to assay (Reyes et al., 2014).

Finally, a recent paper examined methods for accurately determining urinary OT levels in a population of chronically dehydrated elders (Reyes et al., 2014). These researchers found that if creatinine levels reached a cutoff point of 1.4 mg/ml, high levels

of urinary metabolites would saturate the assay's OT antibody and interfere with assay results. Antibody saturation would yield OT levels that were severely inaccurate. They created an acceptable solution to this problem by diluting urine samples by half, and found that results were accurate after this correction. All creatinine levels in this sample were less than 0.5 mg/ml, much lower than the critical value reported by Reyes and colleagues. However, researchers should verify if urine samples meet the cutoff point of 1.4 mg/ml creatinine to ensure that creatinine assays provide accurate results in future studies.

It is possible that in this population, due to the immaturity of the kidneys and urinary system, urine may not be an acceptable candidate for measurement of OT levels in extremely premature infants. Additional research with stricter collection protocols is necessary before drawing any strong conclusions. There have been several studies over that last decade that have shown urine is an acceptable substitute for plasma (R. Feldman et al., 2011; Nagasawa et al., 2009; Polito et al., 2006). Nevertheless, both our urine and plasma data did demonstrate strong stability within infants, as evidenced by our computed ICC. Previous research has also confirmed that OT levels are very stable within individuals over time (Levine et al., 2007).

In order to address our aims, we recruited 37 premature infants over a period of two years, which represents a sample limitation in the study. This was due to high mortality rates for extremely premature infants and a large number of infants meeting our exclusion criteria. This also speaks to the extreme vulnerability of this population, as well as the severe illness acuity and multiple comorbidities that these infants face after birth.

The original expected amount of infants to be screened, based on data provided for the years 2011-2012, was 200 extremely premature infants admitted annually. This meant that we would see at most, 400 infants total over a period of 2 years. We assumed, from the literature available at the time (Gephart, McGrath, Effken, & Halpern, 2012; Vogtmann, Koch, Gmyrek, Kaiser, & Friedrich, 2012), a 25% exclusion rate, for a total of 300 eligible infants screened over a period of 2 years. As shown in Table 1, only 187 infants were screened, and 44% of these infants were excluded from the study—much higher than anticipated from incidence rates of the conditions which qualified infants for exclusion. In addition, 16% of infants who were eligible for the study were not approached for enrollment, due to inconsistency in parent schedules and visitation. This is slightly higher than we anticipated, as we projected a 10-15% rate for this category. It is important to note that 57% of parents who were approached for enrollment actually consented to the study, a percentage which is quite high for this vulnerable population. In summary, the events surrounding a premature birth are extremely stressful for infants and their families, which presents unique challenges during the recruitment period (Behrman, Butler, & Outcomes, 2007).

In addition to the number of subjects affecting our power, there was also significant intermittent missing data present in our analyses, a phenomenon that is common in longitudinal research designs with premature infants. There were numerous factors that contributed to missingness for plasma samples, including labs not being ordered for the week (infants did not experience additional heelsticks for the purposes of the study), low hemoglobin and hematocrit (additional blood was not taken in these

instances due to patient safety), bleeding from the heelstick stopped prematurely (additional heelsticks were not allowed by study protocol), or that labs were ordered at nonstandard times due to changes in patient health status or the plan of care. Factors that contributed to missingness for urine samples include low urine output, movement of the cottonballs out of the diaper, gross contamination with stool or barrier cream, and missed protocols due to the nurse forgetting to place the cottonball in the diaper. All of these missed collection opportunities significantly decreased our power to detect effects. However, because these reasons contributed to missingness completely at random (MCAR), these factors are unlikely to introduce statistical bias into the results in a predictable pattern. Factors that contributed to missingness for both plasma and urine included delay in recruitment. In this instance, observations from the earlier gestational age weeks were more likely to be missing. However, because this missingness of CGA is considered Missing At Random (MAR), and CGA was included in the model, then delay in recruitment should also not introduce bias into the results in a predictable way.

Despite limitations in our sample size and missingness in the data, we were able to detect a significant effect even though our sample was smaller than our original recruitment goal of 50 infants. In addition, a significant strength of our sample was the demonstration of good generalizability for our geographic region, with excellent variability in maternal demographics, including maternal age, education, income, and race. The infants for our sample also demonstrated good variability in birthweight, gender, race, and APGAR score for their gestational age category. Finally, these results provide promise for the use of OT as a biomarker in future studies, as differences

between infants in their relative quantity of OT levels may predict future neurodevelopmental outcomes.

Conclusion

The addition of OT to existing measures of neurobiological processes underlying development represents progress towards a more comprehensive, scientific investigation of human development. As the first study of its kind, more research is needed to determine norms for peripheral levels of OT throughout different gestational ages. However, OT has great potential as a distinctive biomarker of social interactions that provide neuroprotection and promote neuromaturation in premature infants.

Table 1 Recruitment and Enrollment for Study

Percent Excluded from Screened	0.44
Percent Unavailable from Screened	0.16
Percent Approached from Goal	0.34
Percent Consent YES from Screened	0.21
Percent Consent YES from Consented	0.57
Number Screened	187
Number Enrolled	37
Number Approached	68
Number Excluded	82
Number Not Reachable	30

Table 2 Maternal Demographics for Sample

Variable	Mean	Std. Dev	Min	Max
Maternal Age (yrs)	31.2	8.1	18	48
Maternal education (yrs)	14.8	2.8	11	22
Family income \$ (thousands)	55.1	41.7	0	100
Births/Para	1.9	1.6	1	8
Race	81% Caucasian	16% African American		

Marital Status	Frequency	Percent
Single, never married	8	21.62
Married	19	51.35
Partnered, not living together	3	8.11
Partnered, living together	7	18.92

Table 3 Infant Demographics for Sample

Variable	Mean	Std. Deviation	Min	Max	Obs
Gestational age at birth (wks)	27.2	1.14	25.6	28.9	37
Birthweight (g)	1015	242	380	1470	37
APGAR at 5 min	6.8	2.2	2	9	37
Gender	46% male				37
Race	71% Caucasian		26% African American		37
Multiples	90% singleton				37

Table 4 Plasma OT Samples

Plasma OT	Obs	Mean	Std. Deviation	Min	Max
Log Plasma (pg/ml)		3.93	0.67	1.95	5.97
Plasma (pg/ml)	98	63.67	50.49	7.02	392.22
CGA 27 weeks	1	24.53	.	24.53	24.53
CGA 28 weeks	2	57.97	32.39	35.07	80.87
CGA 29 weeks	8	80.52	54.79	7.02	156.91
CGA 30 weeks	15	93.46	95.18	19.19	392.22
CGA 31 weeks	15	69.04	40.69	29.05	194.47
CGA 32 weeks	23	62.08	35.36	20.45	178.36
CGA 33 weeks	19	45.75	24.65	7.32	93.33
CGA 34 weeks	15	48.02	25.76	18.88	100.86

Table 5 Urinary OT Samples

Urinary OT	Obs	Mean	Std. Dev.	Min	Max
Log Urine (pg/ml/ng creatinine)		1.16	5.33	10.84	129
Urine (pg/ml/ng creatinine)	129	9588.75	9695.21	206.25	50838.70
CGA 27 weeks	1	206.25		206.25	206.25
CGA 28 weeks	5	12417.01	12889.36	275.90	32225.83
CGa 29 weeks	9	10086.27	10335.98	774.31	34751.45
CGA 30 weeks	19	9840.20	9741.49	263.57	39198.45
CGA 31 weeks	18	6794.70	9498.97	560.16	42171.63
CGA 32 weeks	31	8459.72	6387.97	214.64	25765.53
CGA 33 weeks	22	9881.67	9221.26	618.31	43974.80
CGA 34 weeks	24	12290.17	12806.24	509.28	50838.70

Table 6 Linear Mixed Model of Logged Oxytocin Trajectories

Log Plasma (pg/ml)	Coef.	P>z	[95% Conf.	Interval]
Corrected Gestational Age	-0.15	0.01	-0.25	-0.04
Intercept	4.72	0.00	4.15	5.29

Random-effects Parameters	Estimate	[95% Conf.	Interval]
Unstructured Matrix			
σ_{b0} (random intercepts)	0.18	0.09	0.35
σ_{b1} (random slopes)	0.98	0.53	1.84
ρ^{\wedge} (correlation of random effects)	-0.99	-1.00	0.22
sd(Residual)	0.56	0.46	0.68

Log Urine (pg/ml/ng creatinine)	Coef.	P>z	[95% Conf.	Interval]
Corrected Gestational Age	0.04	0.58	-0.10	0.18
Intercept	8.44	0.00	7.67	9.21

Random-effects Parameters	Estimate	[95% Conf.	Interval]
Unstructured Matrix			
σ_{b0} (random intercepts)	1.24	0.56	2.76
σ_{b1} (random slopes)	0.23	0.11	0.49
ρ^{\wedge} (correlation of random effects)	-0.91	-0.99	-0.49
sd(Residual)	0.96	0.82	1.14

Table 7 The Covariance Matrix of the Bivariate Random Intercept Model

Parameter	Estimate	Std. Err.	t	p-value
Covariance Matrix				
D_{YY}	0.05861	0.05337	1.1	0.14
D_{YW}	0.04299	0.06978	0.62	0.54
D_{WW}	0.3317	0.164	2.02	0.02
σ_{YY}	0.4102	0.07019	5.84	<.0001
σ_{YW}	0.1333	0.09042	1.47	0.14
σ_{WW}	1.1958	0.1678	7.13	<.0001

D_{YY} , D_{WW} = Variance of the random intercepts, whereas plasma is denoted by Y, and urine is denoted by W. σ_{YY} , σ_{WW} = Variance of the within-infant residuals, whereas plasma is denoted by Y, and urine is denoted by W. D_{YW} = Covariance of the random effects of plasma and urine, from which the correlation of the between-infants estimates ($r = 0.19$ $p = 0.54$) is derived. σ_{YW} = Covariance of the residuals, which with the correlation within-infants' estimates ($r = 0.31$ $p = 0.14$) is derived.

Chapter 5: Oxytocin Trajectories, Infant Social Engagement, and Neurobehavior in Extremely Premature Infants

For all infants, the quality of early life experience is one of the most important predictors in achieving optimal socioemotional health, a key facet of infant development. Our earliest social experiences produce neurobiological changes which shape brain structure and function, and thereby exert lasting effects on neurodevelopmental outcomes (Weber, Harrison, & Steward, 2012). During the last trimester of pregnancy, the rate of growth, pruning and plasticity of the infant brain is at its highest, with almost 50% of neurons in the developing brain experiencing programmed cell death (Volpe, 2009). Extremely premature infants, those born at 28 weeks gestation or less, are thus hospitalized during this critical period of brain growth, and experience severely stressful events during these first months of life in the highly technological Neonatal Intensive Care Unit (NICU).

In addition to painful clinical procedures and distressing sensory stimuli, early life experience in the NICU often encompasses maternal separation and limited parental contact that serve as enormous social stressors for the infant (Dageville, Casagrande, De Smet, & Boutte, 2011; Flacking et al., 2012). Researchers have reported that parents visit their infant in the NICU on average 21 hours per week during hospitalization (Franck & Spencer, 2003; Gonya & Nelin, 2013), and that infants are held on average 2 times per

week (Reynolds et al., 2013). Thus, premature infants experience the vast majority of the first months of life without their mother. Furthermore, previous research shows that increased parent visitation and holding during NICU hospitalization was associated with improved quality of movement and less excitability (Reynolds et al., 2013).

The extremely stressful social experience of maternal separation places the extremely premature infant at high risk for altered brain development and subsequent deficits in socioemotional skills (Montagna & Nosarti, 2016). Extremely premature infants have higher rates of autism, behavioral problems, negative emotional temperaments, inattention, and other various socioemotional problems when compared with their term peers (Mahoney, Minter, Burch, & Stapel-Wax, 2013; Montagna & Nosarti, 2016; Msall & Park, 2008; Peralta-Carcelen, Bailey, Rector, & Gantz, 2013). Research is greatly needed to understand the mediators between early social experiences and subsequent socioemotional health, a critical component of infant development. We propose that the hormone oxytocin (OT) has great potential as a distinctive biomarker of social interactions that promote socioemotional health in vulnerable infant populations. OT is an affiliation hormone released during supportive social interactions (Ruth Feldman, 2012). The purpose of this study is to provide the preliminary evidence to support OT as a reliable, sensitive measure of the effects of early social experience in the NICU on neurodevelopment in premature infants.

Theoretical Framework and Background

Schore's Regulation Theory was the guiding framework for this study. Schore's Theory of Regulation contends that early life experiences result in a cascade of

neurobiological processes which are critical for adequate neuronal network formation, structural brain growth, and brain function (Schoore, 2001). Social interaction provides the infant with exposure to numerous maternal stimuli. If the social interaction is supportive, then maternal stimuli initiate a series of neurobiological processes that contribute to the emergence of infant self-regulation. Examples of these processes include activation of the mesocorticolimbic dopamine system, the HPA and SAM stress response systems, the parasympathetic nervous system, the social engagement system, and the OT system (Weber et al., 2012).

We reviewed the literature on OT to provide a theoretical extension of Schoore's Regulation Theory. OT significantly contributes neurodevelopment through neurobiological events that range from the molecular to multi-organ system levels. Through combinations of somatic, dendritic, and axonal release, OT enhances communication with other neurons through its peptide messaging. OT can also integrate these neurons into developing or existing neural circuits (Ferri & Flanagan-Cato, 2012; Stoop, 2014). Finally, OT-induced giant depolarizing potentials (GDPs) create massive synchronization and organization of neuronal groups, leading to establishment and maturation of infant brain circuits. In this way, the OT system may be an informative biomarker when studying maternal-infant interaction and neurodevelopment.

The OT system contributes to not only the neurobiological events that occur during maternal-infant interaction and promote neurodevelopment, but also the behavioral events. OT coordinates with the social engagement system to produce the physiologic states and engagement behaviors that define a sensitive, supportive social

interaction between the dyad (Porges, 2003). Infant engagement behaviors are elicited by various innervations of the social engagement system. For example, an extremely premature infant might display eyelid opening (eye muscles), vocalizations (larynx/pharynx), head turning towards a mother's voice (neck muscles), calming (vagal nerve), and facial expressions (facial muscles).

OT exhibits powerful, positive, and bidirectional feedback mechanisms with interaction behaviors and neurodevelopment (See Figure 4, Chapter 3). Higher levels of infant OT elicit more socially-engaged infant behaviors during interaction, which enhance infant neurodevelopment through both OT-based (see Figure 4, Stage 7) and non-OT based pathways. Conversely, engaging social behaviors increase infant OT after interaction (Ruth Feldman, Gordon, Influx, Gutbir, & Ebstein, 2013), which itself optimizes infant neurodevelopment (Figure 4, Stages 5-7). Moreover, neurotypical, socially-adept infants are more likely to elicit supportive and engaging maternal behaviors during interaction (Ruth Feldman, 2015a)—completing the positive, bidirectional loop of the relationship between engaging infant behaviors and infant development (Figure 4, Stage 7). Thus, through repeated and consistent daily interactions, the mother exposes her infant to repeated and consistent neurobiological events that significantly contribute to infant brain development.

Importantly, the theoretical relationships described above are being supported with evidence from clinical research studies with human infants. For example, positive associations exist among healthy term infants' salivary OT levels and the infants' socioemotional health (Ruth Feldman, 2015b; Pratt et al., 2015). Increases in infant OT

levels increase infants' social engagement during parent-infant interaction (Weisman, Zagoory-Sharon, & Feldman, 2012). Furthermore, infants with OTR gene alleles categorized as "low-risk" have increased social cognition at 18 months, as well as increased reciprocity, engagement, and empathy (Wade, Hoffmann, Wigg, & Jenkins, 2014).

Researchers must translate findings from healthy infant populations to vulnerable infant populations, so that clinicians can begin to monitor the biological underpinnings of infant socioemotional health and provide early intervention services when infants display altered socioemotional development. To address this gap in the literature, we developed two working hypotheses for our vulnerable infant population of interest, extremely premature infants:

- H1) Higher plasma OT trajectories (higher intercepts and steeper slopes) are associated with greater infant social engagement behaviors during maternal-infant interaction
- H2) Higher plasma OT trajectories (higher intercepts and steeper slopes) are associated with greater infant neurobehavioral organization, an early indicator of infant socioemotional health

This research will significantly enhance our understanding of the neurobiological processes underlying infant social behavior and the associations between OT levels and early indicators of premature infant socioemotional health in the NICU.

Methods

Sample

In order to address the purpose of our study, we recruited premature infants from

three Midwestern Level III NICUs. Inclusion criteria included English-speaking mothers who gave birth to premature infants ranging from 25-28 ⁶/₇ weeks gestation. Exclusion criteria were chosen due to their influence on infant brain development and/or neurobiological processes: history of maternal drug abuse (Ornoy, 2003; Thompson, Levitt, & Stanwood, 2009), presence of major congenital or chromosomal abnormality, grade 3 or 4 intraventricular hemorrhage (Bolisetty et al., 2013), hypoxic ischemic encephalopathy (Logitharajah, Rutherford, & Cowan, 2009), metabolic disorders involving the adrenal system (Khulan & Drake, 2012) and necrotizing enterocolitis requiring surgical intervention (Ta et al., 2011).

Power analysis was conducted to determine adequate sample size for detecting change in OT trajectories in extremely premature infants (See Aim 1, Chapter 4). Thus, because the overall dissertation was powered for Aim 1, the results of Chapter 5 will inform power analyses for future studies.

Procedure

Mothers were recruited during the first two weeks of life by direct approach from the PI. All participating mothers signed a written consent form approved by the institutional review boards of the academic institution and participating clinical sites. Sample collection started approximately on Day of Life 14, then commenced weekly until the infant achieved 34 weeks corrected gestational age (CGA). Sample collections occurred between the hours of 2300 and 0200 to control for the diurnal variation of OT. Urine was collected by cotton ball and placed in the diaper during the previous diaper change prior to data collection. Blood collection occurred by heelstick unless the infant

had an arterial line, and was concurrent with blood draws required for clinical treatment. Blood was collected into chilled EDTA (1mg/ml) BD microtainer tubes (Becton, Dickson, & Company, USA) containing Aprotinin (10 μ L/mL of blood). All samples were collected before feeding as OT levels are reported to be influenced by the act of suckling (Lupoli, Johansson, Uvnäs-Moberg, & Svennersten-Sjaunja, 2001) and digestion (Verbalis, Blackburn, Hoffman, & Stricker, 1995; Verbalis, McCann, McHale, & Stricker, 1986). After collection, samples were immediately placed on ice, processed, and transferred to a locked -80°C freezer.

Infant social engagement behaviors were measured during a videotaped feeding when the infant received at least 25% of daily enteral nutrition through nipple feeding. The first 5 minutes of a video-taped feeding task was scored, because we believed infants would initially display engagement behaviors before becoming fatigued during feeding. Videotaped feedings were scheduled to take place at the infant's bedside, during a time when mothers had already intended to visit and feed their baby in the NICU. Thus, times in which the videotaped feedings took place varied by baby. We also scheduled the videotaped feedings after mothers had already been regularly feeding their infants, to control for differences in maternal experience with premature infant feeding. However, because some mothers were only able to visit their infants a few times during NICU hospitalization, this was not always possible. If deemed safe by the infant's bedside nurse, doors and curtains were closed to maintain privacy of the dyad and minimize distractions.

Infant neurobehavioral organization was measured at 36 weeks CGA, or earlier if the infant was discharged before 36 weeks. Infant examinations were completed by the PI at the infant's bedside before any routine nursing procedures, with doors and curtains (if available) closed to minimize the effect of distractions and noise on the infant. Examinations were scheduled with the infant's bedside nurse on a day when stressful procedures were able to be minimized.

Measures

Plasma and urinary OT were measured using a commercially available kit (Peninsula Laboratories International, Inc., San Carlos, CA, KIT S-1355.0001), and samples were processed as previously described in the literature and in Chapter 4 of this dissertation (R. Feldman et al., 2010; R. Feldman, Gordon, & Zagoory-Sharon, 2011). Samples from the same infant were assayed at the same time to avoid inter-assay variation, and all measurements were performed in duplicate. Samples were assayed in batches to minimize the time spent in storage. Inter-assay and intra-assay coefficients of variation were 15% and 7%, as reported by the manufacturer. Urinary OT levels were normalized to total creatinine, and creatinine concentrations were measured using a commercially available kit (Cayman Chemical Co.). Data were analyzed using GraphPad Prism software.

Infant social behavior was measured using the well-validated Parent-Child Early Relational Assessment (PCERA; Clark, 2010), a 65-item assessment tool with subscales assessing observable behavior and affect of parent, infant, and dyad. The PCERA has been tested, validated, and used with both term and preterm infants (Clark, 1999;

McManus & Poehlmann, 2012; Poehlmann et al., 2011). Each item consists of an ordinal, 5-point Likert-type scale in which 5 is the most positive score (Clark, 2010). Scores of 1 or 2 indicate areas of clinical concern, scores of 3 indicate areas of some concern, and scores of 4 or 5 indicate areas of strength. For the purposes of this study, only the *Subscale: Infant Positive Affect and Behavior* was used, precisely because this scale measures infant social engagement behaviors during interaction. Examples of items include infant alertness during the interaction, social responsiveness, visual contact, and demonstration of exploring the environment. Interrater reliability was assessed by independent viewing of 20% of the tapes by two raters and was calculated at 91% agreement. Raters were blinded and had no interaction with participants. Subscale score was computed as the average of the 11 items in the scale.

Infant neurobehavioral organization was measured by the NICU Network Neurobehavioral Scale (NNNS), designed to evaluate the neurological integrity and behavioral function of high-risk infants (Lester et al., 2004). The NNNS includes assessment of functions such as reflexes, tone, states, and responses to sensory stimulation and stress. Summary scores are given for the areas of Habituation, Orientation, Amount of Handling, State, Self-Regulation, Hypotonia, Hypertonia, Quality of Movement, Number of Stress Abstinence Signs, and Number of Nonoptimal Reflexes. Ranges for each subscale are presented in Table 8. Higher scores on each subscale for the NNNS indicate that an infant possessed more of the quality measured by the subscale (e.g., higher scores on the Hypotonia Subscale means that the infant was more hypotonic). The NNNS has been shown to predict neonatal and medical outcomes

associated with prematurity (El-Dib, Massaro, Glass, & Aly, 2012). Interrater reliability has been shown to be 90% (Lester et al., 2004). Interrater reliability was not calculated for this study, because only the PI was trained and reliable in the NNNS. The PI, trained and reliable in the NNNS, performed and coded 100% of the assessments. The PI was blinded to both OT levels and infant social engagement scores, as these measures were performed by other study personnel. For the purposes of this study, a dichotomous variable was created that indicated normal/abnormal neurobehavioral status. Infants who had 2 or more NNNS summary scores 2 or more standard deviations from the mean of the sample were categorized as having an “abnormal” score on the NNNS. This analysis strategy has been used successfully in previous research (El-Dib et al., 2012).

Potential Confounders

We reviewed the literature for potential confounders that would bias our estimates of associations between infant OT levels, social engagement behaviors, and neurobehavioral organization. It is important to note that no studies of the effects of environmental factors on OT levels exist for infants during the first months of life. Instead, the confounders listed below were chosen based on evidence from theory, animal models, and human adults.

First, distressing early life experiences decrease OT levels in animal models (De Bellis & Zisk, 2014; Meaney, 2001). *Distressing events* were measured by the Neonatal Infant Stressors Scale (NISS), a tool which tracks clinical procedures known to cause distress and pain in the neonate (Newnham, Inder, & Milgrom, 2009). The NISS is designed to cumulatively measure a broad range of distressing events in the NICU such

as painful procedures, mechanical ventilation, handling, and feeding. Events are categorized and weighted by severity. Total score, the sum of all of weighted events an infant has experienced within a specific time period (Newnham et al., 2009), is associated with brain development in preterm infants (Smith et al., 2011). In this study, total score was calculated from the 8 hours prior to each OT collection. We assumed that an 8 hour duration would be both feasible to record and adequate to represent the significant variability in infant stress exposure between infants. Staff nurses were encouraged to record procedures during this time, but the PI also verified the record with the bedside nurse and the EMR.

Second, touch and physical contact have been shown to increase OT levels in animal models and adult humans (Dunbar, 2010; Holt-Lunstad, Birmingham, & Light, 2008). *Exposure of the infant to touch* was measured by total duration (in minutes) of skin-to-skin contact and holding during the previous week, as documented in the EMR. We chose to measure skin-to-skin and holding during a weeklong period in order to capture the inconsistency and variability with parents hold their infants during NICU hospitalization.

Volume of mother's breastmilk consumed per day was measured as mother's breastmilk given in ml/kg/day on the day of sample collection. OT levels may increase with increasing breastmilk volume, as OT is present and slightly variable in breastmilk (Takeda, Kuwabara, & Mizuno, 1986). We recently assayed OT within the breastmilk of 6 mothers, and found OT changes minimally within and between mothers. We also assayed OT in donor milk from 6 mothers before and after pasteurization. OT is

minimally detectable in donor milk, thereby negating the possibility of an effect on infant OT level.

Volume of feeds consumed per day, irrespective of milk/formula source, was measured in enteral volume consumed per day (ml/kg/day) on the day of sample collection. In addition to the OT present in mother's breastmilk, the infant may release additional OT during a feeding regardless of enteral source (i.e. from mother's breastmilk, donor milk, and/or formula) due to the act of digestion. OT levels increase in response to digestion of food and the secretion of cholecystokinin (Verbalis et al., 1995, 1986).

Illness severity was measured weekly using the Score for Neonatal Acute Physiology Perinatal Extension (SNAPPE-II). SNAPPE-II includes items for the lowest mean blood pressure, lowest temperature, lowest pH, respiratory dysfunction (the lowest of PaO₂/FiO₂ ratios at 3 points), low urine output, seizures, low birth weight, low 5-minute APGAR score, and small for gestational age (Richardson, Gray, McCormick, Workman, & Goldmann, 1993). SNAPPE-II is associated with many variables indicative of illness severity, including therapeutic intensity, nursing workload, and mortality (Gagliardi et al., 2004; Lim & Rozycki, 2008; Richardson, Phibbs, et al., 1993). SNAPPE-II has been associated with abnormal structural and functional brain development, including corticospinal tract abnormalities (Zwicker et al., 2013), intraventricular hemorrhage, moderate/severe ventriculomegaly, cerebral white matter lesions, and the Bayley Scales of Infant Development II (Dammann et al., 2010). A weekly SNAPPE-II score is included in the analysis.

Neurobiological risk was measured using the Neurobiological Risk Score (NBRS), a well validated predictor of neurodevelopmental outcomes that is strongly correlated with the Bayley Scales of Infant Development and with abnormal neurologic examination findings (Nunes et al., 1998). Seven items (infection, blood pH, seizures, intraventricular hemorrhage, assisted ventilation, periventricular leukomalacia, and hypoglycemia) are scored as 0, 1, 2, or 4 and summed, with higher scores indicating greater risk for adverse developmental outcomes. Total scores were calculated during the first two weeks of the infant's hospital stay and used for analysis. Infants with a score of ≥ 5 are considered to be at high risk for poor neurological development (Brazy, Eckerman, Oehler, Goldstein, & O'Rand, 1991; Brazy, Goldstein, Oehler, Gustafson, & Thompson, 1993). Presence of steroids in the infant's medication care plan were controlled for since endogenous and synthesized steroids influence the OT system (Chiodera et al., 1992; Lauand et al., 2007). A dichotomous variable (steroids given/not given in the last 7 days) was used in the analysis.

Data Analysis

To control the Type I error rate, an overall alpha level of 0.05 was chosen for analysis. All OT levels were logged, due to the skewed distribution of the data. Intermittent missing data was identified and quantified in the dataset. Six LMMs were ran in order to address the aims, while conserving power due to intermittent missing data. The first two models addressed Aim 1, using infant social engagement behaviors as a predictor of OT levels in plasma and urine, respectively. The next two models addressed Aim 2, using infant neurobehavioral organization as a predictor of OT levels in plasma

and urine. Because our review of the literature determined that no studies of the effects of confounders on OT levels exist for infants during the first months of life, we conducted two exploratory models with infant social engagement behaviors, infant neurobehavioral organization, and key chosen confounders, described below.

We used the Directed Acyclic Graph (DAG) approach to reduce confounding bias when modeling relationships between OT levels and early indicators of infant socioemotional health, namely infant social engagement behaviors and neurobehavioral organization. The DAG approach commonly used in epidemiologic studies was chosen to reduce the number of potential confounders and optimize parsimony of the Aim 2 model (Suzuki et al., 2009). In the DAG approach, relationships are drawn in a causal manner to visually depict the presence of confounding. Confounders can be visually identified by observing “backdoor paths,” i.e. arrows connecting the main independent variable of interest to the dependent variable, or main outcome. The goal of the DAG approach is to block all “backdoor paths” from the independent variable to the outcome, while controlling for the minimum number of covariates in order to preserve statistical power.

The covariates included in our DAG were infant social engagement behaviors, infant neurobehavioral organization, infant demographics, and all potential confounders (Figure 7). Based on our DAG model, it was determined that controlling for distressing events, exposure of the infant to touch, and volume of mother’s breastmilk would adequately control for confounding bias by eliminating all backdoor paths between OT levels and early indicators of neurodevelopmental outcomes (Figure 8).

Therefore, the covariates in the Aim 2 model were infant neurobehavioral

organization, infant social engagement behaviors, distressing events, exposure of the infant to touch, and volume of mother’s breastmilk. The 10% change in estimate procedure was used to confirm appropriateness for excluding all other potential confounders. The Aim 2 model is:

$$Y_{it} = \left(b_0 + \sum_j b_{0j} * X_{ji} + u_{0i} \right) + \left(b_1 + \sum_j b_{1j} * X_{ji} + u_{1i} \right) * Age_{it} + \varepsilon_{it}$$

In the model, X_{ji} is the value of a predictor j for infant i (e.g. gestational age at birth) and b_{kj} is the model parameter for the k^{th} term ($k=0$ or 1) for the j^{th} predictor. Infant social engagement behaviors and neurobehavioral organization were interacted with age in order to determine influence on the developmental trajectory of OT.

Results

Demographic and descriptive statistics for the sample are described in Tables 2 and 3 (See Chapter 4) and Table 8. As seen in Table 8, our sample demonstrated that infant engagement behaviors, as measured by the Infant Positive Affect and Behavior Subscale of the PCERA, were categorized as having “Some Concern” (mean= 3.01). Moreover, only 29 out of 37 infants recruited for the study had scores on the PCERA. This was because some mothers refused to be videotaped during feeding, or were unavailable due to infrequent visitation in the NICU. The average score on the NISS for our sample was 69.92 (17.54), which is lower than published means of approximately 80 (12) from similar cohorts (El-Dib et al., 2012; Smith et al., 2011). Sample variability was large in the NISS, as well as in the weekly amount of touch and daily volume of

breastmilk feeds (ml/kg/day) that infants received in the NICU. Moreover, an extreme bimodal distribution was present in the amount of mother's breastmilk given to the infant.

Our sample also demonstrated for extreme values for summary statistics of Subscales on the NNNS (Table 8), when compared with previously published data for extremely premature infants (El-Dib et al., 2012; Lester et al., 2011). Data for the NNNS were collected at 36 weeks CGA on 31 out of 37 infants, because 6 infants were either intubated or had unstable respirations on CPAP. Out of the 31 infants for whom the NNNS data was collected, 7 were categorized as being "abnormal."

The data for our first aim (Table 9), did not indicate a statistically significant association between infant social engagement behaviors and plasma OT trajectories, as infants with higher social engagement scores did not demonstrate higher OT intercepts, or OT levels at 27 weeks CGA ($b = -0.03$; $p = 0.96$). Nor did extremely premature infants with higher social engagement scores demonstrate steeper OT slopes ($b = -0.08$; $p = 0.45$), as hypothesized in the Aim 1 model. These associations were also true for the intercepts ($b = 0.40$; $p = 0.71$) and slopes ($b = -0.12$; $p = 0.58$) of urinary OT trajectories in extremely premature infants.

The data for our second aim (Table 10), did not indicate a statistically significant association between infant neurobehavioral organization and plasma OT trajectories, as infants with normal neurobehaviors did not demonstrate higher OT levels at 27 weeks CGA ($b = -0.54$; $p = 0.49$) or steeper OT slopes ($b = 0.11$; $p = 0.44$) in the Aim 2 model. Similar insignificant relationships were also found for the intercepts ($b = 1.01$; $p = 0.31$) and slopes ($b = -0.16$; $p = 0.35$) of urinary OT trajectories in extremely premature infants.

These insignificant associations remained, even after controlling for cofounders indicated in our DAG model (Table 11). However, only 25 infants out of the 37 had complete data, and were included in the exploratory DAG models.

Discussion

The purpose of this study was to provide the preliminary evidence of associations between OT levels and early indicators of infant socioemotional health in extremely premature infants hospitalized in the NICU. Our main indicators included infant social engagement behaviors during a maternal-infant feeding interaction, and infant neurobehavioral organization assessed at 36 weeks CGA. We did not see significant differences in OT levels based on infant social engagement behaviors. This finding refutes our original hypothesis that postulated that infants with higher scores on social engagement would tend to have higher OT levels. Interestingly, OT trajectories decreased over time in extremely premature infants, and unexpected result that might impact their ability to display synchronous social engagement behaviors. Feldman and colleagues found that social engagement was related to OT levels in synchronous dyads, but not dyssynchronous ones (Feldman, 2015b). This may explain why we did not find associations in our patient population; however, research that includes comparisons with other infant populations in need to confirm this explanation.

Premature infants are distinctly different from healthy term infants in their ability to exhibit socially engaging behaviors (Barnard, Bee, & Hammond, 1984). Previous research has found that premature infants score lower on the Positive Affect and Behavior Subscale than in normative populations, but these research studies measured the

PCERA at older ages during the first year of life (Clark, 1999). Other measures of maternal-infant interaction have found that premature infants exhibit social engagement behaviors with less frequency and clarity (Barnard et al., 1984).

Clark recommends using feeding as a context for measuring social interaction in young infants (Clark, 1999). It is difficult to observe social engagement behaviors during feedings, because the premature infant is focused the coordination of the suck-swallow-breath mechanism, as opposed to interacting with the parent. The premature infant often attempts to block any extraneous stimuli, such as stimuli from maternal social engagement behaviors, in an effort to maintain physiologic stability during feeding (Thoyre & Brown, 2004; Thoyre & Carlson, 2003; Weber & Harrison, 2014). PCERA scoring places significant emphasis on observable interaction behaviors that require enormous energy expenditure, but can still be seen before term equivalent: eye opening, purposeful motor movements towards caregiver, synchronous gaze, and head turning towards caregiver (Clark, 1999). Thus, the premature infant may not display these behaviors during feeding in an effort to conserve energy, promote physiologic stability, and ensure safe consumption of milk. Changing the context of the interaction to an activity that does not expend a significant amount of energy, such as a diaper change, may mitigate this disadvantage by allowing a premature infant to expend more energy on exhibiting social engagement behaviors during maternal-infant interaction.

Nevertheless, changing the context of measurement would still not address the fact that classic infant “behaviors” listed above typically emerge around 33-34 weeks CGA. An important question to address is the definition and measurement of

“behaviors.” This is because the PCERA rater is coding for “behaviors”, i.e., movements that are coordinated in response to external or internal stimuli (Dugatkin, 2012). The timing of CGA in which an infant will begin to exhibit “behaviors”, as well as the prevalence in which they are exhibited, may vary widely due to infant medical morbidities and experience with the NICU environment (K. Pridham et al., 2005; K. F. Pridham, Brown, Clark, Sondel, & Green, 2002). Previous applications of the PCERA to the preterm infant population measured infant social behaviors after 1 month CGA (i.e. approximately 4 months of life) and thus after NICU hospitalization (Brown, 2007; K. F. Pridham, Brown, Sondel, Clark, & Green, 2001). During NICU hospitalization, a period of time that often equates to the first three months of life, premature infants are making the transition from the physiologic to the sociobehavioral world.

A significant disadvantage of the PCERA in this population is that it assumes infant movements (i.e. behaviors), are purposeful, goal-directed, and thus, socially-motivated. Motor movements in the preterm population can be reflexive, non-purposeful, and physiologically-based due to immaturity of the infant nervous system (Bougle et al., 1990; Weinstein et al., 2014). Furthermore, similar movements may be reflexive in one situation but purposeful in another, based on the internal or external stimuli the premature infant is attempting to process, interpret, and then execute a response (Weinstein et al., 2014). Thus, significant challenges exist in scoring the PCERA for a premature infant during the first months of life, when the infant is learning to exhibit purposeful movement and goal-directed behaviors (Kostović, Judas, Petanjek, & Simić, 1995). It is also during this time that the preterm brain is significantly maturing sensory-motor fiber

tracts that control goal-directed movement, or observable behavior (Weinstein et al., 2014). During the first months of life, our data suggest that the PCERA may not be an appropriate tool for use in this population. Researchers should consider measuring infant engagement behaviors after the first months of life, when the preterm brain has development the sensory-motor fiber tracts that allow for coordinated and purposeful movement—and thus, socially-based behavior.

This does not necessarily mean that premature infants do not socially engage with their parents before this time period, it just means that they interact and engage *differently* than older infants. In other words, we are proposing that premature infants socially interact and engage parents with their *physiology*, as opposed to their *behavior*. This statement is consistent with what we would expect in the developmental timeline of a fetus—a mother and her fetus form a relationship during the pregnancy that is based in physiology. After birth, the dyad is primed to mature the relationship into the behavioral realms. A significant strength of researching the OT system includes that measuring OT means one is measuring the socially-based neurobiological antecedents of the social behaviors (e.g. mutual gaze, therapeutic touch, soft vocalizations) that define social interactions, construct the mother-infant relationship, and promote infant development. Before sociobehavioral rhythms can be established, physiologic rhythms must be intact (R. Feldman, 2006).

This concept is beautifully described by the seminal work of Als, who asserted that the physiologic cornerstones of development (i.e. autonomic nervous subsystem) must be stable before behavioral components of development (motor subsystem,

attention/interaction subsystem) can emerge and then contribute to infant self-regulation (Als, Butler, Kosta, & McAnulty, 2005). Thus, before premature infants interact with their caregivers through their attention, orientation, and social engagement behaviors, they interact with caregivers through their physiology, such as heart rate, respiration, blood pressure, and hormones such as OT (Weber & Harrison, 2014). An excellent example of this principal at work in the NICU would be skin-to-skin contact between a mother and her premature infant (R. Feldman, Weller, Sirota, & Eidelman, 2002). Mothers and their infants may not display any social behaviors during skin to skin, but their heart rate, respiratory rate, and temperature will coregulate and respond to each other (Conde-Agudelo & Díaz-Rossello, 2014; Neu, Hazel, Robinson, Schmiede, & Laudenslager, 2014). Measures that account for physiologic rhythms, and the subtle, yet primitive, motor movements of premature infants would be ideal for research with premature infants. Examples of these measures include the Assessment of Preterm Infant Behavior (Als & McAnulty, 2011)

A significant advantage to measuring OT is that as an ancient and evolutionary hormone, its chemical structure was designed to connect and augment the organism's transition from the physiologic to the sociobehavioral world (Carter, 2014). This is why OT is involved in, seemingly, such a wide range of functions, from the more physiologic-based (blood pressure, hydration, energy balance, heart rate variability), to the socially-based (proximity seeking, mutual gaze, vocalizations). We hypothesize that measuring OT will allow researchers to explore the window of development in which the infant transitions from the physiologic mode of interaction with the environment to the

behavioral mode. OT levels may provide information on the neurobiological antecedents of social behavior, but researchers must determine the best timing of these measures based on theory, previous research, and the scientific question of interest.

We also did not see significant differences in OT levels based on neurobehavior, but saw the possibility of a large effect due to clinically significant coefficients. According to our models, the change in plasma OT levels for an infant categorized as being “abnormal” on the NNNS could have as much as 42% decrease on plasma OT levels. This is consistent with our original hypothesis, which postulated that infants with abnormal scores would tend to have lower OT levels than those infants categorized as “normal” on the NNNS.

A limitation of the data gathered from our NNNS examinations is that it was not possible to calculate inter-rater reliability for the exams, because only one team member, the PI, was trained in the NNNS. We had attempted to videotape the examination and complete reliability scoring by videotape, however, due to the number of position changes required by the examination, it was not possible to obtain a high quality taping without an assistant at the bedside. Scheduling with an assistant was not possible due to the often last minute, and frequent changes, in examination time that were requested by the nurses. The lack of interrater reliability represents a limitation of measurement accuracy in this study.

In addition to larger sample sizes, it will be very effective to compare results of NNNS among extremely premature infants, other preterm infant populations, and term infants. Previous research has noted significant differences in NNNS results between

normative infant populations and high-risk infant populations (Tronick et al., 2004), such as premature infants (El-Dib et al., 2012), drug-exposed infants (Boukydis & Lester, 1999), and infants with mothers suffering from postpartum depression (Salisbury et al., 2011). In our sample, we did see remarkable differences between normative, healthy term infant scores, and also previously published scores for extremely premature infants. However, NNNS data are commonly collected at term-equivalent age (40 weeks CGA), whereas we collected our NNNS at 36 weeks CGA (El-Dib et al., 2012).

The four-week period from 36-40 weeks gestation constitutes an enormous change in brain development, and thus infant neurobehavior. This may explain why our scores indicated lower neurobehavioral organization than previously available data. We collected the NNNS at 36 weeks in order to ensure that all infants would be hospitalized in the NICU, and thus available for data collection. However, we learned from this study that in our extremely preterm population, most infants were still hospitalized at 40 weeks. One solution for future studies may be to measure infant neurobehaviors as late as possible in the design (e.g. 40 weeks gestation, or right before discharge).

As discussed earlier, a considerable challenge in measuring behaviors in this population lies with the extreme immaturity of infant nervous systems, so that the brain often cannot adequately interpret and respond to stimuli during such a physiologically taxing event like feeding or comprehensive neurologic examination (Weber & Harrison, 2014). There are distinct disadvantages of using behavioral methods when studying brain development in neonates and young infants (Mento & Bisiacchi, 2012). Data obtained using behavioral methods are often difficult to interpret with infant populations, as

significant subjectivity in measurement can be introduced due to immaturity of the infant's perceptual and attentive systems (Haith, 1998). Behavioral measures provide no information on the nature, timing, and location of processes occurring within the brain between the introduction of the environmental stimulus and the behavioral response. Moreover, behavioral methods fail to generate critical knowledge on neurobiological mechanisms underlying emerging infant abilities and the relationships between structural and functional brain development (Mento & Bisiacchi, 2012). The common failure to replicate behavioral findings highlights the weaknesses of purely behavioral research. These disadvantages support the notion that behavioral markers alone are not sufficient for descriptive and intervention research focused on brain development.

Given the constraints of behavioral measures, biological measures of neurodevelopmental outcomes which can be safely and efficiently measured in the early period of infant brain development are needed to advance prematurity research. This is precisely why measuring promising biomarkers such as OT constitute as a significant contribution to neurodevelopmental research in premature infants. Biomarkers often give clues pointing to altered developmental processes before behaviors change. Feldman and colleagues asserted that neurobiological rhythms are the foundation for socio-behavioral rhythms in the dyad (R. Feldman, 2006). These social, behavior-based rhythms can be seen at 3 months of life (R. Feldman, 2006). Applying this to our study, we might hypothesize that alteration in preterm infant OT levels would be observed before we measure differences in engagement behaviors and neurobehavioral organization. Future designs may consider measuring infant behaviors during later periods of infant

development, or even longitudinally to determine when reliable patterns of infant social behavior emerge in extremely premature infants.

We conclude that while larger sample sizes are needed to detect the potentially clinically significant results we observed, the research questions and recommendations generated from this dissertation are worth pursuing with future study designs that include larger windows of observation (e.g. until 40 weeks CGA) and comparisons among gestational age groups. Because different infants had missing data on either social engagement behaviors, infant neurobehavioral organization, or both, the exploratory DAG models were executed with 25 infants. The DAG models, although exploratory, certainly reach the limit of what our data can reasonably estimate with completed data on 25 infants. In our experience with longitudinal research designs, intermittent missing data is common with this vulnerable infant population.

While we experienced minimal missing data in the NISS and volume of mother's milk received (due to infant transport off the unit and data entry errors in the EMR), we experienced significant intermittent missingness in plasma and urinary OT levels (See Tables 4 and 5, Chapter 4). For example, most of our infants did not have complete data on plasma OT levels, because either the healthcare provider did not order laboratory tests that week, or the infant's hemoglobin and hematocrit was too low for safe sample collection. Reasons for missingness on urinary OT levels including low urinary output, contamination with stool/diaper cream, or bedside nurses omitting placement of the cotton balls in the infant's diaper. Intermittent missingness in our data represent a limitation of the study, but it is important to remember that the vast majority of

longitudinal designs share this limitation. Future research that incorporates longitudinal designs with the population should plan for missing data in their power analysis (Tu et al., 2007).

Our sample demonstrated wide variability in theoretically important confounders, such as exposure to stressful clinical events as measured by the NISS. Anecdotally, this was most likely due to starkly different clinical courses, comorbidities, and acuity seen in the sample. Furthermore, our sample demonstrated large variability in the amount of weekly touch and volume of breastmilk received from the mother. This was anecdotally due to a wide variability in parent visitation, as each set of parents experienced their own barriers to visiting the NICU, such as access to transportation, childcare for siblings, and paid maternity leave. When parents returned to work, some were able to hold their baby daily, while others only were able to visit during weekends, due to employment obligations. By 34 weeks CGA, most infants received the recommended 150 ml/kg/day of enteral feeds due to their maturation, and thus tolerance, of oral nutrition. By this age, infants whose mothers did not intend to breastfeed were transitioned, or in the process of transitioning, to formula feeds. This common phenomenon in NICUs would explain the extreme bimodal distribution that was present in the variable: volume of mother's breastmilk.

An important finding when viewing models that included either infant social engagement behaviors or infant neurobehavior pertains to the coefficient of CGA (i.e., the effect of age on OT levels). The CGA coefficients were stable in many of the models despite erratically changing p values, and are similar to the coefficients we obtain when

we run models with only CGA as a predictor variable. This statistical observation suggests that while the models are close to being over-parameterized due to limitations in sample size, the large effect of age on decreasing plasma OT levels is an important and consistent finding that warrants replication and future study.

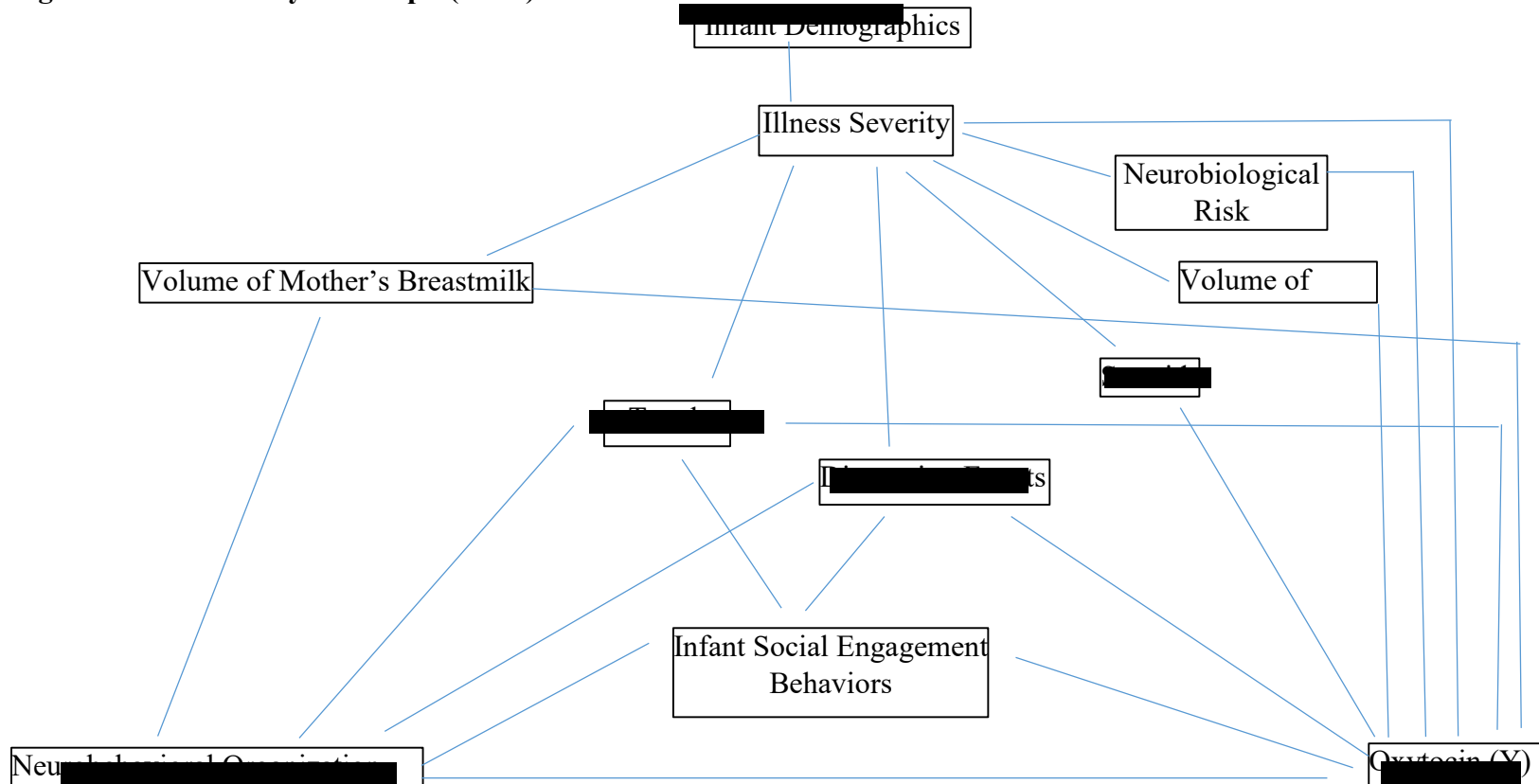
Based on the study findings, we recommend that future research investigate the critical period of infant development that encompasses the infant's transition from the biological to the sociobehavioral world. Given the importance of the caregiver (primarily the mother) in guiding this transition, it is of grave concern that premature infants not only have decreasing OT levels in the NICU, but also display lower social engagement behaviors and neurobehavioral organization than published norms. This may indicate that when the infant is struggling to interact with caregivers in a physiological way, the development of the behavioral interaction also suffers later in life. This hypothesis will inform our next study, which will investigate the impact of the dyad's physiologic relationship on the development of the dyad's sociobehavioral relationship. We will also compare the development of these relationship transitions between different gestational age groups in order to assess the impact that prematurity has on the relationship transition.

Conclusion

This study is the first to investigate associations between OT levels and early indicators of infant socioemotional health in extremely premature infants. This work also provides valuable information for future studies to plan research designs using OT levels in the extremely preterm population. This study also highlights the importance of

incorporating biological measures into prematurity research designs, due to immaturity of this population's nervous system and significant subjectivity of measuring behaviors in the preterm infant population. While our study investigated biobehavioral associations between a social hormone and social behaviors in the early neonatal period, we recommend that in this population, researchers measure behaviors either longitudinally or during a period of infant development in which behaviors are more reliably recorded, such as 3-4 months of life. However, this study represents an important step in creating the foundation for using OT as a biomarker of social processes affecting infant neurobiology and brain development.

Figure 7. Directed Acyclic Graph (DAG) Model: Part II.



113

In the Directed Acyclic Graph (DAG) approach to confounding, all potential confounders are included in the drawing. Lines between variables indicate potential relationships.

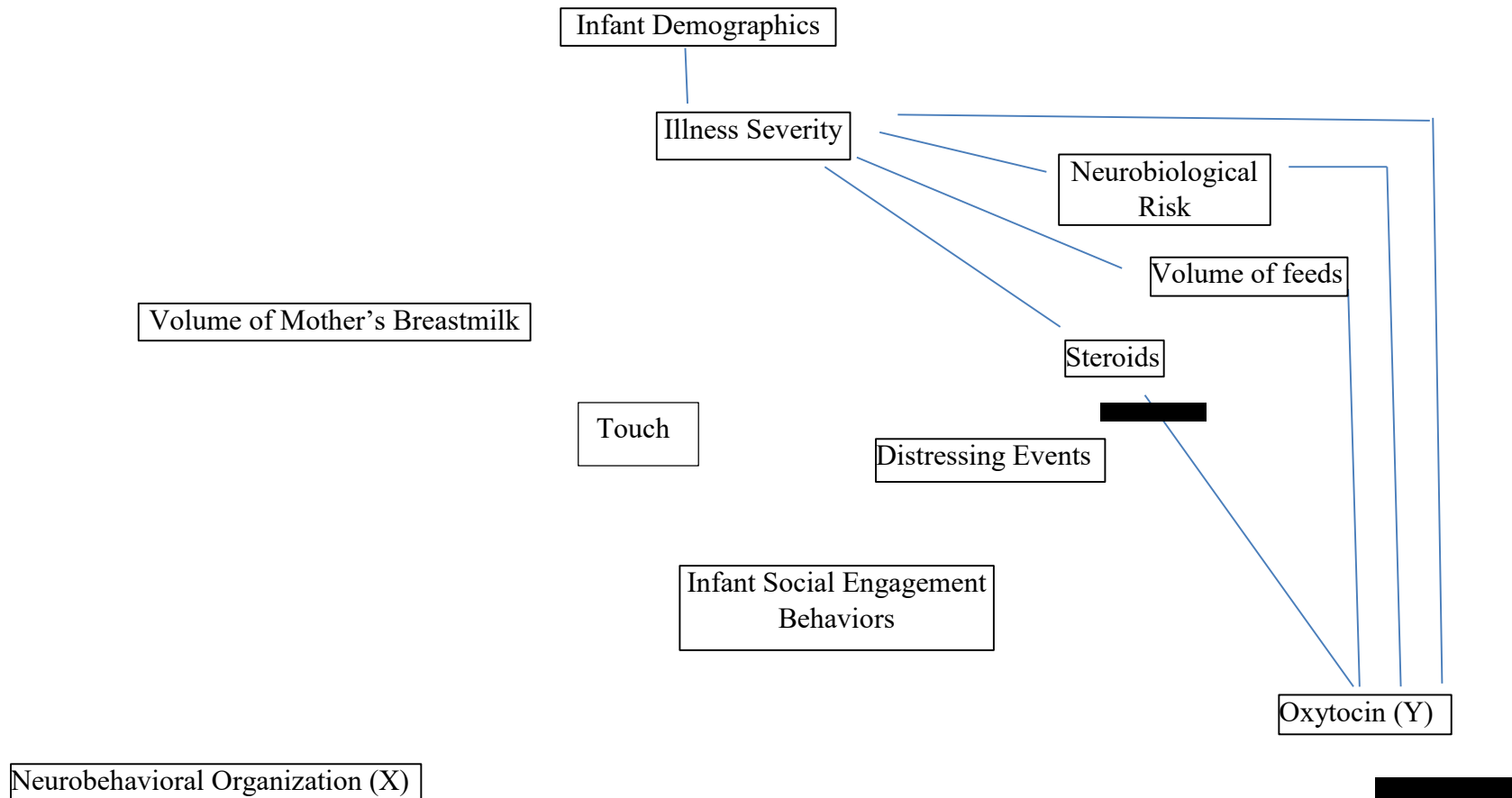
(Continued)

(Figure 7 continued)

The goal of the DAG approach is to control for key variables that will eliminate all confounding pathways between independent variables of interest (Xs: maternal-infant interaction and neurobehavioral organization) and the dependent variable (Y: oxytocin levels). See DAG Model: Part II for the confounding variables that were selected and controlled for to eliminate confounding pathways and bias.

Figure 8. Directed Acyclic Graph (DAG) Model: Part II.

115



This DAG model is the final product in the DAG procedure, which reveals the key variables that will adequately control for confounding bias.
 (Continued)

(Figure 8 continued)

As seen in the model, confounding pathways are eliminated between X's (maternal-infant interaction and neurobehavioral organization) and Y (infant oxytocin levels) when controlling for volume of mother's breastmilk, parental touch, and distressing events. Therefore, those confounders will be included in the Aim 2 Model as they adequately control for confounding bias. All other confounders will be subjected to the 10% change in estimation procedure.

Table 8. Summary for Main Outcomes and Cofounders in the DAG Model

Variable	Obs	Mean	Std. Dev.	Min	Max
Infant Engagement ^a	29	3.01	0.47	2	4
Neonatal Infant Stressor Scale ^b	209	69.92	17.54	33	132
Weekly Touch (hrs)	213	14.46	11.95	0	47
Volume of Breastmilk Feeds (ml/kg/day)	212	95.91	61.53	0	171

a = Measured by the Infant Positive Affect and Behavior Scale on the PCERA

b = Neonatal Infant Stressor Scale (NISS) was measured weekly

NNNS Subscales	Obs	Mean	Std. Dev.	Min	Max	Range
Habituation (nhabit)	14	7.54	1.84	4.00	9.00	1-9
Attention (nattention)	26	4.29	1.38	1.57	6.71	1-9
Handling (nhandle)	28	0.71	0.22	0.25	1.00	0-1
Quality of Movement (nqmove)	31	4.44	0.49	3.33	5.33	1-9
Regulation (nregulation)	31	5.41	0.79	4.29	6.92	1-9
Nonoptimal Reflexes (nnonoptref)	31	4.90	1.66	2.00	8.00	1-15
Physiologic Stress (nstressp)	31	0.58	0.34	0.00	1.00	0-1
Autonomic Stress (nstressa)	30	0.21	0.16	0.00	0.50	0-1
Central Nervous System Stress (nstresss)	31	0.48	0.30	0.00	1.00	0-1
Gastrointestinal Stress (nstressg)	31	0.08	0.14	0.00	0.33	0-1
Skin Stress (nstressx)	31	0.15	0.12	0.00	0.43	0-1
Arousal (narousal)	31	3.80	0.42	3.14	4.71	1-9
Hypertonicity (nhypetone)	31	0.06	0.25	0.00	1.00	0-10
Hypotonicity (nhypotone)	31	1.06	1.06	0.00	4.00	0-10
Asymmetrical Reflexes (nasymref)	31	0.52	0.68	0.00	2.00	0-16
Excitability (nexcitability)	31	2.61	1.33	0.00	5.00	0-15
Lethargy (nlethargy)	31	6.52	2.29	2.00	11.00	0-15

Dichotomous NNNS	Frequency	Percent	Cumulative Percent.
0 = "Normal"	24	77.42	77.42
1 = "Abnormal"	7	22.58	100
Total	31	100	

NNNS = NICU Network Neurobehavioral Scale

Table 9 Models for Determining Associations with Infant Social Engagement

Log Plasma (pg/ml)	Coef.	P>z	[95% Conf.	Interval]
Engagement Behaviors	-0.03	0.96	-1.04	0.99
Corrected Gestational Age	0.16	0.59	-0.43	0.75
Engagement*CGA	-0.08	0.45	-0.27	0.12
CGA at assessment	-0.01	0.18	-0.03	0.01
Intercept	4.43	0.00	1.38	7.48

Random-effects Parameters	Estimate	[95% Conf.	Interval]
Unstructured Matrix			
σ_{b0} (random intercepts)	0.12	0.01	1.87
σ_{b1} (random slopes)	0.00	0.00	0.00
sd(Residual)	0.62	0.51	0.74

Log Urine (pg/ml/ng creatinine)	Coef.	P>z	[95% Conf.	Interval]
Engagement	0.40	0.71	-1.73	2.53
Corrected Gestational Age	0.36	0.58	-0.92	1.64
Engagement*CGA	-0.12	0.58	-0.54	0.30
CGA at observation	-0.02	0.40	-0.05	0.02
Intercept	7.63	0.02	1.20	14.05

Random-effects Parameters	Estimate	[95% Conf.	Interval]
Unstructured Matrix			
σ_{b0} (random intercepts)	1.52	0.77	2.98
σ_{b1} (random slopes)	0.32	0.17	0.61
ρ^{\wedge} (correlation of random effects)	-0.99	-1.00	-0.40
sd(Residual)	0.93	0.77	1.13

Table 10. Models for Determining Associations with Infant Neurobehavior

Log Plasma (pg/ml)	Coef.	P>z	[95% Conf.	Interval]
NNNS	-0.54	0.49	-2.06	0.98
Corrected Gestational Age	-0.19	0.01	-0.32	-0.05
NNNS*CGA	0.11	0.44	-0.17	0.39
Intercept	4.92	0.00	4.16	5.68

Random-effects Parameters	Estimate	[95% Conf.	Interval]
Unstructured Matrix			
σ_{b0} (random intercepts)	1.14	0.63	2.05
σ_{b1} (random slopes)	0.20	0.11	0.37
ρ^{\wedge} (correlation of random effects)	-0.98	-1.00	-0.73
sd(Residual)	0.50	0.41	0.62

Log Urine (pg/ml/ng creatinine)	Coef.	P>z	[95% Conf.	Interval]
NNNS	1.01	0.31	-0.94	2.95
Corrected Gestational Age	0.15	0.08	-0.02	0.32
NNNS*CGA	-0.16	0.35	-0.51	0.18
Intercept	7.91	0.00	6.92	8.91

Random-effects Parameters	Estimate	[95% Conf.	Interval]
Unstructured Matrix			
σ_{b0} (random intercepts)	1.30	0.47	3.60
σ_{b1} (random slopes)	0.20	0.06	0.70
ρ^{\wedge} (correlation of random effects)	-0.91	-1.00	-0.01
sd(Residual)	0.93	0.78	1.12

Table 11 Directed Acyclic Graph (DAG) Models

Log Plasma (pg/ml)	Coef.	P>z	[95% Conf.	Interval]
Corrected Gestational Age	-0.08	0.84	-0.87	0.70
Engagement	-0.43	0.58	-1.95	1.08
NNNS	-0.34	0.68	-1.95	1.26
Engagement*CGA	-0.01	0.96	-0.27	0.26
NNNS*CGA	0.07	0.65	-0.22	0.35
NISS	0.01	0.09	0.00	0.01
Weekly Touch	0.00	0.84	-0.01	0.01
Volume of Breastmilk Feeds	0.00	0.94	0.00	0.00
Intercept	5.36	0.02	0.82	9.91

Random-effects Parameters	Estimate	[95% Conf.	Interval]
Unstructured Matrix			
σ_{b0} (random intercepts)	1.19	0.68	2.07
σ_{b1} (random slopes)	0.20	0.11	0.36
ρ^{\wedge} (correlation of random effects)	-1.00	-1.00	1.00
sd(Residual)	0.47	0.38	0.58

Log Urine (pg/ml/ng creatinine)	Coef.	P>z	[95% Conf.	Interval]
Corrected Gestational Age	-0.03	0.95	-0.94	0.88
Engagement	-0.25	0.77	-1.95	1.45
NNNS	2.19	0.01	-1.95	1.45
Engagement*CGA	0.06	0.71	-0.24	0.36
NNNS*CGA	-0.30	0.05	-0.61	0.00
NISS	0.00	0.57	-0.01	0.02
Weekly Touch	0.01	0.57	-0.01	0.04
Volume of Breastmilk Feeds	0.00	0.50	-0.01	0.00
Intercept	8.39	0.00	3.29	13.50

Random-effects Parameters	Estimate	[95% Conf.	Interval]
Random Intercept			
σ_{b0} (random intercepts)	0.15	0.00	11.69
sd(Residual)	1.01	0.86	1.20

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Appendix A: Data Collection Procedural Timeline

Procedure: Data Collection Timeline.

Data Collection at Corrected Gestational Age (in weeks)										
Gestational age at birth	27	28	29	30	31	32	33	34	36*	Variable Age
25	XOI	OI	OI	OI	OI	OI	OI	OI	N	M
26		XOI	OI	OI	OI	OI	OI	OI	N	M
27			XOI	OI	OI	OI	OI	OI	N	M
28				XOI	OI	OI	OI	OI	N	M

X = Maternal and infant demographic information

O = Saliva, urine, and plasma collection

I = Time-varying infant variables (e.g. volume of feeds, distressing events)

N = Infant neurobehavioral organization

*Measured at 36 weeks or earlier if discharged

M = Maternal-infant interaction—observation will vary by infant feeding readiness