7-2019

Automatic Feature Discrimination of Non-Nutritive Suck Dynamics Among Extremely Preterm Infants.

McKenzie Ann Ochoa
University of Nebraska-Lincoln, ochoam@huskers.unl.edu

Follow this and additional works at: https://digitalcommons.unl.edu/cehsdiss
Part of the Speech Pathology and Audiology Commons

https://digitalcommons.unl.edu/cehsdiss/341

This Article is brought to you for free and open access by the Education and Human Sciences, College of (CEHS) at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Public Access Theses and Dissertations from the College of Education and Human Sciences by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.
AUTOMATIC FEATURE DISCRIMINATION OF NON-NUTRITIVE SUCK DYNAMICS AMONG EXTREMELY PRETERM INFANTS

by

McKenzie Ann Ochoa

A THESIS

Presented to the Faculty of
The Graduate College at the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Master of Science

Major: Speech-Language Pathology & Audiology

Under the Supervision of Professor Steven M. Barlow

Lincoln, Nebraska

July, 2019
AUTOMATIC FEATURE DISCRIMINATION OF NON-NUTRITIVE SUCK
DYNAMICS AMONG EXTREMELY PRETERM INFANTS

McKenzie Ann Ochoa, M.S.
University of Nebraska, 2019

Advisor: Steven M. Barlow

Prematurity is the leading cause of death in the first month of life. As a result of prematurity, neonates are forced to engage the extrauterine environment with an underdeveloped nervous system, with limited connectivity between orofacial sensorimotor anatomy, brainstem pattern-generating circuits, and sensory-driven thalamocortical inputs to layer IV of the neocortex. This is correlated with an inability to produce one of the most complex neuromotor behaviors, oral feeding. In order to overcome these developmental insufficiencies, noninvasive assessment and treatment protocols are needed to promote ororhythmic pattern generation. The NTrainer System is an FDA-approved non-nutritive suck assessment (NNS) and treatment tool that promotes ororhythmic motor patterning in preterm infants though pulsed orocutaneous stimulation. It has been shown to improve NNS motor skills, accelerate the transition to full oral feeds, and reduce the length of hospitalization in preterm infants who exhibit delayed or disordered nipple feeding behaviors.

The present report represents an interim analysis of NNS development among 42 extremely preterm infants (born less than 29 weeks gestational age) who are enrolled in an ongoing randomized control trial (NIH R01 HD086088). Participants were randomized to receive either the pulsed orocutaneous (NTrainer) or non-pulsed (SHAM) treatment beginning at 30 weeks post-menstrual age (PMA). Pulmonary status was
documented throughout the study resulting in the classification and diagnosis of either respiratory distress syndrome or bronchopulmonary dysplasia. Digitized records of NNS dynamics were automatically processed using a new software program known as NeoNNS. This software was coded in Python to extract time and frequency domain features, including minute-rates for NNS performance indicators and NNS burst structure which are considered in the present report.

Linear mixed modeling was used to examine the effect of treatment type among several dependent variables for 817 NNS files sampled from the extremely premature neonates. Significant treatment main effects were shown for NNS cycles/min, NNS cycles/burst, max NNS cycles/burst, and NNS amplitude. Infants’ NNS performance increased for some measures when the SHAM was utilized, while the number of bursts per minute and compression cycle amplitude increased for babies who received the NTrainer therapy. A greater increase in variable measures was observed in RDS infants who received the SHAM. Infants’ diagnosis in combination with treatment (BPD versus NTrainer, BPD vs. RDS) as well as sex (M, F) resulted in no significant effects across all parameters. All measures were highly dependent on PMA (p<.0001).
Acknowledgements

This study was supported by NIH R01 HD086088 (Barlow – PI). The author would like to thank the caregivers who allowed their children to participate in this study and the NICU medical care teams at CHI Health St. Elizabeth (Lincoln, NE), Tufts Medical Center (Boston, MA), and Santa Clara Valley Medical Center (San Jose, CA). Special gratitude toward Chunxiao Liao, MS for NeoNNS software design and Jaehoon Lee, PhD for statistical modeling.
# TABLE OF CONTENTS

Abstract...........................................................................................................................................iii

Acknowledgements..........................................................................................................................v

Table of Contents..............................................................................................................................vi

List of Figures......................................................................................................................................vii

List of Tables......................................................................................................................................viii

Introduction.........................................................................................................................................1

Clinical Advances to Promote Oromotor Rhythms .................................................................2

Background.........................................................................................................................................3

Rhythmic Patterns..............................................................................................................................3

Suck Central Pattern Generator.......................................................................................................4

Lick Central Pattern Generator..........................................................................................................5

Respiratory Central Pattern Generator............................................................................................6

Oxygen Supplementation Diagnoses...............................................................................................6

Swallow Central Pattern Generator ...............................................................................................7

Masticatory Central Pattern Generator...........................................................................................7

Structure of the Non-Nutritive Suck and Nutritive Suck...............................................................8

Suck-Swallow-Breathe.......................................................................................................................9

NNS Disruption................................................................................................................................10

Non-Nutritive Suck and Nutritive Suck Bursts.............................................................................11

Analysis of Non-Nutritive Suck..........................................................................................................12

NNS Therapeutic...............................................................................................................................15
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rationale and Hypotheses</td>
<td>16</td>
</tr>
<tr>
<td>Methods</td>
<td>17</td>
</tr>
<tr>
<td>Participants</td>
<td>17</td>
</tr>
<tr>
<td>Protocol</td>
<td>18</td>
</tr>
<tr>
<td>NTrainer Treatment</td>
<td>19</td>
</tr>
<tr>
<td>SHAM Treatment</td>
<td>20</td>
</tr>
<tr>
<td>Data Collection</td>
<td>21</td>
</tr>
<tr>
<td>Patient Medical Data Management in the Neonatal Intensive Care Unit</td>
<td>21</td>
</tr>
<tr>
<td>Non-Nutritive Suck Data Analysis</td>
<td>22</td>
</tr>
<tr>
<td>Statistical Model</td>
<td>24</td>
</tr>
<tr>
<td>Results</td>
<td>25</td>
</tr>
<tr>
<td>Discussion</td>
<td>41</td>
</tr>
<tr>
<td>References</td>
<td>45</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 1 - Timeline of stable and well patterned central pattern generators ..................8
Figure 2 - Non-nutritive suck compared to nutritive suck bursts.................................12
Figure 3 - Preterm infant receiving PULSED NTrainer stimulation............................16
Figure 4 - Intervention study design.............................................................................19
Figure 5 - NTrainer handpiece.....................................................................................20
Figure 6 - Intervention results layout...........................................................................24
Figure 7 - Marginal means of NNS cycles/minute.........................................................26
Figure 8 – Mixed linear model of NNS cycles/minute..................................................27
Figure 9 – Marginal means of NNS cycles/total oral compressions.............................28
Figure 10 - Mixed linear model of NNS cycles/total oral compressions.........................29
Figure 11 – Marginal means of NNS bursts/minute......................................................31
Figure 12 – Mixed linear model of NNS bursts/minute...............................................31
Figure 13 – Marginal means of NNS cycles/burst.........................................................33
Figure 14 – Mixed linear model of NNS cycles/burst...................................................34
Figure 15 - Marginal means of NNS cycles_{\text{max}}/burst versus disability and treatment.....35
Figure 16 - Mixed linear model of NNS cycles_{\text{max}}/burst versus PMA.......................36
Figure 17 - Marginal means of NNS AMP versus disability and treatment.....................38
Figure 18 - Mixed linear model of NNS AMP versus PMA.........................................38
LIST OF TABLES

Table 1  p-values for NNS parameters.................................................................39
Table 2  Mean values of NNS parameters ...........................................................40
Introduction

The number of premature births peaked in 2009 comprising 12.5% of the total 4.13 million live births in the United States (Glass et al., 2015; NCHS, 2018). In 2012, extremely premature infants (EPIs) (defined as < 29 weeks gestational age (GA)) accounted for 28,861 of all live births in the US. With prematurity being the leading cause of death in the first month of life, these EPIs face a 30% risk for mortality (Glass et al., 2015). In survivors, 20% risk long-term complications including delays and/or long-term impairment in neurodevelopmental outcomes, including sensory, motor, cognition, and communication. Speech articulation, receptive and expressive language have been found to be lower in children who were born premature (Foster-Cohen et al., 2010; Imgrund, Loeb, & Barlow, 2019; Lewis et al., 2002; Sansavini et al., 2010). Researchers Putnik, Bornstein, Eryigit-Madzwamus, and Wolke (2017) state that language scores in preschoolers tend to be fairly stable over time and preterm infants with language deficits are not likely to catch up over time. It has been shown that close to half of children who were born prematurely that were diagnosed with respiratory distress were enrolled in speech-language intervention (Imgrund et al., 2019). This suggests an inverse relation between GA and speech-language outcomes. Negative effects have also been observed in decreased IQs, overall academic ability, emotional stability, and physical ability (Sriram et al., 2018; Twilhaar, de Kieviet, Aarnoudse-Moens, van Elburg, & Oosterlaan, 2018).

These premature infants are also at risk for more pressing short-term complications including an underdeveloped nervous system, inability to thrive without support, and comorbidities such as choking, aspiration, feeding aversions, and developmental difficulties (Poore, Barlow, Wang, Estep, & Lee, 2008). The attainment of
oral feeding is one of the most complex neuromotor behaviors facing preterm neonates early on and a sensorimotor skill that must be sufficiently developed prior to discharge from the neonatal intensive care unit (NICU). The transition to oral feeding occurs during a critical period of brain development associated with neuronal proliferation and extensive pathway formation. This links orofacial anatomy via facial and trigeminal nerves to the ventroposteromedial thalamus, with massive sensory-driven thalamocortical inputs to layer IV of the neocortex to provoke sucking and other motor movements required for feeding (Bosma, 1973). Prematurity and maladaptive experiences can disrupt this critical period and contribute to extended NICU stays and medical costs approaching $500,000 for an EPI (Soilly, Lejeune, Quantin, Bejean, & Gouyon, 2014).

Clinical Advances to Promote Oromotor Rhythms

Despite numerous medical advances in care of the preterm, a need exists for noninvasive assessment tools of oromotor and swallowing function in order to improve feeding and developmental outcomes (Barlow et al., 2017). There are a variety of assessment screenings available for feeding readiness to infants who correct to ≥ 33 weeks’ post-menstrual age (PMA) and have a stable respiratory status (Gennattasio et al., 2015) however, there is no universally accepted criteria for determining feeding readiness (Picker, 2004). NICUs also assess feeding readiness by making adjustments to feedings based on trial and error (Lester, Andreozzi-Fontaine, Tronick, & Bigsby, 2014; Pickler, 2004). This technique may not be safe for EPIs due to their compromised brain and pulmonary systems and may lead to harmful immediate consequences such as fatigue, hypoxia, bradycardia, and agitation (Pickler, 2004). Current approaches are limited and
don’t assess sensorimotor, neurological, or gastrointestinal developmental stages and therefore provide little insight into feeding readiness (Poore et al., 2008a). These approaches were shown to offer “no evidence to inform clinical practice” and indicate that research is needed in this area to establish an evidence base for the clinical utility of instruments to assess feeding readiness and promote oromotor function in the preterm infant population (Crowe, Chang, & Wallace, 2012). By utilizing a technique efficacious for premature infants of all PMAs, it may decrease their risk for future long and short term complications as well as their stay in the NICU.

**Background**

**Rhythmic Patterns**

Ororhythmic function is evident *in utero* (Popescu, E., Popescu, M., Wang, Barlow, & Gustafson, 2008), becomes more robust between 28 and 33 weeks GA and typically stabilizes by 34 weeks (Barlow, Finan, Lee, & Chu, 2008). Infants born prematurely are prone to medical complications beginning at birth due to underdeveloped systems that include rhythmic behaviors essential for feeding readiness. Central pattern generators (CPGs) are premotor interneuron networks which target lower motor neurons that are commonly affected due to prematurity. These circuits coordinate reciprocal muscle groups with bilateral neural representations and interconnections to produce rhythmic motor patterns (e.g., breathing, jumping, walking, running, suck, swallow, respiration) (Barlow, 2009a; Barlow & Estep, 2006; Barlow, Lund, Estep, & Kolta, 2010; Zimmerman & Barlow, 2008). Composed of interneurons that direct patterned output to lower motor neurons, CPGs are generally found in the cerebral cortex, brainstem, and
spinal cord and are part of the premotor neural circuits, and all evolve at various times (Barlow et al., 2010; Barlow & Estep, 2006; Barlow, Rosner, Song, 2018). They are composed of premotor internuncial networks that integrate sensory information to sequence and activate different motor neurons at specified intensities to generate motor patterns (Barlow & Estep, 2006; Barlow et al., 2010). Central pattern generators are subject to neuromodulation which results in behavioral flexibility through an expanded motor repertoire (Barlow et al., 2010). Non-nutritive sucking (NNS) is an oromotor pattern that involves multiple CPGs to coordinate the suck (lips, jaw, tongue), and potentially respiration to achieve safe feeding when progressed to a NS (Barlow, 2009b).

**Suck Central Pattern Generator**

Ororhythmic activity required for feeding is controlled, in part, by a suck central pattern generator (sCPG) located in the brainstem pontine and medullary reticular formation (Barlow & Estep, 2006; Poore et al., 2008a). As one of the earliest motor patterns to occur, the primitive suck appears *in utero* between 15 and 18 weeks GA. By 32 weeks PMA, the NNS, a rhythmic sucking motion with no bolus, becomes stable and well patterned in healthy preterm infants (Barlow et al., 2012). This bilateral network of interneurons output information to cranial nerves V (trigeminal), VII (facial), and XII (hypoglossal) in order to activate the rhythmic suck sequence (Barlow & Estep, 2006). The sCPG is modified by multiple inputs such as pathways from the sensorimotor cortex and reciprocal connections with the cerebellum that control ororhythmic activity (Barlow et al., 2012). Another way the sCPG can be modified is through sensory input from oral and perioral mechanoreceptors that encode oral movements and stimulation (Finan & Barlow, 1998; Barlow et al., 2001; Barlow et al., 2004; Barlow et al., 2012). Suck CPG
patterning can be sensitive to mechanical stiffness of oral devices. Silicone pacifiers which vary in stiffness, due to wall thickness and composition of the silicone nipple, yield significantly different NNS ‘burst-pause’ patterns (Zimmerman & Barlow, 2008). Recently, pacifier compression and longitudinal ‘pull’ stiffness, and nipple shape type yield different NNS dynamics (Zimmerman, Forlano, Gouldstone, 2017). Patterning may also be affected by mild odor which has been shown to increase infants’ NNS abilities (Bingham, Abassi, & Sivieri, 2003).

Modification of the sCPG motor output is evident in a variety of diseases among preterm infants, including respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), infants of diabetic mothers, Down syndrome (Barlow et al., 2014; Gewolb et al., 2001; Gewolb, Vice, Schweitzer-Kenney, et al., 2001; Gewolb, Bosma, Reynolds, Vice, 2003; Gewolb, Vice, 2006; Barlow et al., 2019a, b) and insults to the brain such as hypoxic ischemic encephalopathy and intraventricular hemorrhage (Mizuno, Ueda, 2005). This results in discoordination or omission of the suck leading to poor airway protection, a potential dysphagia diagnosis, and poor state control (Gewolb & Vice, 2006).

**Lick Central Pattern Generator**

Controlled from within several subdivisions of the medullary reticular formation, the lick central pattern generator (lCPG) plays an important role in feeding for control, swallowing, and rejecting aversive gustatory stimuli (Travers, Dinardo, & Karimnamazi, 1997). The motor component of the lick transpires through the hypoglossal nerve or cranial nerve XII. In addition, the cerebellum has been observed to play a role in various rodents by controlling the rate and time of the licking rhythm (Travers et al., 1997).
Respiratory Central Pattern Generator

Chest wall movements during breathing are produced by the respiratory central pattern generator (rCPG) which regulates the phase and amplitude of inhalation and exhalation. The rCPG is located bilaterally in the pre-Bötzinger complex of the ventrolateral medulla (Rybak, Abdala, Markin, Paton, & Smith, 2007). Like other rhythmic motor systems, the rCPG is adaptive and flexible to control breathing throughout multiple internal and external environments in order to meet metabolic demands (Rybak et al., 2007). The rCPG is constantly changing throughout life to adjust to growth and body plan, and evolving task dynamics of speech breathing and song production (Barlow & Estep, 2006; Barlow et al., 2010).

Oxygen Supplementation Diagnoses

Due to an underdeveloped rCPG, premature infants face breathing difficulties. The human lung is not fully developed until around 35 weeks GA (Jobe, 1999). Extremely premature infants are born before this organ can reach maturity. Their lungs are unable to produce enough surfactant causing their lungs to collapse and the potential to suffer from chronic hypoxic injury (Barlow et al., 2019b). They require oxygen supplementation and are diagnosed with RDS if they remain on oxygen for more than 5 days after birth (Loeb, Imgrund, Lee, & Barlow, 2019). If infants remain on oxygen supplementation beyond 36 weeks GA, their diagnosis is then adjusted to BPD (Loeb, Imgrund, Lee, & Barlow, 2018). This adjustment to diagnosis may occur during or after the treatment period. The extensive oxygen therapy these neonates acquire alters their sensory experiences due to nasal cannulas and tape. It has been shown that preterm infants requiring oxygen support demonstrate sucking and feeding difficulties (Gewolb et
al., 2001). In addition, preterm infants requiring extensive oxygen support have been shown to have an increased likelihood of having both motor and cognitive delays when they have a language delay. In the same study it has also been proven that these infants showed the poorest outcomes on language, motor, and cognitive skills compared to healthy infants, infants with RDS, and infants of diabetic mothers (Loeb et al., 2019).

**Swallow Central Pattern Generator**

Two main groups of interneurons within the brainstem have been identified for generating the swallow pattern seen in infants. The swallow central pattern generator (swCPG) is observable at 11 weeks GA and allows the infant to regulate amniotic fluid hundreds of times each day (Barlow et al., 2019a; Bu’Lock, Woolridge, & Baum, 2008; Humphrey, 1971). These internuncial circuits are found within the dorsal and ventral reticular formation in the medulla oblongata and create a complex multi-level oropharyngeal rhythmic pattern generator that is highly modifiable by sensory inputs, including attributes related to texture, bolus size and viscosity, temperature, and taste.

**Masticatory Central Pattern Generator**

By the 6th month postnatally, most infants express a functional masticatory central pattern generator (mCPG) while they transition from liquid nutrient to semi-solid and solid foods (Barlow & Estep, 2006). The pontine mCPG is complemented by a masticatory cortical area in the motor cortex later in development. Coincidently, the mCPG co-emerges with the eruption of dentition and continues to evolve until the permanent teeth have grown in (Barlow & Estep, 2006). In rats, the first masticatory moments appear around postnatal day (P) 12 and the adult pattern is established between P18-P21 (Barlow et al., 2010). Sucking and chewing share basic features of jaw opening
and closing; however, a key difference is when the “power stroke” occurs. During mastication this occurs during jaw closing when breaking down food, whereas during sucking it occurs during jaw opening to produce a high negative intraoral pressure that allows nutrients to flow from the nipple to the oral cavity (Barlow & Estep, 2006).

![Figure 1](image.png)

**Figure 1.** Timeline of stable and well patterned central pattern generators. Representation of patterned ororhythmic development EPI’s do not obtain in utero due to prematurity (Adapted from Barlow, 2009a).

**Structure of the Non-Nutritive Suck and Nutritive Suck**

During early development infants’ transition between two distinct forms of sucking, NNS and nutritive sucking (NS). Non-nutritive sucking behavior is characterized by a repetitive suck cycle pattern known as an NNS bursts which is generated by a combination of mouthing (perioral) and tongue/jaw compressions in the absence of a liquid stimulus (i.e., pacifier or finger) (Lau, 2006; Wolff, 1968). These bursts alternate with pause periods forming a repetitive pattern. The development and stability of this pattern is disrupted in preterm infants with breathing difficulties (Barlow, Burch, Venkatesan, Harold, & Zimmerman, 2012) and tends to make the transition to
oral feeding more challenging (Barlow, 2009a). Preterm infants’ reflexes in the oral region begin around 8.0-8.5 weeks GA with the maxillary and mandibular region developing first (Hooker, 1958; Humphrey, 1971). The sucking reflex is observed as early as 12-18 weeks GA and has been imaged in utero using fetal magnetometry (Popescu et al., 2008). By 30-32 weeks PMA, neurotypical infants without lung disease exhibit well-organized NNS bursts (Barlow, Rosner, & Song, 2019a). Healthy infants transition from NNS to NS by 34-37 weeks PMA. The NS is a continuous motor stream that involves a bolus (i.e., bottle or breast) to evoke tongue movements and trigger oropharyngeal-laryngeal-esophageal reflexive motor patterns for airway protection during a safe swallow (Barlow et al., 2019a; Lau, 2006; Reynolds et al., 2018; Reynolds, 2019).

**Suck-Swallow-Breathe**

During NNS, infants utilize the sucking ororhythmic pattern and occasionally the swCPG for their own secretions. Respiration is independent from these two patterns due to a lack of need to close the tracheal airway to prevent penetration or aspiration of a liquid bolus into the lungs (Lau, 2006). When a bolus is introduced, sucking, swallowing, and respiration are in close succession to create safe oral feeding and airway protection through the closure of the epiglottis, aryepiglottic folds, and vocal folds. This pattern begins in premature infants who have a stable cardiopulmonary function around 33-34 weeks PMA (Lau, 2006). The rhythmic alternation of suction and expression through CPGs begins to coordinate the sucking, swallowing, and respiration pattern and is
obtained when the infant demonstrates a ratio of 1:1:1 or 2:2:1, respectively (Barlow et al., 2019a).

To achieve a safe swallow, infants need to have the proper timing of all rhythmic elements, including bolus form and location and the coordination of laryngeal, pharyngeal, and esophageal muscle systems. During a normal swallow, there is little time between the respiratory cycle and swallow-apnea cycle to successfully integrate a breath. Infants with BPD tend to have abnormally long apnea events during a swallow. This leads to an incoordination of the systems and probable episodes of desaturation, apnea, and/or bradycardia during feedings (Barlow, 2009b). Dyscoordination of the swallow pattern may be associated with aspiration and serious health issues (Lau, 2006). The dysfunction may occur at any phase of the pattern for a variety of reasons, therefore making diagnosis and treatment difficult (Barlow et al., 2019a).

**NNS Disruption**

Most full-term infants are able to achieve a safe swallow and sucking pattern at birth. Prematurity disrupts the process of learning the NNS pattern due to prolonged maladaptive sensory experiences, including extended periods of oxygen supplementation (intubation, continuous positive airway pressure (CPAP), high frequency ventilation, nasal cannulation, etc.) and feeding tubes (orogastric, nasogastric) which are secured to the lower face with tape. These interventions tend to restrict oral movements, limit normal orosensory experiences and challenge the newborn with unexpected maladaptive orosensory inputs which can lead to adverse reactions to any future orofacial intervention, including the introduction of a pacifier or feeding nipple (Barlow et al.,
2019a; Koong Shiao et al., 1996). Preterm infants who fail to attain NNS or successful transition to NS at an appropriate time, may show long-term feeding difficulties (Mizuno & Ueda, 2005). Recent studies show a strong association between feeding difficulties, childhood language, (Adams-Chapman et al., 2013; Malas, Trudeau, Chagnon, & McFarland, 2015; Malas et al., 2017; Zimmerman, 2018), childhood motor abilities, (Wolthuis-Stigter et al., 2017), and overall IQ (Wolthuis-Stigter et al., 2017). Proper development of NNS has been shown to benefit the infant’s behavioral state and accelerate the transition to oral feeding thus enhancing weight gain and shortening hospital stays (Lau, 2006).

**Non-Nutritive Suck and Nutritive Suck Bursts**

The structure of NNS and NS burst cycles varies due to their primary function. Non-nutritive suck bursts typically consist of 2-13 suck cycles that are separated by pause periods of 2-5 seconds to allow for respiration (Barlow et al., 2019a). The number of suck cycles increases during the infant’s development. By 32 weeks PMA, a healthy infant displays, on average, 6-7 cycles with a mean frequency of 2 Hz and a mean peak compression pressure of 17 cm H\(_2\)O (Barlow, Rosner, & Song, 2019b). Studies show that healthy infants on average exhibit 5.67 cycles per NNS burst whereas infants with RDS exhibit 3.87 cycles per NNS burst (Barlow et al., 2012). Nutritive suck bursts consist of a phase where the infant is repeatedly sucking (suction phase) along with an expression phase (Lau, 2006). The expression phase occurs when the infant creates an anterior to posterior stripping motion with their tongue tip along the breast or nipple to express milk (Barlow et al., 2019a; Lau, 2006). Infants are able to feed using the expression phase with
no suction to express milk (Lau, 2006). The suction-expression phase may include more than 40 suck cycles produced at a modal rate of 1 Hz (Barlow et al., 2019a). Examples of NNS and NS bursts are shown in Figure 1.

![Figure 1](image1.png)

**Figure 1.** Examples of NNS and NS bursts.

**Figure 2.** Non-nutritive suck compared to nutritive suck bursts. (Adapted from Barlow et al., 2019a).

**Analysis of Non-Nutritive Suck**

Technological advances in digital signal processing, feature detection, and machine learning offer new approaches for NNS analytics and insight into a neonate’s neurologic status and feeding readiness (Liao, Rosner, Maron, Song, & Barlow, 2019). Physiological recordings of ororhythmic sucking patterns are commonly used in NICUs. Many devices used to record NNS motor patterns rely on readily available pneumatic sensor systems, however, software for data visualization and feature analysis have been limited to relatively simple time domain measures (Bromiker, Medoff-Cooper, Flor-Hirsch, & Kaplan, 2016; Lau et al., 1997; Lau, Alagugurusamy, Schanler, Smith, & Shulman, 2000). A pressure transducer within an artificial nipple has been successfully utilized in previous studies to record NNS waveforms while infants are in a developmentally supportive position (Barlow et al., 2008; Barlow et al., 2012; Barlow, Poore, & Zimmerman, 2011; Poore et al., 2008a, b; Estep et al., 2008; Zimmerman &
Barlow, 2012) Bingham, Ashikaga, & Abbasi, 2010; Drier, Wolff, Berseth, & Nordyke, 1979; Estep, Barlow, Vantipalli, Finan, & Lee, 2008; Gewolb, Vice, Schweitzer-Kenney, Taciak, & Bosma, 2001; Lau et al., 2000; Lau, Sheena, Shulman, & Schanler, 1997). A cribside data acquisition and stimulation system (Finan and Barlow, 1996) was used to record NNS compression waveforms in the NICU. Others have built around the Biopac MP 100 WSP software to decipher the effects of oral sensorimotor intervention that advance NS skills compared to tactile/kinesthetic (Fucile, McFarland, Gisel, & Lau, 2012).

Non-nutritive suck dynamics have been analyzed in various ways. Until recently, most researchers have taken NNS waveforms and analyzed them in MatLAB (Barlow et al., 2012; Bingham et al., 2010; Kugelman, et al., 2016). A peak-picking algorithm (NeoSuck RT©) was developed to discriminate NNS activity during an infant’s most productive two-minute sample (Barlow et al., 2012; Estep et al., 2008).

An approach that has gained considerable interest in recent years involves computation of the NNS spatiotemporal index (NNS STI), which estimates the invariance of the NNS burst structure. The user defines the number of suck cycles within burst, and number of bursts to be processed for any given data set. In essence, this metric is used to quantify pattern stability of a repetitive oromotor sequence by taking the cumulative sum of the standard deviations of the NNS compression signal on time- and amplitude-normalized burst waveforms (Barlow et al., 2012; Poore, Zimmerman, Barlow, Wang, & Gu, 2008b). The NNS STI has been shown to effectively discriminate NNS motor patterns in preterm infants in health and lung disease (RDS) (Poore et al., 2008b).
With the application of these high-speed data acquisition and digital signal processing methods to the study of ororhythmic motor pattern development in preterm infants with significant respiratory and/or neurological conditions, the need arose in 2014 to engineer a new software platform capable of supporting a national network of NICUs to record and process NNS analytics in the time- and frequency-domain, and support a big data pipeline. This new platform included an integrated module for NNS STI calculation. The result is a Python-based program (MS WIN10) known as NeoNNS.exe (Liao et al., 2019). Another essential capability is that NeoNNS be able to extract infant demographics and medical chart history including growth variables, oxygen supplementation, and daily feed mode and volumetrics for co-registration and modeling to develop predictive machine learning models of feeding readiness. The latter has been accomplished with the NICU dBASE (Oh, Barlow, 2016) which is an MS Access data structure to support this research. The data acquisition of NNS activity is completed in the NICUs with the NTrainer System (Innara Health, LLC; Olathe, KS USA). These NNS assessment binary data files serve as the source input for NeoNNS (Liao et al., 2019). This program has baseline correction features, raw file displays, creates NNS burst calculations, displays minutes and seconds, zoom functions, DiscrimStepSize to define the size of the sliding window to find the most active period, and BurstCriterion to identify NNS bursts that consist of two or more NNS peak pressure events. NeoNNS utilizes waveform discrimination methods and feature extraction to identify NNS burst activity in mere seconds, and can be run in single file or batch process mode to process thousands of data files across multiple NICU sites.
NNS Therapeutic

The NTrainer System (FDA-approved 2008) is a therapeutic tool to promote NNS in preterm infants who exhibit delayed or disordered nipple feeding behaviors (handpiece is shown in Figure 3). It is currently being used in over 30 NICUs in the United States. Based on mechanosensory entrainment of the sCPG, this system uses servo-controlled pulsed pneumatic stimuli to drive peri- and intraoral mechanosensory afferents to modulate local reflex activity and produce the targeted NNS oromotor rhythms (Barlow et al., 2008; Barlow et al., 2017).

Previous studies have shown the NTrainer to be an effective form of treatment, when compared to a non-pulsatile pacifier, to advance NNS abilities in pre-term infants (Greene, O’Donnel, & Walshe, 2016; Harding, 2009; White-Traut et al., 2002). One study showed a significant difference in a reduction of length of stay and time to full oral feeds in infants given the NTrainer compared to the control group that had no significant reduction in length of stay at discharge (Song et al., 2019). The study by Song et al. (2019) also provides evidence to show that oral sensorimotor intervention enhances suck-swallow-breath coordination.
Figure 3. Preterm infant receiving PULSED NTrainer stimulation in the neonatal intensive care unit. (Photo courtesy of Innara Health, Inc., Olathe, Kansas USA).

Rationale and Hypotheses

Despite numerous medical advances, there are few physiological assessments and treatments available for one of the most complex sensorimotor behaviors in oral feeding. There is a need for noninvasive assessment and treatment protocols to promote oromotor development in extremely preterm infants faced with significant neurodevelopmental and respiratory challenges during hospitalization in the NICU in order for hospital discharge. There is limited evidence to inform the use of current assessments due to all premature populations not being accounted for and not including orofacial, respiratory, neurological or gastrointestinal development stages, therefore providing little insight into feeding readiness (Crowe et al., 2012; Liao et al., 2019; Poore et al., 2008a). The NTrainer System is a noninvasive medical device used to assess and promote ororhythmic motor patterning and feeding readiness in the NICU (Barlow et al., 2017; Poore et al., 2008b).
The purpose of the current thesis study was to characterize the effects of pulsatile orocutaneous stimulation paired with gavage feedings in EPIs diagnosed as having RDS or BPD on the development of NNS burst production using a new automated Python-based NNS software processing program (Liao et al., 2019) developed in the Communication Neuroscience Laboratories at the University of Nebraska. The work reported here is part of an ongoing multicenter randomized controlled trial at NICUs in Lincoln NE, Boston MA, San Jose CA, and Los Angeles CA with data collection for this NIH project to continue through 2021.

1. In the current study it is hypothesized that infants will show a significant difference in NNS parameters for diagnosis (RDS or BPD).

2. Extremely preterm infants are expected to show a significant effect in NNS parameters based on treatment received (NTrainer or SHAM).

3. The correlation of disability and treatment measures is expected to show a significant effect in NNS parameters.

4. When comparing PMA, it is expected that there will be a significant difference in NNS parameters.

Methods

Participants

Forty-two EPIs [19M/23F] born between 24 0/7 and 28 6/7 weeks’ GA (MEAN[SD] = 188.71[8.32] days), as determined by obstetric ultrasound at <15 weeks or last menstrual period, were eligible to participate in this study. Birthweights ranged (BW) from 590 to 1304 grams (MEAN[SD]: 956.76 [207.04] gm). Twenty-six infants were
diagnosed as RDS and 16 as BPD. Infants were enrolled in the study by beginning at 29 weeks corrected PMA (MEAN[SD]: 235.37 [12.27] days) to limit the number of infants who develop serious sequelae of prematurity. Participant data analyzed for the present thesis study were obtained from three neonatal intensive care units including CHI Health St. Elizabeth (Lincoln, NE), Tufts Medical Center (Boston, MA), and Santa Clara Valley Medical Center (San Jose, CA) (Barlow et al., 2017). Exclusion criteria: (1) chromosomal and congenital anomalies including craniofacial malformation, nervous system anomalies, cyanotic congenital heart disease, gastroschisis, omphalocele, diaphragmatic hernia and other major gastrointestinal anomalies; (2) congenital infection; (3) no documented GA; (4) severe intrauterine growth restriction (IUGR) (3%); (5) abnormal neurological status including head circumference <10th or >90th percentile, intracranial hemorrhage grades III and IV, seizures, meningitis, neurological examination showing abnormal tone or movements of all extremities for PMA; (6) history of necrotizing enterocolitis (stage II and III); and (7) culture-positive sepsis at the time of study enrollment (Barlow et al., 2017).

**Protocol**

Following informed consent, each infant was randomly assigned to a treatment. Twenty-six infants received the NTrainer pulsed pneumotactile and 16 infants received the controlled SHAM ‘non-pulsatile’ intervention. Both were paired with tube feedings (gavage) up to 3 times per day (Monday-Friday). The interventions began at 30 weeks PMA and continued for 4 weeks. The first 2 weeks included NTrainer stimulation (2 x 3-minute blocks) with a 1-minute stimulus “off-period” between the stimulation blocks.
The stimulus dose was then increased for the subsequent 2 weeks (3 x 3-minute blocks of NTrainer pulsed stimulation) with a 1-minute stimulus “off-period” between the stimulation blocks to mimic the natural progression of an infant’s NNS. Infants who received the SHAM intervention were given a regular silicone pacifier simultaneously during tube feedings for 4 weeks. Care procedures were the same for infants in either group. To ensure blinding, only study site PIs or co-investigators as well as the neonatal study coordinators were informed of infants’ group assignments. Physicians, nurses, and other NICU care staff remained blinded about the study infants’ treatment assignment. Based on a standardized cue-based feeding schedule utilized by each site known as Infant Driven Feeding (Ludwig, Waitzman, 2007; Waitzman, Ludwig, Nelson, 2014), EPIs will advance their feeding abilities leading to full nipple feeds. Infants remained connected to their bedside monitors at all times for observation of respiration, heartbeat and oxygen saturation.

**Figure 4.** Intervention study design for EPIs and schedule for sampling NNS behavior in the NICU. (Adapted from Barlow et al., 2017).

**NTrainer Treatment**

Infants assigned to the PULSED treatment group received 3-minute periods of pulsed orocutaneous stimulation using the NTrainer System coupled to a standard
silicone pacifier (e.g. WeeSoothie or Soothie). This pattern of stimulation mimics the spatiotemporal dynamics of NNS bursts and is correlated to rapid organization of an infant’s suck. The pneumatic bursts delivered by the NTrainer to the lumen of the pacifier are frequency modulated (FM) from 2.8 to 1.6 Hz across the 6-cycle structure, with a 2-second pause period between bursts. Individual pressure cycles have a 31 millisecond (ms) rise/fall time to ensure salient stimulus spectra with significant energy from DC-16 Hz. Frequency modulation is a physiologic feature of the NNS in preterm infants. A total of 34 pneumotactile bursts are presented through the pacifier in a 3-minute block. A 1-minute rest period (no stimulation) occurs between stimulation blocks. Criteria for initiation of orocutaneous therapy include the following: (1) stable vital signs and not on continuous vasopressor medications, (2) tolerating enteral feeds in previous 48 hours, and (3) not intubated and mechanically ventilated. If the infant is on nasal intermittent positive pressure ventilation, continuous positive airway pressure or nasal cannula >2 liters per minute, then the fraction of inspired oxygen (FiO2) must be <40%.

![NTrainer handpiece](Image courtesy of Design World).

**Figure 5.** NTrainer handpiece. (Image courtesy of Design World).

**SHAM Treatment**
Infants assigned to the control group were given a non-pressurized Soothie silicone pacifier that was not modulated or driven by the NTrainer System. The SHAM was administered during tube feedings over the same schedule, and the NICU care team remained blinded throughout the study.

**Data Collection**

EPIs were assessed 3 times a week for NNS performance in addition to the oral stimulation intervention. The NNS compression waveforms, taken by the NTrainer, were digitized at 3,000 samples per second (16-bits ADC) during a 3-minute session while the infant latched on a silicone pacifier immediately preceding a tube feeding not associated with an intervention condition.

**Patient Medical Data Management in the Neonatal Intensive Care Unit**

The Neonatal Oromotor Database was maintained by each NICU study coordinator for EPIs enrolled at their respective sites. This database is a custom software developed in the Barlow laboratory specifically for NTrainer studies (Barlow et al., 2017). It is compatible with Microsoft WIN10 and includes password-protected security systems through Microsoft Access 2013. This software is a paperless and efficient way for NICU study personnel to log daily information. Parameters include birthdate, birth order, sex, birthweight, body length, head circumference, GA, apnea bradycardia and desaturation (ABD), medications, retinal integrity, imaging results, growth parameters; pulmonary status, supplemental oxygen requirements; medical procedure log, history of feeding volumes and mode of intake, and comments (Barlow et al., 2017). Records will
be compared to NNS assessment files in order to find correlations of suck dynamics among EPIs.

**Non-Nutritive Suck Data Analysis**

The amplitude and temporal dynamics of NNS behavior is automatically extracted and processed using a new terminal application known as NeoNNS (Liao et al., 2019). This Python-based NNS waveform discrimination and feature extraction software outputs a variety of measurements for single session and/or repeated sessions within and between infants and supports batch processing among multiple NICU sites (Liao et al., 2019). The NeoNNS software accesses a database file known as the Neonate Oromotor Database to retrieve and co-register the NNS assessment file with key information about a given infant’s PMA, diagnosis, NTrainer intervention, and oral feed variables. The NeoNNS software imports the source NNS binary file originally sampled at 3 kHz (16-bit ADC) for 3 minutes on the NTrainer and converts to human-readable ASCII format. The resulting 540,000 samples are down-sampled to 100 samples/second and saved in an intermediate file to improve memory resource management, graphical display, and computational efficiency while preserving the integrity of NNS waveform features for reliable waveform discrimination (Liao et al., 2019). In batch mode, NeoNNS can process 11 NNS binary source files per minute.

The NNS pressure waveforms are preprocessed with a low-pass Butterworth digital filter (f<sub>c</sub> @ 50 Hz) to remove high frequency noise. The NNS pressure signal has an inherent thermal drift due to the heat conducted by the neonate’s mouth to the pacifier nipple air volume. Thus, baseline correction of the pressure signal over the full 3-minute waveform was automatically calculated by Asymmetric Least Squares Smoothing
baseline correction algorithm (ALSS) (Eilers & Boelens, 2005). This computation was iterated 10 times to find the best baseline fit of the NNS data (Liao et al., 2019).

Non-nutritive suck pressure peaks >1.6 cm H$_2$O are subjected to feature extraction criteria, including suck cycle symmetry, cycle duration, and burst identification defined as two or more NNS events occurring within 1200 ms. This algorithm permits objective identification of NNS burst activity distinct from non-NNS mouthing compressions or tongue thrusts against the pacifier in order to create a high reliability measure. NNS bursts are automatically extracted and labeled with a green cross (as seen in Figure 6) and non-NNS cycles are in red. NNS bursts are identified and highlighted in pink. Five measures were automatically extracted from each NNS assessment data file in a batch file process using NeoNNS (Liao et al., 2019). Measures included minute-rates for (1) NNS suck cycle events, (2) NNS bursts (2 or more suck compression cycles), (3) non-NNS compressions (jaw, tongue thrust), (4) ratio NNS/total compressions (%), and (5) NNS cyclic compression pressure amplitude (cmH$_2$O) (Barlow et al., 2017).

Parameters measured included NNS cycles/minutes (how many NNS per minute), NNS cycles/total compressions (the number of true NNS events over total oral compressions that may include jaw movements, thrusts, etc.), bursts/minute (bursts are classified as 2 or more cycles), NNS cycles/burst, maximum NNS cycles/burst, and NNS amplitudes (cmH$_2$O).
Figure 6. Intervention results layout for Extremely Premature Infants of nonnutritive suck assessment. The graphical user interface of NNS includes five analysis pages: (1) NNS View, (2) Pan View, (3) Results View, (4) Power spectrum View, and (5) STI View. (Adapted from Liao et al., 2019).

Statistical Model

The primary endpoint was the longitudinal comparison of NNS cycles/min, ratio of NNS per total mouthing events, NNS bursts/min, NNS cycles/burst, max NNS cycles/burst, and NNS amplitude performance between two stimulus types (NTrainer, SHAM), each consisting of two preterm infant groups (RDS, BPD). Linear mixed modeling (LMM) was used to examine the effect of stimulus type, the effect of infant
group, and their interaction while accounting for infants’ GMA, PMA, sex, as well as dependency among performance observations repeatedly measured at multiple time points. The model parameters were estimated via restricted maximum likelihood (REML), which often produces unbiased parameter estimates with an unbalanced sample and/or incomplete data. When the interaction between stimulus type and infant group was significant at 0.05 alpha level, adjusted means of the four conditions (RDS in NTrainer, BPD in NTrainer, RDS in SHAM, BPD in SHAM) were pairwise compared at a Bonferroni-corrected alpha level (i.e., 0.05/6 = 0.008). A proper error covariance structure was determined in a preliminary analysis (i.e., intercept-only model) based on model fit (e.g., adjusted Akaike Information Criterion, Bayesian Information Criterion). All analyses were conducted using SAS 9.4 (SAS Institute, 2002-2012). A one-way analysis of variance (ANOVA) was utilized to determine significance of GA and BW between diagnoses.

Results

The LMM was completed using the factors sex (M, F), respiratory diagnosis (RDS, BPD), and treatment (NTrainer, SHAM) for the dependent variables NNS cycles/min, Ratio of NNS per total mouthing events, NNS bursts/min, NNS cycles/burst, max NNS cycles/burst, and NNS amplitude based on a sample of 817 NNS compression pressure waveforms sampled from 42 EPI neonates. As expected, the mean GA for RDS was significantly greater than GA for BPD (191.32 versus 184.75 days, respectively; p<.05). Average birthweights for RDS babies were somewhat greater compared to BPD (1004 and 883 grams, respectively) although this difference did not reach statistical
significance (p<.067). The longer GA and higher birthweight among RDS correlates to a less severe respiratory diagnosis compared to preterm infants with BPD that require O₂ supplementation past 36 weeks PMA.

For the dependent variable NNS cycles/min, the main effects for respiratory status were not significant, whereas orosensory treatment type was significant (p<.05). No significant interaction between lung status and treatment was found (p=.37). Infants who received the NTrainer treatment were observed to have fewer NNS cycles/min regardless of respiratory diagnosis compared to SHAM (26.90 vs 31.12 NNS cycles/min, respectively for BPD, and 27.97 versus 38.12 NNS cycles/min, respectively for RDS) (Figure 7).

![Figure 7](image)

**Figure 7. NNS Cycles/min** versus disability and treatment (Tr 1= NTrainer, Tr 2= SHAM). Pairwise comparison at a Bonferroni-corrected alpha level that is adjusted for sex and age.

A comparison of raw means and polynomial trendlines (marginal means) for NNS cycles/min by sex and age is shown in Figure 8. Positive growth in the dependent variable, NNS cycles/min, is shown for preterm infants from 209 to 270 days PMA with age exhibiting a significant effect (p<.0001). Sex was found to not be significant (p=.23). An analysis of growth rates using a simple linear regression model for male infants
(N=19) shows NNS cycles/min increases by 0.861 cycles/min per PMA day (F=17.84, p<.001, R²=51.21%) and is described by the expression,

\[
\text{♂} \text{NNS cycles/min} = -172 + 0.861x, \text{ where } x \text{ equals PMA.}
\]

A polynomial fit to the male data, shown in Figure 8, resulted in an improved fit (R²=60.75%) and is given by the expression,

\[
\text{♂} \text{NNS cycles/min} = -0.001x^3 + 0.6919x^2 - 160.36x + 12296.
\]

Female infants (N=23) show a lower rate of growth in NNS cycles/min during the intervention phase. The slope associated with linear regression is 0.338 (F=2.31, p=0.143, R²=9.92%) and is described by the expression,

\[
\text{♀} \text{NNS cycles/min} = -52.1 + 0.338x.
\]

A polynomial fit to the female data, shown in Figure 8 also resulted in an improved fit (R²=19.69%) and is given by the expression,

\[
\text{♀} \text{NNS cycles/min} = 0.0015x^3 - 1.1049x^2 + 267.66x - 21577.
\]
Figure 8. Mixed linear model of NNS cycles/min versus PMA (N=42 EPI infants). NNS cycles/min values were averaged for each preterm participant across multiple measurements of NNS activity in the NICU. The dotted lines are marginal means estimated as a polynomial function of PMA in cubic regression.

No significant main effects were found for the dependent variable Ratio of NNS cycles/total oral compressions. The main effects for respiratory status was not significant (Figure 9).

Figure 9. NNS cycles/total oral compressions versus disability and treatment (Tr 1= NTrainer, Tr 2= SHAM). Pairwise comparison at a Bonferroni-corrected alpha level that is adjusted for sex and age.
A comparison of raw means and polynomial trendlines (marginal means) for the ratio of NNS cycles over total compressions by sex and age are shown in Figure 10. Positive growth in the dependent variable is shown for preterm infants from 209 to 270 days PMA. Sex was found to not be significant (p=.55). Oral compressions discriminated as NNS cycles increased significantly with age, showing a significant dependence on PMA (p<.001). An analysis of growth rates using a simple linear regression model for male infants (N=19) shows ratio of NNS cycles/total increases by 0.004 per PMA day (F=10.51, p<.01, R²=38.21%) and is described by the expression,

\[
\hat{\frac{\text{NNS cycles/total}}{\text{total}}} = -0.8878 + 0.004926x,
\]

where x equals PMA. A polynomial fit to the male data, shown in Figure 10, resulted in an improved fit (R²=45.07%) and is given by the expression,

\[
\hat{\frac{\text{NNS cycles/total}}{\text{total}}} = -5E-06x^3 + 0.0033x^2 - 0.7602x + 57.942.
\]

Female infants (N=23) did not show a significant growth in ratio NNS cycles/total during the intervention phase. The slope associated with linear regression is -0.000609 (F=0.14, p=0.716, R²=0.6%) and is described by the expression,

\[
\hat{\frac{\text{NNS cycles/total}}{\text{total}}} = 0.4108 + 0.000609x.
\]

A polynomial fit to the female data, shown in Figure 10, also resulted in an improved fit (R²=3.03%) and is given by the expression,

\[
\hat{\frac{\text{NNS cycles/total}}{\text{total}}} = -3E-06x^3 + 0.0026x^2 - 0.6336 + 52.149.
\]
Figure 10. Mixed linear model of ratio NNS cycles/total versus PMA (N=42 EPI babies). These ratiometric values were averaged for each preterm participant across multiple measurements of NNS activity in the NICU. The dotted lines are marginal means estimated as a polynomial function of PMA in cubic regression.

For the dependent variable NNS bursts/min, a marginal effect was found for respiratory diagnosis (p=.063) shown in figure 11. On average, infants with BPD produced fewer NNS bursts/min compared to infants with RDS (5.86 vs 6.44 bursts/min, respectively for NTrain, and 5.18 vs 6.58 bursts/min, respectively for SHAM babies). The treatment effect was not significant, as were the interaction between respiratory diagnosis and treatment.
**Figure 11. NNS bursts/min** versus disability and treatment (Tr 1= NTrainer, Tr 2= SHAM). Pairwise comparison at a Bonferroni-corrected alpha level that is adjusted for sex and age.

A comparison of raw means and polynomial trendlines (marginal means) for NNS bursts/min by sex and age are shown in Figure 12. Positive growth in the dependent variable NNS bursts/min is shown for preterm infants from 209 to 270 days PMA. The number of NNS bursts/min increased significantly as a function of PMA ($p<.0001$). Sex was found to not be significant ($p=.44$). An analysis of growth rates using a simple linear regression model for male infants ($N=19$) shows NNS bursts/min increases by 0.1006 per PMA day ($F=17.60$, $p<.001$, $R^2=50.9\%$) and is described by the expression,

\[
♂ \text{NNS bursts/min} = -17.80 + 0.1006x,
\]

where $x$ equals PMA.

A polynomial fit to the male data, shown in Figure 12, resulted in an improved fit ($R^2=66.97\%$) and is given by the expression,

\[
♂ \text{NNS bursts/min} = 0.0001x^3 - 0.1079x^2 + 26.064x - 2090.7.
\]

Female infants ($N=23$) showed a significant growth in NNS bursts/min during the intervention phase. The slope associated with linear regression is $+0.07172$ ($F=5.97$, $p<.05$, $R^2=22.1\%$) and is described by the expression,

\[
♀ \text{NNS bursts/min} = -10.87 + 0.07172x.
\]
A polynomial fit to the female data, shown in Figure 12, also resulted in an improved fit (R²=35.72%) and is given by the expression,

\[
♀ \text{ NNS bursts/min} = 0.0002x^3 - 0.13x^2 + 31.935x - 2609.6.
\]

Figure 12. Mixed linear model of NNS bursts/min versus PMA (N=42 EPI babies). These ratiometric values were averaged for each preterm participant across multiple measurements of NNS activity in the NICU. The dotted lines are marginal means estimated as a polynomial function of PMA in cubic regression.

For the dependent variable NNS cycles/burst, a significant main effect was found for orosensory treatment (p<.05). On average, infants with BPD and RDS produced fewer NNS cycles/burst during orosensory entrainment compared to infants who received the SHAM treatment (4.27 and 4.26 NNS cycles/burst, respectively during entrainment treatment versus 5.50 and 6.13 cycles/burst, respectively during SHAM).
Figure 13. **NNS cycles/burst** versus disability and treatment (Tr 1= NTrainer, Tr 2= SHAM). Pairwise comparison at a Bonferroni-corrected alpha level that is adjusted for sex and age.

A comparison of raw means and polynomial trendlines (marginal means) for NNS cycles/burst by sex and age are shown in Figure 14. The number of NNS cycles/burst was highly dependent on PMA (p<.0001). Sex showed no significance in NNS cycles/burst (p=.08). Positive growth in the dependent variable NNS cycles/burst is shown for preterm infants from 209 to 270 days PMA (p<.001). An analysis of growth rates using a simple linear regression model for male infants (N=19) shows NNS cycles/burst increases by 0.1069 per PMA day (F=8.38, p<.01, R\(^2\)=33.0%) and is described by the expression,

\[
♂ NNS \text{ cycles/burst} = -20.15 + 0.1069x
\]

A polynomial fit to the male data, shown in Figure 14, resulted in an improved fit (R\(^2\)=49.99%) and is given by the expression,

\[
♂ NNS \text{ cycles/burst} = -0.0002x^3 + 0.1698x^2 - 39.692x + 3077.1
\]

Female infants (N=23) showed a non-significant growth in NNS cycles/burst during the intervention phase. The slope associated with linear regression is +0.01554 (F=0.19, p=.665, R\(^2\)=0.91%) and is described by the expression,

\[
♀ NNS \text{ cycles/burst} = -0.708 + 0.01554x
\]
A polynomial fit to the female data, shown in Figure 14, also resulted in an improved fit \( (R^2=8.52\%) \) and is given by the expression,

\[
♀ NNS \text{ cycles/burst} = 0.0003x^3 - 0.1865x^2 + 44.811x - 3580.6.
\]

**Figure 14.** Mixed linear model of NNS cycles/burst versus PMA (N=42 EPI babies). These ratiometric values were averaged for each preterm participant across multiple measurements of NNS activity in the NICU. The dotted lines are marginal means estimated as a polynomial function of PMA in cubic regression.

The trend was similar for the maximum number of NNS cycles/burst with a significant main effect for Treatment type \( (p<.05) \) in which BPD and RDS infants receiving orosensory entrainment showed shorter NNS Burst maxima, 9.78 and 9.56 cycles respectively, during NTrainer compared to 12.27 and 13.96 cycles respectively, during SHAM shown in figure 15.
A comparison of raw means and polynomial trendlines (marginal means) for MAX NNS cycles/burst by sex and age are shown in Figure 16. Positive growth in the dependent variable MAX NNS cycles/burst is shown for preterm infants from 209 to 270 days PMA. This variable also was highly dependent on PMA (p<.0001). Sex was found to not be significant (p=.09) An analysis of growth rates using a simple linear regression model for male infants (N=19) shows MAX NNS cycles/burst increases by 0.2883 per PMA day (F=8.68, p<.01, R²=33.8%) and is described by the expression,

\[ \mathop{♂}\limits^{MAX \text{ NNS cycles/burst}} = -56.41 + 0.2883x, \text{ where } x \text{ equals PMA.} \]

A polynomial fit to the male data, shown in Figure 16, resulted in an improved fit (R²=49.60%) and is given by the expression,

\[ \mathop{♂}\limits^{MAX \text{ NNS cycles/burst}} = -0.0005x^3 + 0.3847x^2 - 89.486x + 6898. \]

Female infants (N=23) showed a non-significant growth in MAX NNS cycles/burst during the intervention phase. The slope associated with linear regression is +0.06722 (F=0.57, p=.458, R²=2.65%) and is described by the expression,

\[ \mathop{♀}\limits^{MAX \text{ NNS cycles/burst}} = -6.21 + 0.06722x. \]
A polynomial fit to the female data, shown in Figure 16, resulted in an improved fit ($R^2=10.27\%$) and is given by the expression,

\[
\text{MAX } NNS \text{ cycles/burst} = 0.0007x^3 - 0.4972x^2 + 119.3x - 9524.
\]

**Figure 16.** Mixed linear model of NNS cycles$_{\text{max/burst}}$ versus PMA (N=42 EPI babies). These ratiometric values were averaged for each preterm participant across multiple measurements of NNS activity in the NICU. The dotted lines are marginal means estimated as a polynomial function of PMA in cubic regression.

A relationship was found for the dependent variable NNS amplitude expressed in cmH$_2$O. Significant main effects were found for both respiratory diagnosis ($p<.05$) and orosensory treatment ($p=.03$) shown in figure 17. Infants diagnosed with BPD showed higher NNS compression pressures during entrainment treatment compared to SHAM, 24.07 versus 20.42 cmH$_2$O, respectively. Infants diagnosed with RDS showed an opposite pattern with higher NNS compression pressures during the SHAM compared to orosensory entrainment treatment, 33.93 versus 26.07 cm H$_2$O, respectively.
A comparison of raw means and polynomial trendlines (marginal means) for NNS amplitude by sex and age are shown in Figure 18. Positive growth in the dependent variable NNS amplitude is shown for preterm infants from 209 to 270 days PMA. The amplitude of NNS compression pressure was highly dependent on PMA ($p<.0001$) with a robust growth in peak pressures from 1.6 to 54.1 cmH$_2$O between 210 and 270 days PMA. Sex showed no significance in MAX NNS cycles/burst ($p=.79$). An analysis of growth rates using a simple linear regression model for male infants (N=19) shows NNS amplitude increases by 0.6490 cmH$_2$O per PMA day ($F=11.18$, $p<.001$, $R^2=39.7\%$) and is described by the expression,

$$\text{NNS Amplitude} = -126.3 + 0.6490x,$$

where $x$ equals PMA.

A polynomial fit to the male data, shown in Figure 18, resulted in an improved fit ($R^2=50.15\%$) and is given by the expression,

$$\text{NNS Amplitude} = -0.0007x^3 + 0.4911x^2 - 112.83x + 8574.9.$$
Female infants (N=23) showed a non-significant growth in NNS amplitude during the intervention phase. The slope associated with linear regression is +0.3588 (F=2.41, p=.135, $R^2=10.3\%$) and is described by the expression,

$$♀ \text{NNS Amplitude} = -59.33 + 0.3588x.$$  

A polynomial fit to the female data, shown in Figure 18, resulted in an improved fit ($R^2=19.59\%$) and is given by the expression,

$$♀ \text{NNS Amplitude} = 0.0006x^3 - 0.4346x^2 + 110.5x - 9317.7.$$ 

**Figure 18.** Mixed linear model of NNS AMP (cmH$_2$O) versus PMA (N=42 EPI babies). These ratiometric values were averaged for each preterm participant across multiple measurements of NNS activity in the NICU. The dotted lines are marginal means estimated as a polynomial function of PMA in cubic regression.
<table>
<thead>
<tr>
<th></th>
<th>NNS Cycles/min</th>
<th>NNS/total compressions</th>
<th>Bursts/min</th>
<th>NNS Cycles/burst</th>
<th>Max NNS cycles/burst</th>
<th>NNS AMP (cmH₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>P = .23</td>
<td>P = .55</td>
<td>P = .44</td>
<td>P = .08</td>
<td>P = .09</td>
<td>P = .79</td>
</tr>
<tr>
<td><strong>Disability Effect</strong></td>
<td>P = .27</td>
<td>P = 0.79</td>
<td>P = .07</td>
<td>P = .54</td>
<td>P = .54</td>
<td>P = .02</td>
</tr>
<tr>
<td><strong>Treatment Effect</strong></td>
<td>P = .03</td>
<td>P = .29</td>
<td>P = .80</td>
<td>P = .02</td>
<td>P = .03</td>
<td>P = .03</td>
</tr>
<tr>
<td><strong>Disability and Treatment</strong></td>
<td>P = .43</td>
<td>P = .79</td>
<td>P = .36</td>
<td>P = .60</td>
<td>P = .56</td>
<td>P = .08</td>
</tr>
<tr>
<td><strong>PMA</strong></td>
<td>P &lt; .0001</td>
<td>P &lt; .001</td>
<td>P &lt; .0001</td>
<td>P &lt; .0001</td>
<td>P &lt; .0001</td>
<td>P &lt; .0001</td>
</tr>
</tbody>
</table>

**Table 1.** P-values for all NNS parameters and dependent variables.
<table>
<thead>
<tr>
<th></th>
<th>NNS AMP (cmH₂O)</th>
<th>Max NNS cycles/burst</th>
<th>NNS Cycles/burst</th>
<th>NNS/total compressions</th>
<th>NNS cycles/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPD/NTrainer</td>
<td>26.90 (SE = 3.15)</td>
<td>9.78 (SE = 1.37)</td>
<td>5.86 (SE = .37)</td>
<td>2.4% (SE = 2%)</td>
<td>26.90 (SE = 3.15)</td>
</tr>
<tr>
<td>BPD/SHAM</td>
<td>31.12 (SE = 5.09)</td>
<td>12.27 (SE = 2.22)</td>
<td>5.18 (SE = .61)</td>
<td>2.9% (SE = 4%)</td>
<td>31.12 (SE = 5.09)</td>
</tr>
<tr>
<td>RDS/NTrainer</td>
<td>27.97 (SE = 2.85)</td>
<td>9.56 (SE = 1.24)</td>
<td>6.44 (SE = .34)</td>
<td>2.7% (SE = 2%)</td>
<td>27.97 (SE = 2.85)</td>
</tr>
<tr>
<td>RDS/SHAM</td>
<td>38.12 (SE = 3.58)</td>
<td>13.96 (SE = 1.56)</td>
<td>6.58 (SE = .44)</td>
<td>3.0% (SE = 3%)</td>
<td>38.12 (SE = 3.58)</td>
</tr>
</tbody>
</table>

Table 2: Mean values for NNS parameters compared to treatment type and diagnosis.
**Discussion**

The attainment of oral feeding is a complex neuromotor behavior that is significantly underdeveloped in EPIs due to prematurity. Their fragile structures have not yet acquired CPGs essential for oral feeding. This results in an inability to thrive without support, in combination with co-morbidities and long-term complications (Glass et al., 2015; Poore et al., 2008a). Infants and their families will face extensive medical bills, delayed language skills, decreased IQs, emotional instability, and decreased academic performance (Foster-Cohen et al., 2010; Imgrund et al., 2019; Lewis et al., 2002; Sansavini et al., 2010; Sriram et al., 2018; Twilhaar et al., 2018). In addition to complications due to prematurity, infants are exposed to maladaptive experiences, such as oxygen supplementation for BPD and RDS, that disrupt the development of orofacial movements required for sucking (Barlow et al., 2018; Koong Shiao et al., 1996).

Prematurity was also observed to have an effect on respiratory diagnosis with younger GAs demonstrating more severe diagnosis (BPD). Lower BW, often associated with younger GAs, were shown to correlate with a severe respiratory diagnosis (BPD).

Premature infants are in need of a noninvasive assessment and treatment tool to advance oromotor and swallowing function in order to improve feeding and development outcomes (Barlow et al., 2017). The NTrainer System is a tool that has been shown to modulate local reflex activity and support infants in producing the targeted NNS oromotor rhythms (Barlow et al., 2008; Barlow et al., 2017). It can significantly increase developmental gains in oral feeding proficiency (Barlow et al., 2017; Poor et al., 2008a). A need also exists for an efficacious analytic software for NNS waveforms. Previous research shows many devices used for NNS measures have well designed mechanics;
however, the software used for data analytics has limited evidence on efficacious output measures across repeated treatment sessions (Bromiker et al., 2016; Lau et al., 1997; Lau et al., 2000).

The current study is part of an ongoing randomized control trial with the purpose to characterize the effects of pulsatile orocutaneous stimulation paired with gavage feedings in EPIs using the automated Python-based NNS software processing program (Liao et al., 2019). It was hypothesized that infants will show a significant difference in NNS parameters between diagnoses RDS and BPD, treatment will show a significant effect, the correlation of disability and treatment will show a significant effect, and PMA will not show a significant effect.

Treatment (NTrainer vs. SHAM) exhibited a significant difference in NNS parameters among the 42 infants whereas there were no significant effects within disability (RDS and BPD). Infants’ performance increased for most measures when the SHAM was utilized versus the NTrainer, while the number of bursts per minute and compression cycle amplitude increased for infants who received the NTrainer therapy. A greater increase in variable measures were observed in RDS infants who received the SHAM due to less severe respiratory systems and on average higher PMAs in this group. No significant difference was demonstrated with the correlation of disability and treatment. Both BPD and RDS infants demonstrated similar outputs for treatment types, resulting in disability having no effect on treatment type. All parameters were highly dependent on PMA and resulted in a significant difference in NNS parameters. Sex did not have a significant effect on all dependent variables.
The interim results described in the present report are based on a relatively small sample of EPI’s, and show that the NeoNNS.exe Python batch processor is an effective tool to accurately assess infants’ NNS abilities. It is also an efficacious software due to output quality and timeliness. This software output confirms that disordered NNS dynamics are associated with extreme prematurity, with BPD infants manifesting more significant impairments in NNS parameters compared to RDS infants.

Previous studies have stated the importance of needing an accurate and evidence-based instrument to assess feeding readiness in the preterm infant population. This instrument demonstrates consistent results among disability type and sex, providing the instrument with high reliability throughout the study (Liao et al., 2019).

Future research is required in order to assess specific parameters of infants’ NNS in response to treatment throughout the 4-week treatment protocol. Data collection for the NIH RCT is ongoing and will continue through 2021. The larger sample size will increase the power and effects size of the results, and will make it possible to stratify infants according to GA (24 0/7 - 26 6/7 weeks GA, and 27 0/7 - 28 6/7 weeks GA) to determine potential effects of immaturity on NNS performance and oral feeding progression in the NICU. Demographics of participants were also limited to three NICUs in the United States. NICUs were widespread throughout the country; however, they may still have an effect on the results.

In summary, the results of the present investigation are an important step in determining interim results for a larger study. They provide future direction suggestions as well as point out potential limitations. Based on the current findings, the NTrainer showed a significant difference in NNS parameters, disability did not show a difference
in NNS parameters nor did it when paired with treatment, and sex did not have an effect on results. Further research in the area of NNS parameter analysis is needed.
References


Zimmerman, E. (2018). Do infants born very premature and who have very low birth weight catch up with their full term peers in their language abilities by early school age? *Journal*
