Medical University of South Carolina
Protocol

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Study Title: **Noninvasive Brain Stimulation to Improve Oromotor Function In Neonates**

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A. SPECIFIC AIMS

Preterm infants and term infants who suffer hypoxic ischemic encephalopathy (HIE) are at high risk for motor problems, which primarily manifest as feeding delays during their neonatal hospital admission. Oromotor dyscoordination is very common in both groups of infants, and typically takes 3-6 weeks of working on oral feedings in the hospital before the infant may take enough breast milk or formula to sustain adequate growth for discharge. Occupational therapy usually works with infants once a day to ensure that the feeding particulars, such as nipple choice, frequency of oral feeding, do not tax infant physiology too greatly and to guide learning this motor skill. Feeding difficulty is the primary reason for delayed discharge of preterm or HIE infants. Many of these infants will not be able to master this motor skill before term age (40-42 weeks gestation) and will receive a gastrostomy tube (G-tube) for direct gastric feeding, in order that they may finally be discharged from the hospital to home. Over the past 5 years in our MUSC NICU (2012-2016), we have placed an average of 40 G-tubes per year. This procedure requires general anesthesia for both insertion and eventual take down, and leaves scars in the epigastric area. The G-tube also reinforces the parental perception that their child is not normal and that he or she has a more limited developmental potential than a 'normal' child.

Even with significant brain injury, we know that neuroplasticity in infants may lead to improved, and even near normal outcomes. This neuroplasticity involves stimulating neurogenesis and reparative inter-neuronal connections to improve motor skills in neonatal animal models and in adults after stroke. In addition, we know that rehabilitative training may be enhanced by brain stimulation using a variety of modalities.

In recent years scientists have discovered that plasticity increases if you pair a stimulus or behavior with stimulation of the vagus nerve. We think that the vagus signal likely activates norepinephrine fibers coming from the locus ceruleus. This time-dependent signal to the cortex and other parts of the brain 'tells it' to pay attention to or be alert for other novel signals. By pairing a stimulus or behavior, scientists and clinicians can improve processes that involve neuroplasticity, like learning or rehabilitating a motor skill. Precisely targeted neural plasticity accomplished through Vagal Nerve Stimulation (VNS) and stimulus pairing has so far been shown to improve treatment of neurological disorders associated with motor function, tinnitus, and stroke. In motor studies in rats, VNS paired with movement led to a doubling in size of the primary motor cortex associated with the paired movement, whereas rats receiving identical motor training without VNS pairing did not exhibit motor cortex plasticity (1). In stroke, patients who received VNS paired with rehabilitation demonstrated a significant increase in their Upper Extremity Fugl–Meyer score compared with patients who received rehabilitation without VNS (2). In a different model of tinnitus in noise-exposed rats, VNS paired with auditory tones completely eliminated the physiological and behavioral symptoms of tinnitus (3), and the same treatment in humans seemed to exert a beneficial effect in nonmedication-taking patients, both with regard to perceived sound and distress (4). There are now ongoing FDA-pivotal studies involving pairing VNS with rehab to improve stroke rehabilitation (NCT03131960)
or pairing VNS with sounds to improve tinnitus (NCT01962558). These pivotal studies are funded by a small startup company (MicroTransponder) whose patent portfolio involves surgically implanted cervical VNS.

Cervically implanted vagus nerve stimulation (VNS) is an FDA approved treatment for epilepsy and major depression, with the first implant in humans in 1988 (5,6). Although cervical VNS is relatively safe and effective in seizure prevention (7,8) the risks involved in surgical implantation as well as its high cost (about $30-50k) make it less appealing and less available as a treatment modality.

Recently it has been shown that VNS can be administered non-invasively through stimulating the auricular branch of the vagus nerve located in the ear (4, 9,10) with a transcutaneous electrical nerve stimulation (TENS) device. TENS is a very common, FDA-approved therapy for pain management. Researchers in the brain stimulation lab here at MUSC have conducted initial safety and feasibility trials of transcranial auricular VNS (taVNS) in healthy adults. In these studies, there were no minor or major adverse effects observed throughout the duration of their trial. Their work validates ideal parameters for delivering taVNS, suggesting that one-minute taVNS periods at 200% perceptual threshold delivered at 500μs 10Hz are safe and tolerable and produce regional brain changes consistent with activating the vagus nerve related brain regions involved in neuroplasticity (10).

Surgically implanted cervical VNS is expensive, risky, and would not likely be used for transient boosts of normal motor development. However, if we can use noninvasive taVNS rather than implanted VNS, this promising research modality may be translated to more fragile infants. Such a safe, bedside treatment would be ideal for preterm and HIE neonates trying to master motor skills that are reflexive for normal near term neonates, but must be learned for those infants born prematurely or relearned for those with brain injury. The added benefits of enhancing accomplishment of this primary motor task would be earlier discharge, lower hospital costs, improved parental perception of the developmental potential of their infant, and finally reduced stress and better bonding with parents, both in and out of the hospital.

Feeding in neonates involves a sequence of sucking, swallowing, and breathing that requires coordination of the face, head, and neck muscles with the myelinated vagal regulation of the bronchi and the heart (11). In preterm neonates, the muscles needed to feed are underdeveloped, resulting in the need for OT rehabilitation to ‘learn’ feeding patterns. Preterm neonates’ inability to feed effectively is the primary reason for prolonged hospital stays (12). In neonates with HIE, development of cortex and basal ganglia is interrupted, and depending on the severity, normal developmental plasticity is hindered, further contributing to their inability to feed (13). Both types of feeding difficulties involve complex motor learning, which requires integration of sensory and motor pathways. Treating oromotor difficulties during the learned task of feeding with noninvasive brain stimulation that promotes plasticity, poses a highly novel application of taVNS. Our major premise is that in babies at high risk for motor problems, simultaneously delivered brain stimulation via taVNS will boost motor cortical plasticity involved in a learned feeding task, leading to better feeding.

This protocol has 2 specific aims that will be investigated:

Specific Aim 1: Improved Motor Learning - We aim to investigate whether pairing taVNS with rehabilitation training of feeding behavior in preterm and HIE neonates will enhance and accelerate the learning of effective feeding behavior in a dose responsive manner. We call this treatment “taVNS-paired feeding.” The primary outcome measures of taVNS-paired feeding will be daily oral milk feeding volume changes from before to during treatment, and number of infants reaching full oral feeds

between once and twice daily taVNS-paired feeding, duration to full feeds, as well as general motor functional impairment as measured by Dr Coker-Bolt's Specific Test of Early infant motor Performance (STEP). Our hypothesis is that taVNS-paired feeding for preterm neonates is feasible, safe and effective at reducing length of stay solely for feeding reasons, compared to historical controls. As part of our safety investigations with separate funding, we may perform taVNS during a clinical modified barium swallow study with pediatric speech language pathologists performing the study and compare 10 swallows with stimulation on and off to determine if taVNS affects swallowing parameters.

Specific Aim 2: Brain Changes - We aim to determine if white matter fiber tracts show improved integrity as measured by diffusion imaging (DKI) before, after a 2-week treatment taVNS-paired feeding course, and whether treatment improves neuronal energetics in the basal ganglia as measured by magnetic resonance spectroscopy (MRS). Unsedated MRS and DKI imaging will last approximately 40 minutes, and will be done before and after the two-week taVNS-paired rehab treatment session. The changes in white matter tract diffusion parameters and N-acetylaspartate (NAA) and glutathione (GSH) concentration in the basal ganglia and thalamus will be compared with published changes of these parameters over gestational age in preterm infants (14, 15) and with our own MRS data in preterm and HIE infants who did not receive taVNS.
We will perform DKI to determine the effects of taVNS on plasticity, and to identify areas of the brain involved in afferent vagal activation. We propose utilizing the concurrent taVNS/fMRI method developed by Dr. Badran at MUSC to determine whether taVNS activates the CNS and afferent vagal network in a subset of preterm and term infants in the study.

B. BACKGROUND AND SIGNIFICANCE

Traditional cervical VNS involves surgically implanting a stimulator into the left chest wall. Bipolar electrodes are then wrapped around the left vagus nerve in the neck with wires connected to these electrodes running inside the neck and connecting to the stimulator. Cervical implanted VNS is FDA approved in patients with intractable epilepsy (5-8). Serendipitously in initial epilepsy studies, it was noticed there were associated mood improvements in implanted patients even in patients who had no seizure decrease (16,17). PET studies at the time also showed that VNS caused changes in mood regulating regions. These two pieces of evidence led Dr. George to begin exploring VNS as a treatment for depression (18, 19) and ultimately being approved for the long-term treatment of chronic or recurrent depression. MUSC was the site of the first depressed patient ever implanted with cervical VNS. Aside from the anti-epileptic and positive mood effects in humans, VNS has been studied extensively in animal models, with applications ranging from attenuation of inflammatory response (20, 21), increased survival rate in heart failure (22), reduction of infarct size in cerebral ischemia (23, 24), motor rehabilitation after stroke (25, 26, 27) and tinnitus VNS (28, 31). VNS is an emerging area of interest in the neuroscience field and has many translatable applications. The ability to pair VNS with stimuli and motor behavior and promote learning and plasticity is the most germane aspect of VNS for this specific proposal.

The vagus nerve spans from the gut up to the nucleus track solitaris (NTS) of the brainstem. From there, projections span to many different regions of the brain, each with its own resulting effect. A small branch of the vagus nerve, known as the auricular branch, innervates the ear. This branch also has afferent projections to the brain and may elicit similar effects when stimulated as does traditional

cervical vagus stimulation in the neck. The concept of transcutaneous auricular vagus nerve stimulation (taVNS) as a noninvasive alternative to the conventional implantable VNS is relatively new and was first developed in 2000 (2). Since then, there have been several groups that have conducted studies on this novel form of neuromodulation (4,9,10).

At MUSC, Dr Bashar Badran and the brain stimulation division have conducted three focused studies examining the safety, feasibility, and effectiveness of taVNS in adults. They found that during taVNS, participants showed minor but statistically significant immediate heart rate decreases followed by a modest sympathetic rebound upon termination of stimulation. The group developed and conducted a concurrent taVNS/fMRI trial to determine the neurobiological effect of taVNS. Findings from the taVNS/fMRI trial demonstrated that the neurobiological effect of taVNS mimics that of cervically implanted VNS and targets several cortical and subcortical vagus afferent pathway targets. It additionally suggested that taVNS for short time periods activates the vagus nerve (10-25Hz, average 1.8mA). The group saw no minor or major adverse events during the treatment sessions and no spontaneously reported event following termination of the trial. No rapidly accelerated or sustained drops in HR were seen during the one-minute taVNS stimulation periods. Minor, temporary, and light redness was seen at the sight of stimulation but disappeared within five minutes of stimulation completion. A follow-up study determined that the optimal parameter to modulate the parasympathetic response activated via taVNS was 500μs pulse width, 10Hz for 60s duration (10).

VNS has been shown to rescue the brain if stimulation begins immediately after trauma, such as immediately after strokes. Rats that received vagal stimulation immediately after focal cerebral ischemia had not only significantly better neurological scores compared to non-VNS rats, but these rats also had significantly smaller infracts (24). A large group out of Dallas, TX has conducted extensive research on the effects of VNS in both animal and human models. In treating tinnitus, the group tested VNS, tones, and paired VNS-tones (4, 28). When VNS alone was delivered to the rats in a tinnitus model, there was no decrease in tinnitus symptoms. When tones were delivered alone there were no reductions. This suggests there is a synergistic effect of VNS when combined with a paired stimulus that is directing plastic changes to occur in the cortex. This concept is called “targeted plasticity” (2,3,4) or use- or state-dependent plasticity, in which various cortical targets can be selectively changed depending on the paired stimulus. This group is exploring VNS induced targeted plasticity as a treatment for other neurological disorders in animal models involving cortical reorganization, including stroke (25, 26) and have successfully moved into human clinical trials for both these treatments. Most notably, this pairing of VNS and rehabilitation paradigms has been shown to restore motor behavior in stroke. It is conceivable that these targeted plasticity findings can be seen with taVNS, without the cost or risk of surgical implantation.

If we can use taVNS rather than implanted VNS, the prior wealth of promising research can potentially be translated to more fragile patients noninvasively. While surgical implantation VNS has produced a large body of positive results, it would be far more cost effective and easier to conduct VNS noninvasively. In looking to the future of VNS, noninvasive taVNS poses significant applications in populations such as preterm neonates, who are too unstable to undergo surgery and might benefit from VNS for a specific motor task of feeding. This population represents an ideal group for testing this novel use of taVNS. To ingest milk properly and efficiently, neonates must have the neural integration to coordinate the complex sequence of sucking, swallowing, and breathing. This sensorimotor sequence

requires coordination of the face, head, and neck muscles with the myelinated vagal regulation of the bronchi and the heart. Since the structures involved in feeding are linked to the myelinated vagus, the ability to feed may provide an early indicator of the functional status of a neuromuscular system that will later be involved in social engagement behaviors. Prematurity and illness affect the developmental trajectory of many neural circuits, including the vagal circuit. Abnormal development of the vagus is reflected in myelination of the vagus, interneuronal connections in the brainstem that form the face-heart connection, and/or corticobulbar regulation of the brainstem circuits regulating both vagal activity and the striated muscles of the face, head, and neck. The consequences in typical neural maturation seen frequently in older preterm infants would be expressed as RSA (Respiratory sinus arrhythmia) due to lower vagal tone and brainstem immaturity (11). RSA has been associated with poor behavioral state regulation common in preterm or HIE infants, and too little or too much autonomic reactivity (37). Such reactivity and neural immaturity make coordinating and learning a complex motor task even more difficult, explaining why the feeding mechanism must be taught through OT rehabilitation, when it should be a normal reflex. Interestingly, the RSA also associates with behavioral reactivity problems in older children, conditions for which both preterm and HIE infants are at increased risk, and vagal regulation deficits are reported in adult psychiatric disorders (38).

Although postnatally the developing brain is more plastic than the adult brain, and thus would be expected to have better recovery mechanisms following injury, the immature or injured neonatal brain has some of the worst developmental outcomes following significant insult. Injury, oftentimes occurring in the difficult labor associated with preterm birth and conditions associated with HIE, trigger excessive stimulation of particular inflammatory pathways normally involved in shaping the developing brain circuitry. In the immature brain outgrowth of neural projections resulting from injury actually generate abnormal connections and circuitry as well as a paucity of myelinating cells and inter-neurons that subsequently lead to motor and cognitive impairment. Around the time of birth, the cortex and basal ganglia undergo significant integrative connectivity associated with shaping of central motor pathways. A HI or other injury event around this time interrupts these processes and can affect normal developmental plasticity through altering neurotransmission and changes in cellular signaling leading to abnormal connectivity (13).

The data in both animals and adult humans is convincing that VNS paired with motor training improves motor functioning (2,3,4). A multisite, FDA-pivotal, industry-sponsored trial of implantable cervical VNS to treat upper motor hemiplegia after stroke in adults is currently underway, built on this promising data. Activity dependent plasticity that is enhanced by electrical stimulation, has become a common tenant of neuromodulatory research, but has not yet been translated to infants or neonates after global brain injury of prematurity or HIE. The justice of holding back investigational drugs and therapies from children and infants who may benefit simply because they are children, has come into question the past years, as we clinicians have to acknowledge that virtually NONE of the treatments we use in infants have actually undergone testing or are FDA approved in the childhood populations routinely used. We have an obligation as pediatric clinical researchers to remedy this extreme discrepancy in innovative research in children.

The data and safety supporting the study of non-invasive TENS in neonates and young infants is represented in studies that investigated TENS for specific purposes such as pain reduction or treatment of torticollis or brachial plexus injuries (39-42). There were no adverse effects reported at various HZ

from 2-10 and for 0.5 – 5 mA. Moreover, the safe use of TENs for the iontophoresis necessary for the sweat test in neonates to confirm cystic fibrosis, supports its minimal risk designation in neonates (43). The investigators in these neonatal and infant studies as well as research conducted by Drs. Badran and George in adults at this institution, used the same frequency and intensity (10Hz, 0.5-5mA), and no painful or ill effects have been observed. Not only is TENS safe in all populations and is FDA cleared, in the case of VNS, we already have substantial evidence of dose response in adults (Badran, George) as well as efficacy and safety. The dosing parameters chosen in this study were found to be safe and have positive brain imaging results.

The purpose of this study is primarily to determine feasibility of the proposed taVNS dose and duration with a paired feeding, that has minimal negative effects and confers positive functional and CNS structural benefits. As this is a pilot study, we do not know the exact intensity of the stimulus that our infants will perceive. We have built in a dose determination during rest and during feeding for perceptual threshold. We also have an immediate reduction built in for safety if it causes discomfort, and we have planned for a gradual increase in the duration of treatment over the first feeding sessions to determine the tolerability. We have included the protocol for the perceptual threshold determination for clarification.

We hope to verify that the protocol for delivering taVNS-paired feeding will effectively align with regular, pre-existing OT rehabilitation performed in nearly all pre-term neonates, and that taVNS-paired feeding improves their ability to feed. We anticipate that taVNS-paired feeding treatments can be conducted in a feasible, time and cost effective manner. Our secondary purpose is to understand with brain imaging how taVNS may improve the CNS circuitry and metabolism, which correlates with the specific early motor task of learning to feed. As long term developmental data is always important in studies of infants, we will also look at preliminary data to see if this effect can be linked to long-term functional ability.

Diffusion MRI (dMRI) may determine effects of taVNS on plasticity.
Rapid changes in diffusion have been noted with electrical stimulation accompanied by a 20-45% increase in extracellular water in animals and human volunteers(3-5). On a cellular level, the diffusion changes are believed to indicate increased Na+/K+ATPase activity(6), and ionic and water movement through neuronal and glial membranes(5). These diffusion changes in white matter (WM) tracts have been proposed as an alternative to BOLD fMRI(3), and may be particularly applicable to measuring direct changes after taVNS in this neonatal and infant population after global HI brain injury. In addition, we will use DKI which is more sensitive in neonates and infants, who generally have lower FA and higher kurtosis values. This is a novel application of DKI as an indication of vagal-mediated central effects induced by taVNS paired feeding.

This research proposal will further develop a noninvasive vagus nerve stimulation as an assistive tool in the rehabilitation of neonates born with feeding deficits. We will confirm the safety of this new technique using fluoroscopic imaging as well as its effect on brain plasticity using advanced structural and functional neuroimaging.
We hypothesize, based on the paired VNS studies in animals and humans to date as well as human noninvasive VNS studies conducted here at MUSC, that taVNS-paired feeding in pre-term neonates will feasible, safe and not worsen swallowing problems and perhaps improve them. Additionally, we hypothesize that ta-VNS-paired feeding will accelerate pre-term neonates' ability to learn feeding

sensorimotor skills, which will be apparent in increased volume of milk consumed and longer oral feeding durations. If feeding behavior is improved, then we further expect decreased levels of functional impairment as compared to baseline STEP tests after receiving taVNS-paired feeding. These hypotheses regarding neonatal health and improvement, while vital in understanding the impact of taVNS-paired rehab in pre-term neonates, come second to the primary hypothesis that this novel treatment is safe and feasible.

C. PRELIMINARY STUDIES

The principal investigator, Dr. Jenkins, is a neonatologist. Her clinical research focuses on the management and treatment of hypoxic ischemic and neuroinflammatory diseases in neonates. She conducted a phase II multicenter randomized controlled trial of hypothermia to treat hypoxic-ischemic encephalopathy (HIE) in neonates. She developed other neuroprotective treatments in animal models, which she is currently translating to critically ill neonates with HIE undergoing hypothermia treatment in conjunction with N-acetylcysteine (NAC) and Vitamin D. In this IRB approved study (HR#31254), she is using magnetic resonance spectroscopy (MRS) to quantify participants' GSH levels before and after NAC and Vitamin D, using glutathione as a biomarker for oxidative stress and NAC pharmacodynamic CNS effect. The hope is that improving oxidative stress in neonates with HIE will improve neuronal growth and development. She has also safely performed other cutting edge studies using NAC maternal chorioamnionitis to cross the placenta and protect the fetal brain before delivery (HR#16708). She is well suited to conduct these studies safely in infants.

Dr. Mark George, MD, is a neurologist and psychiatrist and is an expert in brain imaging in mood disorders and is a pioneer in using brain stimulation, particularly TMS and VNS, for treating depression. In his earlier research at MUSC, he and his group were the first to use VNS to treat depression, and also were the first group to perform VNS within an fMRI scanner. He is currently chair of the DSMB for a multisite, FDA-pivotal, industry-sponsored trial of implantable cervical VNS to treat upper motor hemiplegia after stroke in adults, which is built on promising data in both animal and humans that VNS paired with motor activity improves motor learning. He is also the editor in chief of the major journal in the field of brain stimulation (Brain Stimulation). In the past 7 years he has become more interested in how and when to use brain stimulation devices to improve stroke rehabilitation and is a part of the MUSC COBRE involving brain stimulation and stroke recovery.

Dr. Bashar Badran, Ph.D, obtained his doctorate under Dr. George, and has extensive experience and background in clinical TMS and taVNS, especially in the development of novel protocols for neurological disorders. His thesis work involves systematic elucidation of the dose response of taVNS on heart rate, use of heart rate as a biomarker of taVNS effect, and the use of taVNS with fMRI in determining pathways and regions affected by taVNS in adults. He determined that the 'on' pulse heart rate change was a mean of -3 beats per minute (bpm) while the 'off' pulse heart rate change was +6 bpm. Although these are statistically significant and measurable changes, they are physiologically insignificant. Nevertheless, this will be an important biomarker for infants, but is not expected to result in safety concerns. Dr Badran's careful and thorough studies lay the safety and efficacy groundwork for the current proposal.

D. RESEARCH DESIGN AND METHODS (including data analysis)

Due to the novelty of these studies, Dr. Dorothea Jenkins will be present for each taVNS-paired feeding session conducted in the first participants, and as needed thereafter. Dr George and or Dr Badran will monitor taVNS effects. If any adverse events are noticed which are not described in the consent or are serious, we will inform the IRB before continuing with the next enrolled subject. Importantly, all of these methods have been done before, and published by our and other groups in adults, and we believe these extra precautions will assure the safety and well-being of any participants enrolled.

taVNS-paired feeding to enhance feeding behavior in neonates

Screening

Prospective participants will be identified by the PI (Jenkins) at the MUSC neonatal intensive care units (Level II and III), and checked for potential inclusion (please see the uploaded taVNS screening form). Paige Merrill, and other clinical OTs based in the nursery may also mention the study to parents and refer them to Dr. Jenkins if they are interested in participating.

Participants

Inclusion criteria:

Infants must be clinically stable, on minimal respiratory support (nasal cannula, or room air), in discussions about Gtube placement for poor po feeding and are currently greater than or equal to 39weeks post-menstrual age and

1) If born premature have been working on oral feeding for 30days;

or

2) If term Infant with significant medical issues that have precluded oral feeding, such as hypoxic ischemic encephalopathy (HIE), have been working on oral feeding for 14days.

Exclusion criteria:

1) Unstable infants or those requiring respiratory support involving positive pressure.
2) Major unrepaired congenital anomalies
3) Anomalies/conditions that limit feeding volumes
4) Cardiomyopathy
5) Repeated episodes of autonomic instability (apnea or bradycardia) which are not self-resolving *

*Preterm infants commonly have short periods of shallow or absent breathing or lower heart rate termed apnea and bradycardia, respectively, and most are being treated for these physiologic manifestations of prematurity with caffeine, an effective central stimulant. Infants are on cardiorespiratory monitors through the nursery stay to capture events, many of which are self-resolving. Infants who require repeated episodes of tactile stimulation to come out of these events are defined as *unstable*.

Neonates who are beginning oral feeds after medical treatment for critical illnesses, such as HIE brain injury, will be included as these neonates represent a population in which taVNS-paired feeding could present the largest success in overcoming impaired brain development. Congenital syndromes may be included if the infants do not have major, unrepaired anomalies or anomalies that limit feeding volumes.

Written informed consent will be obtained from the mother if available and if she has custody or decision making authority for the participant, otherwise a parent or legal guardian, prior to participation in the experimental paradigm. Department of Social services may have custody of infant but require the parent or foster parent to consent for all medical procedures.

Electronic Consent will be used for situations when the parent (or Legally Authorized Representative) is at another hospital and cannot travel to MUSC to provide consent in person. We will use the approved REDcap system to obtain consent. Dr. Jenkins or other approved study personnel will go over the entire consent document on the phone with the parent to ensure comprehension and to confirm identity. Parent will be given a copy of the completed, signed consent form in person or by email or fax.

Study Design

Up to 40 preterm/HIE neonates will be enrolled in this prospective, open-label safety and feasibility trial, with a dose response. The experimental paradigm consists of 2 to 3 weeks of once or twice daily taVNS-paired feeding.

Our control group was investigated by Drs. Allison Chapman and Rita Ryan at MUSC, who have researched all infants failing feeding and getting Gastrostomy tubes in our nurseries over the past 5 years. We can compare our data to this historical group at our own institution.

All consented participants will receive the active stimulation condition.

taVNS Stimulation (in Detail)

After obtaining consent and describing this procedure to the parents and all members of the baby's care team, we will first, with a 500μs, 25Hz pulse, gradually increase the intensity of the TENS unit until the baby can first feel something in their ear. We will use the facial expression change and fidgety movements to determine when the infants feel the stimulation. This intensity will be recorded as the perceptual sensory threshold. The stimulation intensity we will use for this study on taVNS-paired feeding is a small decrement from the perceptual sensory threshold for each infant (-0.1mA), a point at which they should not react to the stimulation. We will retest the sensory threshold before every session.

Determination of Perceptual Threshold

1. Electrode clip will be placed on the left tragus in an enrolled infant at rest. Starting at 0.2mA at 25Hz, we will deliver 5 seconds of pulses, and increase by 0.1mA until the perceptual threshold is achieved by observation of the infant's facial expression, fidgety movements, or NIPPS score. This will be termed the perceptual threshold.
2. We will then deliver this level of microcurrent while the infant is feeding on the first day to determine if the perceptual threshold changes with feeding. Current will then be held constant while pulse width and frequency will be set at 500us and 25Hz, respectively.

3. Finally, we will decrease the microcurrent by 0.1mA *below the perceptual threshold* and administer this taVNS treatment during the feeding and deliver this only while the infant is actively sucking from the bottle.
4. The total duration of this 'on-and-off' taVNS stimulation treatment will continue for 30 minutes or the duration of the feed.
5. We may manually trigger the taVNS stimulation or place a gel EMG electrode over the temporomandibular joint area to trigger the taVNS impulse.

Once the daily taVNS intensity has been determined, the treatment will continue to be administered in conjunction with the daily OT feeding once or twice daily.

As the baby begins to suck from the bottle or breast, stimulation will be triggered and a timer will be started. Sometimes the infant has non-nutritive sucking and it is difficult for the operator to discern between these lip motions and real suck/swallow. We wish to stimulate during sucking motions that include swallowing but may occasionally pair with ineffective and incomplete lip sucking, characteristic of more immature patterns. We may use manual triggering by a research assistant or triggering by the EMG at the buccinator muscle to ensure accurate delivery of impulses and pairing of impulse with sucking/swallowing.

The stimulation will continue until the baby stops sucking, at which point the stimulation will be stopped and the timer will stop, until the baby begins to suck again. The typical feeding OT session lasts up to 30 minutes. Physiological data will be monitored continuously as measured by the echocardiogram (ECG). Neonatal Infant Pain Scale (NIPS) data will be collected at beginning, middle and the end of treatment session. The NIPS rating scale currently in use in the nursery is included in this submission. These studies will be conducted in both level II and III Neonatal Intensive Care Units (NICUs), depending on the location and of the enrolled participants.

taVNS Device

tVNS will be delivered to the left ear only using an active (tragus) location for the taVNS clip using either the Digitimer Type DS7AH (Digitimer Ltd, Hertfordshire, England) or Soterix 0125-LTE stimulator customized for our infant trials. Both are TENS units and are cleared by the FDA for electrical nerve stimulation in research trials (See uploaded documents from Soterix). We also recently received FDA Breakthrough device designation for our BabySTrong taVNS infant feeding system, based on a customized 0125-LTE Soterix taVNS unit. TENS units are widely used in children with overactive bladder and dysfunctional voiding and stooling (44-45) and for analgesia in children, and as a treatment for chronic pain, including cancer (46). A custom electrode, embedded within a carbon matrix will be used to stimulate the auricular branch of the vagal nerve at the tragus. We will then trigger the taVNS unit to deliver the microcurrent or to stop the microcurrent when the infant is actively sucking and swallowing.

Safety Monitoring

Prior to determination of the daily sensory threshold of the infant, an initial pain level will be recorded using the NIPS. Previous studies using pain as a marker for safety in neonates check for pain halfway through the respective treatment (39-40). TENS treatments in studies examined in creating our protocol

typically lasted 30 minutes (41-43). Because the duration of the taVNS-paired feeding varies daily and will ultimately exceed 10 minutes, we will record pain levels before, at the end of treatment, and additionally at 5 and 10 minutes for longer sessions. This is shown in more detail in the *In Session Form* included.

In an effort to standardize delivery of taVNS-paired feeding treatment, the taVNS device will placed in the left ear as default. Skin redness at the site of taVNS attachment to the ear is possible after treatment, but is expected to be transient. In order to prevent injury to the skin in contact with the device, each day the left ear will be first examined for redness prior to attaching the taVNS device However, if there is skin redness at the site attachment to the ear prior to each day's treatment, we will switch the stimulation that day to the right ear. If these symptoms are persistent, we will decrease the stimulation.

ECG will be monitored during each treatment session. The HR measures will provide valuable data for safety analysis as well as immediate markers for physiological safety during taVNS-paired feeding. Bradycardia will be defined by NICU standards of <80bpm for 5 seconds.

In all sessions of taVNS-paired feeding, Dr. Jenkins or research staff trained in taVNS will be present. At any point during taVNS-paired feeding, the research staff will decrease stimulation intensity, if they feel the participant is in mild distress.

Developmental follow-up

Developmental follow-up is routine in all preterm and HIE infants, with clinical visits occurring every 3 months at Neonatal High-risk clinic. If parents do not bring their infants back for standard of care developmental testing, and if more convenient for parents, the developmental testing will be performed by Drs. Coker-bolt and/or Aljuhani in the home or other location of the parent's choosing. Developmental testing in the home will be free of charge, and test information will be discussed with parents during the testing session and then uploaded into EPIC for other care providers.

Payment

No payment will be provided in this trial.

Data Analysis

For safety we will compare HR changes from baseline to during taVNS within subjects. We will compare treatment before and after the taVNS-paired feeding treatment for the rate of feeding volume increase, and days to full po feeds. We will use repeated measures ANOVA to analyze repeated measures such as HR over 5 minutes and mean daily feeding volumes in 7-10 day epochs. We will analyze the number of infants who reach full feeds vs requiring G-tube during taVNS-paired feeding, between once and twice daily stimulation by Chi-squared. We will assess differences in kurtosis parameters from pre-to post taVNS scans by paired t-test, and between those that reach full oral feeds vs those that require a Gtube by unpaired t-test.

Estimated Difficulties, Limitations and Time Frames

Estimated Difficulties. Many of the initial parameters for taVNS in humans, such as frequency and pulse width, were accomplished in trials at MUSC by Badran et al. that were approved by the IRB. In one sense, we will be shrinking many of the components into miniature form to deliver stimulation to neonates and infants. In spite of the clear differences in size and skin between the neonatal and adult ear, the custom electrodes have been fitted to several different weights of infants (2.5-4.0 kg).

Limitations. We will not be delivering a SHAM stimulation paradigm (electrodes and Digitizer with appearance of taVNs treatment without actual current). Our goal is to determine safety and feasibility of active treatment with a dose response in order to investigate whether this should be administered in a larger randomized control trial. In this respect, the comparison we lose in not having a SHAM stimulation is offset by the validation we will receive in the safety and feasibility of active treatment. We will compare our overall results with characteristics of infants who required a Gtube from Dr Chapman and Ryan's publications.

Estimated Time Frames. The duration of the study will likely be ~4 years, with 2 years of active enrollment and 18 months of follow-up.

E. PROTECTION OF HUMAN SUBJECTS

1. RISKS TO THE SUBJECTS

The human subjects involved in this research will be preterm neonates or infants with critical illnesses that preclude oral feeding, such as HIE. Inclusion criteria involves 2 main groups. The first is comprised of typical pre-term, high risk neonates who are in the convalescent near and post-term period and learning to po feed. The second is comprised of neonates that present after severe medical illnesses or brain injury. In both cases, the infants can only be enrolled if they are stable, not making progress with OT training, and are in discussion for Gtube placement. Participants will be excluded if they have major unrepaired congenital abnormalities that limit volume of feeds, cardiomyopathy, significant autonomic instability, such as repetitive, unresolved bradycardia or apnea, or use a continuous positive airway pressure (CPAP) device or other form of high respiratory support preventing them from breathing without assistance.

Targeted/Planned Enrollment Table

Total Planned Enrollment: 40 babies (active)

TARGETED/PLANNED ENROLLMENT: Number of Subjects receiving active taVNS			
	Sex/Gender		
Ethnic Category	Females	Males	Total
Hispanic or Latino	4	5	9
Not Hispanic or Latino	15	16	31
Ethnic Category: Total of All Subjects*			

Racial Categories			
American Indian/Alaska Native			
Asian			
Native Hawaiian or Other Pacific Islander			
Black or African American	8	7	15
White	12	13	25
Racial Categories: Total of All Subjects*	20	20	40

**The "Ethnic Category: Total of All Subjects" must be equal to the "Racial Categories: Total of All Subjects".*

Volunteers of all ethnicities and racial categories will be accepted into this study protocol. No preference will be given based on race, gender or ethnicity. No vulnerable populations or special classes of subjects will be considered for participation.

b. Sources of Materials

Participants will be screened for eligibility in person by the PI (Jenkins). All participants enrolling in the study will be given an alphanumerical code that will be used to identify them. This code will be used to link participants to any identifying demographic information that is collected. Only the principal investigator and appropriate study staff will have access to the documents linking identifying information with alphanumerical codes. Infant data on medical conditions, significant clinical events including birth history, and postnatal course, as well as medications, and surgeries will be collected from EPIC.

c. Potential Risks

taVNS has been conducted in individuals under the age of 18. Moreover, TENS has been used in several studies in neonates and young infants as detailed below. A summary of potential risks in infants include the risk of skin irritation, discomfort, slightly decreased heart rate, and loss of confidentiality. The potential risks have been clearly outlined in the informed consent document. There are no alternative treatments, except the standard infant feeding with OT, speech or nurses.

<u>Potential Risks of taVNS</u>

taVNS is transcutaneous electrical nerve stimulation (TENS) of the auricular branch of the vagus nerve (ABVN) that innervates the ear. Although this novel therapeutic modality is still in the development and optimization process, risks are a combination of those to be expected by both the peripheral TENS and implantable cervical VNS.

TENS devices are FDA approved for pain relief and are available over the counter. The main risks associated with TENS are electrical hazards that may result in user discomfort or injury. The unit used

in these studies (Digitimer DS7AH) is a 510(k) cleared electrical stimulator that meets the rigorous electrical standards of the FDA. The Soterix© taVNS unit has been customized for our infants with a maximum current delivery of 2 mA and automatic shut-off after 120 seconds (See uploaded documents and letter from Soterix). The customized Soterix unit will be used in our BabyStrong device which has received Breakthrough Device designation from the FDA 12/8/2020. Skin irritation, redness, or inflammation may occur under the stimulating electrodes if TENS current is delivered for a prolonged period of time.

Two studies have used TENS on neonates to try to prevent or mitigate painful procedures (39-40). The TENS was administered at acupuncture sites with increasing current from 1-3.5 mA, and Hz from 2-10 in 30 healthy infants <3 days old. (39) In a subsequent study, this investigative team randomized 162 term neonates to TENS (3.5mA, 10Hz) or usual care to prevent pain from lancet heelsticks for blood sampling (40). These TENS parameters were safe and did not cause discomfort in the neonates.

TENs has also been used with passive stretching in neonatal torticollis at 8Hz, 0.2mA for 30 minutes continuously (41) and in brachial plexus nerve injuries starting at 6 weeks of age to improve motor function of the hand, in conjunction with constraint induced movement therapy (42). Neither study reported adverse effects of electrical stimulation. TENS has also been used in peroneal and sacral nerve stimulation for dysfunctional voiding and stooling (44-45). Finally, the routine sweat test employed in newborns and infants to confirm the diagnosis of cystic fibrosis uses transcutaneous electrical stimulation to deliver pilocarpine via iontophoresis (43). From the product brochure of the Nanoduct™ Neonatal Sweat Analysis System (Discovery Diagnostics, Canada), the nominal current is 0.5 (± 0.02) mA up to 5 mA for 2.5 minutes (± 0.2 Sec.).

Implantable cervical VNS is FDA approved for the treatment of treatment resistant depression and intractable epilepsy in children as young as 8 months (47-49). Cervical VNS has risks associated with the procedure of implanting the nerve, and the surgery. None of those apply here. VNS does have some minimal risks that are due to the actual stimulation of the nerve within the neck such as skin irritation. tVNS also has associated risks that may arise from the direct brain effects stimulating the vagus nerve. These theoretical risks associated with neuromodulation of the parasympathetic nervous system would also be applicable in the administration of noninvasive tVNS. They are the following: reduction of heart rate, blood pressure, and vasovagal syncope.

There have been dozens of studies in which tVNS has been used on humans, none of them reporting adverse events. A simple PubMed search for “transcutaneous vagus nerve stimulation” shows 24 peer-reviewed articles, 15 of which have been published within the past 3 years. See below table for 10 most recent publications on PubMed involving tVNS on the auricular branch of the vagus in humans:

Author, Year	**Aim (subject number)**	**Side effects/Risks/AEs**
Aihua et al. 2014	Epilepsy (n= 60)	Dizziness, drowsiness
Capone et al, 2014	Cortical excitability (n=10)	No modification of instantaneous HR, systolic BP, diastolic BP, and mean BP
Kreuzer et al, 2014	Tinnitus (n=50)	Twitching and pressure at electrode site
Rong et al, 2014	Epilepsy (n=144)	None reported

Kraus et al, 2013	tVNS/fMRI (n=16)	A bright, prickling sensation, twinge or stabbing pain
Rong et al, 2012	Depression (n=120)	None Reported – trial still under progress
Kreuzer et al, 2012	Safety Study (n=24)	In those subjects with no known pre-existing cardiac pathology, preliminary data do not indicate arrhythmic effects of tVNS
Busch et al, 2013	Pain (n=48)	No relevant alterations of cardiac or breathing activity or clinical relevant side effects were observed during t-VNS
Stefan et al, 2012	Epilepsy (n=10)	Hoarseness, headache, or constipation
Polak et al, 2009	Far field potentials (n=20)	Slight pain at electrode site

An extremely through review of all tVNS literature has been performed and no harm or adverse events have been observed and any side effects were resolved by decreasing current intensity. There also is currently a commercial tVNS device available for purchase on the European market (Cerbomed - Nemos device; www.cerbomed.com) that is marketed as a take-home treatment for epilepsy.

Not only does the current literature show a lack of harm done by tVNS, implantable cervical VNS also has a good safety record. According to Cyberonics, the company that supplies the cervical VNS implantable devices, there has been over 100,000 implanted patients being monitored by over 3,000 providing physicians. Most side effects range from alteration of voice, coughing, pharyngitis, hoarseness, headache, and nausea. Cardiac evaluations have been made on hundreds of VNS patients with no changes in cardiac function (Handforth et al, 1998; Sackeim et al, 2001b; Morris and Mueller, 1999) with long-term safety confirmed in recent large sample retrospective studies (Menascu et al, 2013; Ryvlin et al, 2014; Choi et al, 2013).

Bradycardia: Dr. Badran's own safety studies in adults and this pilot study in infants designate a small, measurable change in heart rate which is not clinically significant (-3 and+6 bpm). Given the minimal risk of both of these already FDA approved methods, we intend to show that taVNS will continue to be a very safe procedure in neonates.

2. ADEQUACY OF PROTECTION AGAINST RISKS

a. Recruitment and Informed Consent

This information is listed on the eIRB website.

b. Protection against Risk

The following risks are present with this study: Risk of skin irritation, risk of discomfort, risk of slightly decreased heart rate, and risk of loss of confidentiality.

Risk of skin irritation: we will monitor the skin on the inner aspect of the left ear before and after each session, and will switch to the right ear if redness is present, and decrease the stimulation if redness persists.

Risk of discomfort: We expect to be able to use the pain ratings scales to identify the perception of tingling versus pain with taVNS in these infants. We will use facial expression change, fidgety movements and infant behavioral discomfort using our standard neonatal pain scale (NIPS) with the bedside nurse at the start and after 5 minutes of taVNS, at 10 minutes and at the end of treatment. We will protect against the risk of discomfort by close monitoring and by the determination of perceptual threshold using the protocol outlined above.

We will hold the treatment for a NIPS score of >3, until discomfort subsides, and resume at a lower stimulus level if this level of discomfort recurs. We do not expect the infant to cry at this level, but show discomfort in brow furrowing, grimace, etc. Feeding is generally comforting for infants, and we expect to see minimal pain response to treatment. Infants are fed sidelying in the nursery. We will position the baby so that the left ear is facing up, and we can monitor the site and pain without disturbing the feeding process unduly.

Risk of slightly decreased heart rate: we will monitor HR, respiratory rate and oxygen saturation continually during the feeding to monitor these physiologic responses to taVNS-paired feeding. The infants' vital signs are constantly monitored as standard of care while in the nursery. We will print out values at baseline, and changes with feeding and taVNS-paired feeding. Normal heart rate is 120-170 beats per minute in preterm infants feeding, and 110-140 bpm in term HIE infants. As HR usually accelerates with motor activity of feeding, we do not expect significant bradycardic HR changes during the 'on' pulse. However, if the HR decreases to <100bpm during 'on' pulse, or if HR rebound is > 190bpm during 'off' pulse, we will hold treatment and start again at a lower stimulation level.

Risk of loss of confidentiality: We will assign study numbers to each participant, keep CRF and consent forms in locked cabinets in locked offices. Database files with names and contact information will be password protected and stored on the MUSC Pediatric server. All other data files related to this study will be identified by participant number only, without link to standard identifiers. We will publish the aggregate data only. MRI files are stored by participant study number and date of scan.

3. POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECTS AND OTHERS

taVNS paired with oromotor feeding training may enhance feeding skills, and enable the infants to learn this task more quickly and be discharged from hospital sooner. The treatment could potentially help avoid placement of a G-tube, but we will not be able to determine this from this dose-finding study. The treatment may also help with development of other motor skills, which we will assess by neurodevelopmental tests. We may also discover that taVNS-paired feed improves swallowing function, but even if we do not show any change in swallowing function, this data will support the safety of taVNS in neonates and infants. However, there may be no direct benefit to the participant, but we will gain knowledge about taVNS in infants, and strategies to improve oromotor skill training.

4. IMPORTANCE OF THE KNOWLEDGE TO BE GAINED

This trial could demonstrate the concept of “targeted plasticity” that could be used to enhance deficient networks in the preterm neonatal central nervous system. Behaviors integral to development such as feeding are impaired in this cohort, and if we can accelerate the learning of this behavior, we would greatly impact the recovery times and growth abilities of preterm or injured neonates. A positive connection between taVNS and learned motor function at such a critical time of development would contribute significantly to the field of neuronal plasticity and learned behaviors in neonates.
If taVNS is shown to improve swallowing function, then this research may open up an entirely new treatment modality for these and other patients, such as post-stroke patients, who have swallowing problems.

5. SUBJECT SAFETY AND MINIMIZING RISKS (Data and Safety Monitoring Plan)

Introduction

The participants who receive active taVNS involved with this proposal will be neonates whose participation is agreed to by their guardians in written consent. The principal investigator will be responsible for monitoring the safety of the proposed experiments. She will also execute the Data Safety Management (DSM) plan and provide any necessary progress reports to the IRB, including but not limited to subject demographics, recruitment rates, retention rates, quality assurance issues, and adverse events or significant adverse events.

Data and Safety Monitoring Plan

Dr. Jenkins or her designee, should she be unavailable, will be responsible for reporting all unanticipated problems or AEs to the IRB. The PI will be present for each patient and record AE's real time. Dr. George will be responsible for reporting any unanticipated device-related AE's to FDA. All screening data will be kept in a binder in the locked office in Dr Jenkins office. Screening data collected from participants who do not qualify for the study will be securely destroyed.

Dr. Mark George, who is a board certified neurologist and psychiatrist, and Dr Badran will oversee the use of taVNS. Dr. Jenkins will also personally attend the sessions to monitor safety and adverse events.

An independent Safety Monitoring Committee (SMC) will be formed to advise the study investigators. The SMC will review and evaluate accumulated study data to ensure safety. They also will make recommendations concerning continuation, modification, or termination of any of the taVNS studies. It will be composed of Dr. David Annibale, neonatologist; Dr. Jeff Borckardt, MUSC associate professor and assistant provost with extensive VNS, TMS, and tDCS experience. Patty Coker-Bolt, OT PhD, infant feeding expert, will be available to discuss subjective and objective measures of infant response during the taVNS experiments. Paige Merrill, clinical OT and infant feeding expert, is not involved with the study, but will be giving many of the feeds during the tVNS sessions, and may also be called upon for impressions of safety and infant response, if the safety committee requires it.

Data will be discussed after the first five infants, and then again after 10 infants. The SMC will be notified immediately of any and all SAE's. We will report to the IRB the number of treatments held for

HR or redness, discomfort.

Dr. Jenkins will obtain informed consent, during which participants' guardians are fully advised on the research procedures to be used, the amount of time required of them, the possible risks and benefits of the procedures, their right to refuse their infant's participation in the study without prejudice, their right to terminate participation of their infant at any moment without prejudice, and the name and telephone number of the principal investigator.

Electronic Consent will be used for situations when the parent (or Legally Authorized Representative) is at another hospital and cannot travel to MUSC to provide consent in person. We will use the approved REDcap system to obtain consent. Dr. Jenkins or other approved study personnel will go over the entire consent document on the phone with the parent to ensure comprehension and to confirm identity. Parent will be given a copy of the completed, signed consent form in person or by email or fax.

Legal guardians will give informed consent. However, the Department of Social services may have custody of infant, but require the parent or foster parent to consent for all medical procedures.

Regarding confidentiality, subjects' guardians are informed that the information they provide, as well as participation in the study, will be kept strictly confidential, with access limited to the research staff. All paper records (consents, CRFs, study tools) will be kept in Dr Jenkins' locked office, in a locked cabinet. The identity of subjects in databases will be protected with alphanumeric codes. All data will be kept in locked file cabinets or on secure servers designed for use and access by Brain Stimulation and Neonatology Lab members only.

*Clinical Trials
Unless required by a funding agency, this study will not be registered on clinicaltrials.gov, as it will be considered a Phase 0 trial, used for assuring safety and feasibility of the taVNS-paired feeding treatment. The potential for intellectual property development and patent application by MUSC will preclude disclosure of this invention.

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G.CONSULTANTS

N/A

H. FACILITES AVAILABILITY

These studies will be conducted in the NICUs, clinical units on the 5th floor of Shawn Jenkins Children's Hospital. The portable taVNS devise will be brought to the nursery room for each taVNS session. The Brain Stimulation Lab (BSL) is a series of labs and offices (>3000 square feet) primarily located on the 5th floor of the Institute of Psychiatry (IOP). BSL studies use electromagnetic approaches as either research tools investigating neuroscience questions or as investigational or FDA approved treatments for brain diseases. Techniques actively being used by BSL researchers and

their collaborators include: transcranial magnetic stimulation (TMS), vagus nerve stimulation (VNS), transcranial direct current stimulation (tDCS), electroconvulsive therapy (ECT), deep brain stimulation (DBS) and epidural cortical stimulation (epCS). ECT, VNS and TMS are clinical services offered within the BSL. BSL researchers at MUSC were the first in the world (in 1998) to implant VNS devices in patients with major depression who had not adequately responded to traditional antidepressants.

I. INVESTIGATOR BROCHURE

N/A

J.APPENDIX

N/A