Published in final edited form as:

Adv Neonatal Care. 2018 February ; 18(1): E12–E23. doi:10.1097/ANC.0000000000000452.

Associations between Nurse-Guided Variables and Plasma Oxytocin Trajectories in Premature Infants during Initial Hospitalization

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Abstract

Background—Oxytocin (OT) is a social hormone that may help researchers understand how nurse-guided interventions during initial hospitalization, such as supporting human milk expression, promoting comforting touch, and reducing exposure to stressors, affect preterm brain development.

Purpose—To determine if factors related to human milk, touch, or stressor exposure are related to plasma OT trajectories in premature infants.

Methods—Plasma from 33 premature infants, born gestational ages 25–28 6/7 weeks, was collected at 14 days of life, then weekly until 34 weeks corrected gestational age (CGA). Variables related to feeding volumes of human milk and formula; touch, as indexed by skin-to-skin contact (SSC) and swaddled holding; human milk and clinical stressors were extracted from the electronic medical record. Linear mixed models tested associations between nurse-guided variables and plasma OT trajectories.

Results—Same-day SSC was positively related to plasma OT at 27 weeks CGA ($\beta = 0.938$, $p = 0.002$), but also related to a decline in plasma OT over time ($\beta = -0.177$, $p = 0.001$). Volume of enteral feeds (ml/kg/day), its interaction with CGA, and number of stressful procedures were not

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Original Research Category: Work occurred at The Ohio State University

The authors declare no conflicts of interest or commercial interests in this work.

statistically significant ($\beta = 0.011$, $p = 0.077$; $\beta = -0.002$, $p = 0.066$; $\beta = 0.007$, $p = 0.062$, respectively).

Implications for Practice—Nurse-guided interventions are associated with infant plasma OT, suggesting nurses may impact the neurobiology of the developing premature infant.

Implications for Research—Replication with larger sample sizes and randomized controlled trial designs is needed to test effects of specific nursing interventions on infant OT.

Keywords

oxytocin; premature infant; stress; NICU; kangaroo care; physiology; feeding; holding; oxytocin trajectory; skin-to-skin contact

As neonatal nurses, we take care of infants during a critical and sensitive period in our patients' brain development.¹ Perhaps the most vulnerable population under our care are infants born at earlier gestational ages, e.g., extremely/very preterm infants. These infants are hospitalized in the Neonatal Intensive Care Unit (NICU) for roughly the duration of the last trimester of pregnancy, when malleability of the infant brain is at its highest.² During this time the preterm brain is dependent upon developmentally-supportive experiences in order to properly organize, differentiate, and function in the extra-uterine NICU environment.³ In our nursing practice, we observe how our patients positively respond to developmentally-supportive experiences, such as skin-to-skin contact (SSC),⁴ and nursing researchers have shown that developmentally-based care improves structural and functional brain development.^{5,6} However, it is unclear *how* developmentally-supportive experiences are able to improve brain development.

The development of a reliable biomarker of infant responses to developmentally-supportive experiences would provide nursing researchers with a more immediate indicator of intervention effects. Moreover, the use of biomarkers would enhance nurse researchers' ability to develop new interventions that promote developmentally-supportive experiences by increasing understanding of the neurobiological mechanisms driving infant neurodevelopmental outcomes. We propose that oxytocin (OT), the "social" or "bonding" hormone,⁷ has the potential to be a biomarker of infant responses to developmentally-supportive and socially-based experiences.

Background and Significance

OT is produced by the hypothalamus, the center of the OT system. The structure of the OT system includes vast connections within the brain as well as diverse and far-reaching peripheral targets such as the heart, gastrointestinal tract, and kidneys.⁸ This foundational structure allows the OT system to efficiently relay and respond to somatosensory stimuli provided by infant experiences, the NICU environment, or therapeutic nursing interventions. The OT system interacts with many other crucial biological systems, including the hypothalamic-pituitary-adrenal axis,^{9–11} the autonomic nervous system,^{12–14} and the dopaminergic system.^{15–17} These interactions produce biological changes in hormonal release which significantly influence neurologic processes and shape infant development.¹ Importantly, OT is involved in social processes such as bonding^{18,19} and attachment,^{20,21}

and in neurologic processes related to social cognition and emotion regulation.^{22–26} However, research on the development of the OT system has not been conducted in premature infants. Moreover, published norms for plasma OT levels do not exist for human infants and children, although norms have been published in adults.²⁷ Neonatal nurses interact more than other health professionals with newborns and their families, occupying a key position for teaching and modeling how to provide developmentally-supportive social experiences that promote bonding, attachment, and socioemotional development. When families are not present at the bedside, nurses assume the responsibility of providing social experiences for the infant. Incorporating measurement of OT into neonatal research offers the opportunity to test effects of newly-developed nursing interventions aimed at supporting the parent-infant relationship and neurobiological processes that promote infant socioemotional development. By validating newly-developed nursing interventions with reliable biomarkers and understanding the neurobiological pathways through which effective nursing interventions work, new interventions can be translated into practice in less time, and nurse clinicians can better understand and implement those interventions with a more comprehensive evidence base.

Literature Review

Factors known to activate the OT system in humans are primarily nurse-guided, such as 1) expression of human milk,^{28,29} 2) touch,³⁰ and 3) exposure to stressors,^{31,32} and are highly relevant to neonatal interventions that influence infant brain development.

Human milk

A mother's milk is critical for the optimal health, nutrition, and growth of her premature infant. As the people who can best provide the safest and most effective source of nutrition to our patients, mothers are the most important providers in the provision of milk for preterm infants. To fulfill this critical role, mothers commonly receive guidance from bedside nurses and lactation consultants to be successful. Neonatal nurses are instrumental in supporting and guiding these mothers during the processes involved in providing human milk for their infants and in supporting direct breastfeeding as soon as possible. Nurses provide lactation education and interventions that assist mothers in initiating and maintaining the expression of human milk for the optimal development of their infants. OT is present in fresh, refrigerated, frozen, and thawed human milk,^{33,34} and researchers have hypothesized that the provision of human milk is one mechanism through which mothers influence the neurobiological environment of the infant brain.³⁵ Salivary and plasma OT levels are higher in human breastfeeding mothers than formula-feeding mothers,³⁶ and the concentrations of OT in human milk are also higher after breastfeeding.³⁴ Whether the OT levels of preterm or term neonates are responsive to breastfeeding is unknown (See Box 1). Only one study has compared plasma OT levels in breastfed and formula-fed infants,³³ and these researchers did not find significant differences in a sample of 25 term neonates over the first four days of life. Whether human milk OT is transferred intact and readily absorbed by the digestive system in human neonates is also unknown. No human *in vivo* studies have investigated the absorption of OT by the infant's intestinal tract.

Box 1**Did you know?**

Studies using buffalo³⁷ and dairy cow milk³⁸ showed that the OT in exogenously spiked milk was resistant to freezing and pasteurization processes. OT in animal milk was also resistant to boiling and to gastric pepsin digestion, but readily degraded by intestinal proteases, such as pancreatin.³⁷ The findings of these researchers, in addition to others using simulations of human intestinal digestion of OT,³⁹ have led some to conclude that OT in human milk cannot be absorbed due to rapid degradation by pancreatic juices in the intestinal tract. However, simulation studies using human intestinal tracts ex vivo found that OT can be absorbed when levels of pancreatic enzymes are low.³⁹ Premature infants are known to have pancreatic insufficiency, due to the immaturity of the infant gastrointestinal tract.^{40–43} Absence of pancreatic juices has been found in the guinea pig⁴⁴ and in the human intestinal tract³⁹ to increase the intestinal absorption of OT. In neonatal mice lacking genes necessary for OT secretion, plasma OT levels are normal until cessation of nursing, leading one group of researchers to conclude that the offspring must have been receiving exogenous OT from mother's milk.⁴⁵ Finally, in a rat model, radiolabeled OT given intravenously to the mother was found in the plasma of the neonates.³⁴ Thus, the data from these animal and human ex vivo studies suggest that it is possible for OT in mother's milk to be absorbed by the infant's developing intestinal system.

Regardless of enteral feeding source, OT is released in response to food digestion^{29,46,47} and acts as a key satiety hormone.⁴⁸ OT also plays a role in the regulation of food intake, energy balance, and body weight via complex physiologic mechanisms, including activation of afferent vagal neurons.^{49–51} Thus, the volume of an infant's enteral feeds, regardless of milk source, may stimulate OT release. No human or animal studies have investigated differences in OT levels (either before or after feeding) between infants who receive increasing volumes of enteral feeds.

Touch

In daily practice, neonatal nurses either provide or encourage numerous forms of comforting touch, including hand containment, swaddled holding, or SSC. Touch is a stimulator of peripheral OT release in humans.^{30,52–54} We, along with others,³⁵ hypothesize that infants who experience increased physical contact will have increased OT release and peripheral OT levels. Both human parents and their infants/children have increased salivary OT levels after physical contact.^{52,56–58} Importantly, both mothers and fathers increase their salivary OT levels after SSC with their preterm infants,⁵⁹ but it is not known whether their preterm infants exhibit the same OT response. Nurses are often the primary partner in either providing or facilitating touch experiences for premature infants. Research is needed to determine if nursing interventions that provide comforting touch (e.g. SSC, holding, hand containment) increase infant OT levels, whether provided by parents or by other caregivers.

Stressors

Nurses play a key role in minimizing infant stress, especially during clinical care and procedures. Premature infants are exposed to numerous stressors during hospitalization,^{60–62} such as diaper changes, suctioning, handling, and mechanical ventilation. OT is an important peptide in the reduction of the human stress response.⁶³ In animal models is released in response to stress exposure (e.g., acute restraint).⁶⁴ However, animal models suggest that chronic stress (e.g., chronic cold exposure) may dampen OT secretion in response to stressors over time.⁶⁴ Human studies investigating the role of OT administration,^{65,66} OT genetic risk,^{67–69} and endogenous OT levels found more optimal functioning of the OT system is associated with buffering of the stress response,^{31,70} e.g. reduced adrenocorticotrophic hormone and cortisol levels.⁷¹ These researchers used stressors such as the Trier Social Stress Test,^{31,65,67} public speaking,⁶⁶ negative social environments and economic stress,⁶⁹ and the context of war⁶⁸ in their research methods, which are different from the clinical stressors infants experience. Whether the OT levels of human neonates are responsive to clinical stressors is unknown (See Box 2). Further research is needed to determine the relationship between premature infant exposure to stressors and endogenous OT levels.

Box 2

Did you know?

In animal models, OT is released in response to stress exposure (e.g., acute restraint).⁶⁴ However, animal models suggest that chronic stress (e.g., chronic cold exposure) may dampen OT secretion in response to stressors over time.⁶⁴

Nursing interventions that increase consumption of human milk, provide comforting touch, or reduce stressful experiences could enhance functioning of the infant's OT system and promote infant socioemotional development. However, no studies of the effects of these nurse-guided variables on OT levels exist in human premature infants. The purpose of this study was to determine if factors related to volume of human milk and enteral feeds, touch, or stressor exposure were related to plasma OT trajectories in premature infants during NICU hospitalization. Our hypotheses were:

1. Higher plasma OT trajectories (higher intercepts and steeper slopes) are associated with:
 - A. Increased enteral feeding volumes of mother's human milk
 - B. Increased enteral feeding volumes regardless of milk source (i.e., volumes of human milk, donor milk, and/or formula).
2. Higher plasma OT trajectories (higher intercepts and steeper slopes) are associated with increased hours in SSC and swaddled holding by any caregiver.
3. Higher plasma OT trajectories (higher intercepts and steeper slopes) are associated with exposure to a higher number of clinical stressors.

Methods

Design

The study used a prospective, longitudinal design with an observational cohort of premature infants <29 weeks gestation at birth.

Sample

Our sample of 33 premature infants was derived from a larger sample of infants recruited from three Midwestern Level III NICUs. This subset was used because these infants had complete data on the covariates required for analysis. Study eligibility criteria included English-speaking mothers who gave birth to premature infants ranging from 25–28 ⁶/₇ weeks gestation at birth. Exclusion criteria were characteristics having potential impact on infant neurobiology and brain development: history of maternal drug abuse,^{72,73} presence of major congenital or chromosomal abnormality, grade 3 or 4 intraventricular hemorrhage,⁷⁴ hypoxic ischemic encephalopathy,⁷⁵ metabolic disorders involving the adrenal system,⁷⁶ and necrotizing enterocolitis requiring surgical intervention.⁷⁷

Power analysis was conducted for the larger study to determine adequate sample size for detecting change in OT trajectories in premature infants, the primary study aim.⁷⁸ Adequate power was achieved with 37 infants following a 2 year recruitment. A priori power calculations were not conducted for the secondary aim of the larger study, which is presented in this paper. Thus, we did not determine a priori whether these analyses with 33 infants had adequate power.

Procedure

Mothers were recruited during the first two weeks of their infants' lives by direct approach from the principal investigator (PI). All participating mothers signed a written consent form approved by the hospitals' institutional review boards. Plasma collection started on approximately day of life 14, then continued weekly until the infant achieved 34 weeks corrected gestational age (CGA). We started data collection on day 14 to eliminate aberrant OT levels resulting from birth and/or exposure of the infant to Pitocin during labor. We completed OT measurements at 34 weeks CGA to ensure all infants would still be available for sample collection. Plasma collections occurred between the hours of 2300 and 0200 to control for potential diurnal variation of OT, as diurnal patterns of OT release in human infants are unknown. Plasma collection occurred as a capillary sample by heelstick or an arterial sample if the infant had an arterial line, and was concurrent with blood draws required for clinical treatment. No central or peripheral venous samples were used. Blood was collected into chilled EDTA (1mg/ml) BD microtainer tubes (Becton, Dickson, & Company, USA) containing Aprotinin (10 µL/mL of blood). All samples were collected before feeding as OT levels are reported to be influenced by the act of suckling⁴⁶ and digestion.^{79,80} Moreover, none of the infants in the study received continuous feeds. After collection, plasma was immediately placed on ice, processed, and transferred to a locked –80°C freezer. All nurse-guided variables were extracted weekly from the electronic medical record (EMR).

Measures

Plasma OT was measured using an extraction-free, commercially available kit (Peninsula Laboratories International, Inc., San Carlos, CA, KIT S-1355.0001). Roughly 0.3ml of blood was collected and immediately placed on ice, transported, then centrifuged at 4°C at 2000g for 10 min within one hour of collection. Supernatants were collected and stored at -80°C until assayed. Samples from the same infant were assayed simultaneously to avoid inter-assay variation. All measurements were performed in duplicate. Intra-assay coefficients of variation were 12%.

Human milk was conceptualized as a nurse-guided variable in which human milk expression is supported and encouraged by nursing staff. Volume of mother's milk consumed by the infant per day (i.e., a 24-hour period) was measured by mother's milk (i.e., not donor milk) given in ml/kg/day on the day of plasma collection, as documented in EMR. Irrespective of milk/formula source, volume of feeds consumed per day (i.e., 24-hour period) was measured in enteral volume consumed (ml/kg/day) on the day of plasma collection, as documented in EMR.

Touch was conceptualized as a nurse-guided variable including both SSC, encouraged and promoted by nursing staff, and swaddled holding by any caregiver (e.g. parents, grandparents, nurses, volunteers, etc.). We measured total hours the infant was positioned in SSC and total hours the infant was held in a swaddled position during the 8 hours prior to each plasma collection, as documented in the EMR. We chose the period of 8 hours prior to sample collection to capture a reasonable timeframe in which parents were likely to perform SSC or swaddled holding, i.e., in the afternoon and evenings. We theorized that the specimens would likely reflect OT released during touch occurring within the 8 hours prior to plasma collection. In addition, we recorded the hours of SSC and swaddled holding over the entire previous week to explore whether touch exposure earlier in the week could have residual effects on infant plasma OT.

Exposure to stressors was measured by the Neonatal Infant Stressors Scale (NISS), a tool which tracks clinical procedures known to cause distress and pain in the neonate.⁸¹ The NISS is designed to cumulatively measure a broad range of distressing events in the NICU such as painful procedures, mechanical ventilation, and handling. 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, is associated with brain development in preterm infants.⁸² This study used the total NISS score from the 8 hours prior to each infant's plasma collection, which coincided with the measurement period of SSC. Staff nurses recorded procedures, and the PI verified the record with the bedside nurse and the EMR.

Data Analysis

An overall alpha level of 0.05 was chosen for the final model. All OT levels were log transformed, due to the skewed distribution of the data. Each nurse-guided variable and its interaction with age were included in their own linear mixed model to assess potential associations between infant plasma OT (i.e., the dependent variable) and candidates for the final model. The model was:

$$Y_{it} = \beta_0 + \beta_1 (\text{RN-guided variable}) + \beta_2 (\text{Age}_{it} - 27) + \beta_3 (\text{RN-guided variable}) * (\text{Age}_{it} - 27) + b_{0i} + b_{1i} * (\text{Age}_{it} - 27) + \varepsilon_{it}$$

In this model, i indexes the infant, t the corrected gestational age. The β terms are fixed effects. The two b terms (b_{0i} , b_{1i}) are random effects, which adjust the mean parameters for infant-specific variation in OT trajectory. Age_{it} is the corrected gestational age for infant i when plasma OT was measured at time t , modeled as a continuous variable and centered at the earliest age we could expect data: 27 weeks CGA. All nurse-guided variables were centered at their means. Variables with a p -value less than 0.1 were included in the final linear mixed model.

Results

Demographic and descriptive statistics for the sample are displayed in Tables 1, 2, and 3. Total volume of enteral feeds (132.42 ± 36.02 ml/kg/day) was higher than the total volume of mother's milk (77.29 ± 58.45 ml/kg/day) received on the day of plasma collection, indicating supplementary use of formula or donor human milk (Table 2). The average number of hours an infant was either positioned in SSC or swaddled holding during the 8 hours prior to plasma collection was less than 1 hour (Table 2 & Figure 1). Average number of weekly hours of SSC was 7.59 ± 5.25 and swaddled holding 6.91 ± 6.48 , and several infants experienced weeks in which they were not held swaddled or in SSC for the entire week (Table 2 & Figure 1). In the institutions used for this study, nurses document when and for how long they hold infants. Because our sample consisted of extremely premature infants, many of whom were small for gestational age and also on ventilators, it would not be possible for nurses to swaddle-hold these infants. These infants would only be able to maintain their temperature and airway in kangaroo care. It is not a standard of care for nurses at these institutions to kangaroo their patients. Finally, average total score on the NISS during the 8 hours prior to plasma collection was 70.69 ± 14.05 , the equivalent of receiving about 14 severely stressful procedures (Table 2). Figure 2 displays the distribution of NISS scores by CGA. Table 3 shows the summary statistics of plasma OT levels by CGA, which were much lower than published adult norms (mean plasma OT = 375.78 pg/ml; $SD = 264.03$; range = 51.40–2752.30),²⁷ possibly due to use of different assays.

Among the individual linear mixed models, volume of enteral feeds (ml/kg/day) ($b = 0.013$; $p = 0.037$; 95% CI: 0.001 – 0.025) and its interaction with age ($b = -0.003$; $p = 0.038$; 95% CI: -0.002 – -0.001) were statistically significant in relation to plasma OT. However, volume of mother's milk (ml/kg/day) ($b = 0.004$; $p = 0.414$; 95% CI: -0.005 – 0.012) and its interaction with age ($b = -0.001$; $p = 0.468$; 95% CI: -0.002 – 0.001) were not related to plasma OT, and the insignificant association remained after removing the interaction from the model. SSC during the 8 hours prior to plasma collection ($b = 1.054$; $p = 0.001$; 95% CI: 0.448 – 1.660) and its interaction with age ($b = -0.192$; $p = 0.001$; 95% CI: -0.301 – -0.084) were statistically significant in relation to plasma OT. However, swaddled holding ($b = 0.219$; $p = 0.708$; 95% CI: -0.924 – 1.361) and its interaction with age ($b = -0.032$; $p = 0.726$; 95% CI: -0.209 – 0.146) were not related to plasma OT, and the insignificant association remained after removing the interaction from the model. To determine whether

weekly exposure to SSC or holding had effects on plasma OT (as opposed to exposure during the 8 hours prior to sample collection), we fit two additional models with these variables and their interactions, but the parameters for the individual model with weekly infant exposure to SSC ($b = 0.0004$; $p = 0.68$; 95% CI: $-0.001 - 0.002$) and its interaction with age ($b = -0.0001$; $p = 0.504$; 95% CI: $-0.004 - 0.0002$), and the individual model with weekly infant exposure to swaddled holding ($b = 0.039$; $p = 0.38$; 95% CI: $-0.05 - 0.13$) and its interaction with age ($b = -0.0005$; $p = 0.46$; 95% CI: $-0.02 - 0.008$) were not statistically significant. Finally, the interaction of age with exposure to stressors was nonsignificant ($b = -0.0002$; $p = 0.91$; 95% CI: $-0.005 - 0.005$), so we dropped the interaction term for the individual model, leading to statistical significance of the main effect of stressor exposure ($b = 0.007$; $p = 0.05$; 95% CI: $0.001 - 0.015$).

The final model, shown in Table 4, included the main effects of volume of enteral feeds and SSC, their interactions with age, and the main effect of stressor exposure. As seen in Table 4, while the coefficients remained largely the same for volume of enteral feeds, the interaction of enteral feeds with age, and exposure to stress, the p-values changed to statistical nonsignificance. However, the main effect of SSC and its interaction with age remained statistically significant.

Discussion

The premature infants enrolled in this study were exposed to low levels of touch and high numbers of stressors during NICU hospitalization. Our results are consistent with other researchers, who found that premature infants participated in SSC about once a week and in swaddled holding approximately twice a week.⁸³ Our findings that premature infants were exposed to a large number of stressors also echoes previous findings, such as reports of infants experiencing 10 painful procedures per day⁶⁰ and 38 handling episodes over a 24 hour period.⁸⁴ The finding that several infants experienced weeks in which they did not receive any form of holding is concerning for the impact on the developing preterm brain and subsequent socioemotional development.⁸⁵ Anecdotally, some infants were not held due to severity of infant illness, but a portion of them were not held due to lack of parental visitation. Given that parental separation is a significant stressor for the infant, and researchers have widely documented the critical contribution of sensitive parent-infant interactions to infant socioemotional development,^{62,86–88} we believe that clinicians should be concerned about the quantity and quality of social interaction that premature infants are receiving in the NICU, especially when infants experience little family visitation. Future research should determine whether nursing interventions such as alternative female SSC (e.g., SSC performed by a grandmother, aunt, or healthcare personnel),⁸⁹ infant massage, or continuous hand containment can provide supplemental social experiences and minimize the detrimental effects of parent-infant separation.

Volume of mother's milk consumed was not related to infant plasma OT levels. This finding is consistent with previous researchers finding no difference in OT levels between formula-fed and breastfed infants.³³ Given the novelty and heterogeneity of existing studies on this topic, future research should use animal models to compare infant plasma and brain OT reactivity to ingestion of fresh mother's milk, pasteurized milk, or formula. This experiment

could be supplemented with studies using ingestion of radiolabeled OT in milk. Future research involving human infants could measure and control for OT present in mother's milk fed to the infant. Volume of enteral feeds and its interaction with age were not significant in the final model. Given that the coefficients changed minimally in the final model, the lack of significance most likely represents loss of statistical power due to the presence of additional covariates. OT is released in response to food digestion and signals the attainment of satiety.^{29,79,80} Perhaps plasma OT is decreasing in response to the need for the infant to consume increasing enteral volumes to maintain appropriate growth. Thus, decreasing OT levels may be an adaptive response, promoting increased appetite, display of hunger cues, and food intake as the infant matures.

We found a significant main effect of same-day SSC at 27 weeks CGA (i.e., we centered CGA at 27 weeks), in addition to a significant effect of the interaction of SSC with CGA. The parameter estimate of the main effect of same-day SSC, 0.938, translates to a 17% increase in plasma OT for each additional 10 minutes of SSC (Table 4). This exciting finding supports SSC as a nursing intervention related to OT-based neurobiological processes. Previous researchers have hypothesized that SSC increases both parent and infant peripheral OT levels,⁹⁰ and one study has demonstrated that salivary OT in parents increases after SSC.⁵⁹ Our results are consistent with this work. In contrast to SSC, swaddled holding before sample collection was not related to infant plasma OT. This finding could have occurred because elicits greater preterm infant autonomic stability⁹¹ and maturation^{4,92} through activation of afferent vagal nerves. In animal models, vagal nerve stimulation increases plasma OT levels.^{93,94} Exogenous OT administration is also associated with greater vagal activity in human adults⁹⁵ and infants.⁹⁶ Future studies could measure premature infant plasma OT and vagal reactivity to SSC.

The significant interaction effect of SSC with age (Table 4) indicated that more hours of SSC over time was associated with decreasing plasma OT trajectories. In addition, the estimate of the decline of the OT trajectory associated with SSC suggests that one additional hour of SSC above the average amount of SSC received for our sample nearly doubles the decline of OT (Table 4). In the larger study, our team found a significant decline in plasma OT with age.⁹⁷ In the current study, we found that SSC is associated with the steepness of that decline, suggesting that SSC is enhancing a maturational decrease in plasma OT (Figure 3). Physiologic explanations for the decrease in plasma OT warrant future research. It may be that, over time, the immature infant OT system gains greater control over peripheral OT release from the pituitary, allowing buildup of OT in the brain and decline of circulating OT in the periphery. This phenomenon may be facilitated by SSC. In animal models, increased physical contact and social interaction during the early period of infancy increases infant brain OT gene expression, OT receptors, OT release, and greater regulation of behavioral responses to stress and novelty.⁹⁸ SSC is associated with enhanced maturation of neurobiological and neurobehavioral systems in premature infants;^{4,92,99} and perhaps SSC is also facilitating maturation of the infant's OT system. Future research designs investigating relationships between interventions and peripheral OT levels could measure OT during the immediate intervention/exposure period. OT reactivity (i.e. change in OT levels pre/post intervention or stimulus), instead of basal OT levels, may be a better indicator of OT system

function in humans. Future research should compare these two measurement methods in their responsiveness to interventions and ability to predict infant outcomes.

Higher levels of exposure to stressors were not related to higher plasma OT levels. The coefficient for stress exposure changed minimally in the final model, suggesting a loss of statistical power due to the presence of additional covariates. In adult women, higher plasma OT levels were associated with relationship stress,¹⁰⁰ and in maltreated girls urinary OT levels increase following a social stress test.¹⁰¹ To our knowledge, this is the first study to investigate associations between stressor exposure and OT in human infants. Our findings suggest plasma OT may be released in premature infants when exposed to clinical stressors, supporting the physiologic role of OT as a stress-buffering hormone in this population. Future research can build on these findings in premature infants by determining if OT reactivity is inversely related to hormonal, behavioral, and autonomic responses to stressors. Research with larger sample sizes investigating plasma OT reactivity to an immediate clinical stressor would greatly enhance our understanding of premature infant OT responses to stressors.

This study had several limitations. First, our study involved a convenient, small sample from one geographic region. We did not have the statistical power to control for maternal or infant characteristics in the final model. Second, we did not measure plasma OT reactivity in direct response to feeding, SSC, or stressor exposure, because we felt it would be unethical to pierce the infant multiple times for plasma collection (no infants experienced heelsticks for the sole purposes of this study). Third, we did not directly conduct post-hoc power analyses and future analyses could include post-hoc sample size calculations (however we did report the beta coefficients and confidence intervals required for these calculations). Finally, the study design was observational; hence, we did not randomize infants to receive varying levels of human milk, SSC, or stressful procedures.

Despite these limitations, we detected a significant association between SSC and plasma OT. A strength of our study was good sample generalizability for our geographic region, with excellent variability in maternal and infant demographics. This study is a pivotal first step in using OT as a biomarker for infant populations by demonstrating relationships between plasma OT and nurse-guided variables known to influence the premature infant's neurodevelopment. An association between plasma OT and same-day SSC is an exciting finding that brings additional research questions about the reactivity and function of the OT system in premature infants. In sum, these findings provide a knowledge base from which to build future interventions that use the infant's natural OT systems to reduce stress and promote social development. Nurse clinicians can better understand and implement newly-developed nursing interventions to support parent-infant relationships when those interventions are based on solid neurobiological evidence.

Given the novelty of OT measurement in premature infants, there are many areas of inquiry ripe for future research. Future studies could control for OT levels present in human milk, as OT levels may differ based on pasteurization, freezing, and thawing processes. Researchers could also continue measuring infant plasma OT levels until term equivalent to investigate the effects of oral infant feeding and breastfeeding on infant plasma OT. Future research

could also measure OT as a neurobiological antecedent to social engagement, especially in premature infants, where observable behaviors may be limited due to developmental or medical conditions. Future research should also consider OT as a biomarker for early socioemotional development.

Conclusion

Nurses are the healthcare clinicians who most consistently interact with infants and their families. Thus, nurses are in a unique and privileged position to shape biological pathways of the developing infant brain. Through facilitating OT-promoting interventions such as SSC and minimization of stressful procedures, the nurse is instrumental in providing families with the social, emotional, and educational resources to expertly care for their infant and provide a socially supportive environment during the first critical years of life. In addition, the nurse, through hands-on patient care, may also directly affect infant OT through the provision of comforting touch, holding, sensitive caregiving, and a developmentally-appropriate healthcare environment. Understanding the neurobiological mechanisms through which caregiver behavior is related to infant neurodevelopment can contribute to providing efficient and effective assessment and intervention services. Our study is a first step toward understanding OT-based pathways that minimize stress and promote neurodevelopment in premature infants.

Acknowledgments

Funding: This work was supported by the National Institutes of Health NINR (F31NR-014985; T32NR015433-01, UL1TR001070), AWHONN and the Every Woman, Every Baby Campaign, the Epsilon Chapter of Sigma Theta Tau, Ohio Nurses Foundation, Sigma Theta Tau International and the Midwestern Nursing Research Society, National Association of Neonatal Nurses Small Grants program, Nurses Educational Funds, the Ohio Perinatal Research Network (OPRN), and The Ohio State University Graduate School's Alumni Grants for Graduate Research and Scholarship (AGGRS). The authors thank Dr. Mary Cismowski for generously providing her time to assay the samples required for this research. The authors would also like to thank Dr. Courtney DeVries for her mentorship and critical appraisal of this research project.

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What This Study Adds

- The study is the first assessment of associations between plasma OT trajectories in premature infants and nurse-guided variables that are hypothesized to activate the OT system and are known to influence the premature infant's growth and neurodevelopment.
- This study is the first to detect an association between skin-to-skin contact and plasma OT in premature infants.
- The study's findings provide a knowledge base from which to build future interventions that harness the infant's natural OT systems to reduce infant stress and promote social development.
- Nursing interventions to support parent-infant relationships are more likely to produce intended outcomes when those interventions are based on solid neurobiological evidence.

Summary of Recommendations for Practice and Research

What we know

- Our ability to develop neuroprotective nursing interventions is hindered by a lack of understanding of neurobiological mechanisms that drive infant development.
- Neonatal nurses are crucial in teaching and modeling developmentally supportive social experiences that promote bonding, attachment, and socioemotional development—all processes theorized to be OT-based.
- Peripheral OT levels are associated with indicators of socioemotional health and development in term-born infants and children.^{14,57,58,102}

What needs to be studied

- Animal models that compare infant plasma and brain OT reactivity to ingestion of fresh human milk, pasteurized milk, and formula.
- Comparisons between plasma OT and vagal reactivity to SSC in premature and term infants.
- Whether OT reactivity or basal OT levels are better predictors of infant outcomes.
- If, and how, nursing interventions can induce peripheral OT release and contribute to the premature infant's neurobiology.

What we can do today

- Promote key nursing interventions that are likely to be OT-based: breastfeeding, SSC, infant stress reduction, and minimal maternal-infant separation.
- Provide social supportive experiences to our patients, especially when parents are not able to be present at the bedside.
- Neonatal nurses have the potential to impact the infant's neurobiology and brain development. Continue to support each other and our families in meeting each baby's unique developmental needs with our nursing practice!

Figure 1. Distributions of a) minutes of SSC during the 8 hours prior to plasma collection and b) hours of SSC during the week prior to plasma collection by Corrected Gestational Age.

Figure 2.

Distribution of Neonatal Infant Stressor Scale (NISS) scores calculated during the 8 hours prior to plasma collection by Corrected Gestational Age.

Figure 3. Plasma OT Trajectories in Premature Infants, grouped by SSC status: infants were categorized as either receiving no SSC during the 8 hours prior to sample collection, or some SSC during the 8 hours prior to sample collection.

Table 1

Maternal-Infant Demographics

Maternal Demographics		Mean	Std. Dev.	Min	Max	N
Age (yrs)		30.56	7.91	18	43	33
Education (yrs)		14.56	2.51	11	20	31
Family income (ten-thousands \$)		5.25	4.14	0	10	31
Births/Para		1.59	1.13	1	7	33
Race	82% Caucasian (n = 27)					33
	18% African American (n = 6)					
Marital status	50% Married (n = 17)					33
Infant Demographics		Mean	Std. Dev.	Min	Max	N
GA at birth (wks)		27.32	1.14	25.57	28.86	33
Birthweight (g)		1013.24	255.00	380.00	1470.00	33
Gender	55% Male (n = 18)					33
Race	76% Caucasian (n = 25)					33
Multiples	9% (n = 3)					33

Table 2

Descriptive Statistics of Nurse-Guided Variables

Nurse-Guided Variable	Mean ^c	Std. Dev. ^c	Min ^c	Max ^c
Enteral Feeds (ml/kg/day)	132.42	36.02	1.50	161.67
Mother's Milk (ml/kg/day)	77.29	58.45	0.00	160.00
SSC ^a (hrs during prev. 8 hrs)	0.61	0.91	0.00	4.00
Holding (hrs during prev. 8 hrs)	0.59	0.77	0.00	3.00
SSC (hrs during prev. Week)	7.59	5.25	0.00	18.14
Holding (hrs during prev. Week)	6.91	6.48	0.00	23.00
NISS ^b Total Score	70.69	14.05	47.50	102.00

N = 33

^aSSC = Skin-to-Skin Contact^bNISS = Neonatal Infant Stressor Scale^cSummary Statistics were derived by first averaging across timepoints for each infant, then statistics were computed on the infant averages.

Table 3

Summary Statistics for Plasma Oxytocin (OT) Levels in Premature Infants

Plasma OT	Obs	Mean	Std. Dev	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

CGA = Corrected Gestational Age

Table 4

Final Linear Mixed Model of Nurse-Guided Variables

Log Plasma OT (pg/ml)	Coef.	P>z	[95% Conf.	Interval]
Corrected Gestational Age (wks)	-0.160 *	0.009 *	-0.280	-0.040
Enteral Feeds (ml/kg/day)	0.011	0.077	-0.001	0.023
Enteral Feeds * Age	-0.002	0.066	-0.005	0.000
SSC ^a (hrs during prev. 8 hrs)	0.938 *	0.002 *	0.344	1.532
SSC * Age	-0.177 *	0.001 *	-0.283	-0.071
NISS ^b Total	0.007	0.062	-0.001	0.013
Intercept	4.783	<0.001	4.114	5.452

Random-effects Parameters	Estimate	[95% Conf.	Interval]
Unstructured Matrix			
σ_{b1i} (random slopes)	0.217	0.131	0.361
σ_{b0i} (random intercepts)	1.228	0.752	2.004
$\hat{\rho}$ (correlation of random effects)	-0.994	-1.000	-0.517
sd(Residual)	0.507	0.412	0.624

^aSSC = Skin-to-Skin Contact^bNISS = Neonatal Infant Stressor Scale* Significant at $\alpha = 0.05$

N = 33

Note: Corrected gestational age, SSC, and the interaction of SSC with corrected gestational age (i.e. SSC*Age) are all statistically significant in the final model. Thus, these variables have significant associations with infant plasma OT trajectories.