PROTOCOL TITLE:

BabyStrong taVNS-Paired Bottle Feeding to Improve Oral Feeding

PRINCIPAL INVESTIGATOR:

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1.0 Objectives / Specific Aims

Feeding difficulty is the primary reason for prolonged hospitalization for preterm and term infants in the US,and often requires significant interventions including gastrostomy tube (G-tube) placement to attain adequate nutrition for discharge home.[1] Preterm infants who have CNS dysmaturation or other brain injury and term infants who have had hypoxic ischemic brain injury (HIE) commonly have oromotor dyscoordination, resulting in feeding difficulty. Suck-swallow-breathe is a complex sensory-motor sequence that infants with brain injury must learn and master to take all feeds by mouth (po) and be discharged home. Current standard of care involves daily oral stimulation or feeding practice with therapists, nurses or parents. The scope of the problem is significant. A recent study by the Neonatal Research Network of 6017 moderately preterm infants shows that 37% remain hospitalized beyond 36 weeks due to feeding difficulty.[2] Correspondingly, G-tube placement has doubled from 2002 to 2012 .[3] Among preterm infants born between 23-33 weeks gestation, 5.5% of had G- tubes placed for feeding difficulties.[4] Novel therapies are needed to improve oromotor learning and decrease hospital length of stay for these infants at high risk of developmental delay.

Transcutaneous auricular vagus nerve stimulation (taVNS) is a non-invasive form of vagus nerve stimulation, which facilitates activity-dependent neuroplasticity and improves functional motor outcomes when paired with behavioral interventions in adults post stroke.[5, 6] In a first-in-infants pilot study we paired stimulation (taVNS) with bottle feeding to enhance motor learning of feeding in preterm or term HIE infants, who were failing oral feeds.[7] Out of 30 infants to date whose clinical treatment plan was to have immediate placement of a G-tube, 52% were able to achieve full oral feeds with taVNS.[8] We observed no adverse effects of pain or bradycardia. Additionally, infants treated twice daily required less time to full po feeds than infants treated oncedaily, without safety risk. Heart rate (HR) decreased transiently at stimulation onset, which served as a reliable marker of vagus target engagement. taVNS-paired feeding was well accepted by parents and care providers.

During this pilot trial, our team developed a custom closed-loop taVNS-paired feeding system: a bulky analog system consisting of an EMG system paired with a computerized electrical stimulation system, mountedon a cart, and connected to wired electrodes.[9] For further clinical trial testing and commercialization, we need aless expensive compact set-up that is easy to use. The company, FRD Accel, will collaborate with Soterix, a manufacturer of electronic pulse generators (EPG) to develop this product with the taVNS pioneers at Medical University of South Carolina and test it in infants: Dr. Jenkins, a clinical neonatologist and translational scientist, Dr. Badran, an expert in the technical development of taVNS, and Dr. George, a mentor in early taVNS development with a record of moving multiple neuromodulation-based therapies through FDA approval and market acceptance. FRD Accel will develop and gather clinical data on a portable, switch-triggered taVNS system in this phase I proposal to include in our FDA application.

Aim 1. Develop the BabyStrong taVNS-paired bottle system. The device will feature an on/off switch on a sleeve designed to slip onto any bottle, a wired connection to a small, programmable EPG to deliver pulsedelectrical stimulation in 0.1mA increments, and wired ear electrodes. In aim 1 we will:

1) **Bench test the device for accuracy** of microcurrent delivery in small (0.1mA) increments;
2) **Verify target engagement** with reproducible HR decrease with active stimulation in adult volunteers;
3) **Refine the system** with feedback from parent advisor and nurses on the ease of

operability of theswitch-on-sleeve design, and the EPG and ear electrodes.

Criteria for success for Aim 1: Confirmation of reliable waveform (0.1mA increments) using benchtop oscilloscope, & passing electrical stress tests on all components; HR decrease of 5-10bpm with onset of active stimulation in adult volunteers, indicating target engagement; Approval of design by parent advisor and nurses.

Aim 2. Conduct a small-scale safety and efficacy study of the BabyStrong portable taVNS feeding system. We will test the prototype BabyStrong feeding system using active (n=10) and sham (n=10) taVNS ininfants with twice daily A or B treatment for 10 days, with cross over to B/A treatment if there is no progress with feeds. The treatment assignment will be blinded to care providers and parents. We will compare daily po volumes over 10 days prior and the 10 days of treatment. Safety measures will be bradycardia and discomfort.

Criteria for success of BabyStrong feeding system: No sustained increase in discomfort scores or bradycardia; attainment of full oral feeds in 50% ofinfants.

Success of Phase I aims will position us well for Phase II and a pivotal RCT clinical trial with cross-over design, to determine if the BabyStrong system allows preterm and term infants with feeding failure to be discharged sooner and avoid costly complications as well as prolonged hospital stays. If successful, the BabyStrong device could have significant impact on the infants, their families, and hospital costs, as well as set the stage for greater use of taVNS targeted plasticity in neurodevelopmental disorders like cerebral palsy.

2.0 Background

Oromotor dyscoordination is common in preterm infants and in term infants who suffer HIE. Current standard of care consists of an occupational therapist working with infants once daily to encourage early feeding behaviors as early as 31 weeks gestational age (GA) or within a week of HIE birth. Therapists safely guide learning this motor skill and ensure that nipple flow rate and frequency of oral feeding do not tax infant physiology too greatly. Even with this therapy, feeding difficulty is the primary reason for delayed discharge [1]. In a recent Neonatal Research Network study, 37% of 6017 moderately preterm infants remain hospitalized beyond 36 weeks GA due to feeding difficulty [2]. These infants lose valuable developmental time in the hospital and have the highest risk for adverse neurodevelopmental outcomes.

If infants do not master this motor skill by late term age (42 weeks GA), they receive a G-tube for direct gastric feeding in order to be discharged to home. For example, in the Medical University of South Carolina (MUSC)'s NICU, an average of 40 G-tubes per year were placed from 2012-2016. MUSC's experience mirrors national data, which shows a doubling of G-tube insertion rates in infants from 2002 to 2012 [3]. In another recent study, 5.5% of 12,621 preterm infants born between 23-33 weeks gestation had G-tubes placed for feeding difficulties[4]. This procedure exposes very young infants to anesthesia risks and has a high complication rate with recurrent emergency room visits and hospitalizations, adding to the cost burden for both the family and hospital. The G-tube also reinforces parental perception that their child is not normal and has a more limited developmental potential than a 'normal' child.

There is a clear unmet clinical need for new therapies that increase feeding motor development, accelerate time to discharge, and ultimately obviate G-tube insertion.

Any therapy that facilitates motor learning and enhances feeding skills would have a significant impact for infants who fail feeding rehabilitation. In addition, decreasing time to full oral feeds would offer significant costs savings for both hospitals and families. Decreasing gastrostomy tube placement (cost $15,000) and length of stay ($3000/day) due to feeding delays are major objectives of this technology.

With feeding delays in approximately 20% of 380,000 preterm infants and 4,000 term infants born with HIE in the US per year, as well as 76% of late preterm infants [10], this therapy may translate to a large number of infants and impact their outcomes. Moreover, if the portable taVNS system is successful in the targeted motor behavior of feeding, we plan to extend use of these devices and early neuromodulatory therapy in high-risk infants to prevent or mitigate other life-long motor problems, such as cerebral palsy. Thus, developing a commercial taVNS system for neonates and infants will provide a foundation for applying these devices in infants at high risk for motor problems who have few alternative treatments.

Etiology of feeding failure and relevance to our approach: Why do neonates with brain injury and some preterm infants fail to master oral feeding? Feeding involves sensorimotor integration of a complex sequence of sucking and swallowing, within a respiratory rate of 20-40 times a minute. Furthermore, feeding is the first sequential motor task that neonates must learn. The vagus and other nerves innervate muscles of the mouth, mandible, pharynx, and esophagus involved in feeding [11-14]. CNS injuries from brain dysmaturity in preterm infants[15] or HIE injury in term infants, interrupt the developmental trajectory of vagal and other neural circuits[15,]

Abnormal connections[16], along with decreased populations of myelinating cells and inter-neurons, result in less myelination and fewer brainstem-cortical connections, and reduced corticobulbar regulation of both vagal activity and the muscles of the face, head, and neck[14, 17]. In spite of developmental plasticity, the immature brain still has some of the worst outcomes following significant insult, and difficulty feeding is frequently the first manifestation of later motor and cognitive impairments[16]. Additionally, key sensorimotor cortical connections require activation during critical developmental windows, as for vision and hearing/language. A critical window is also postulated for oromotor skills [18]. If illness delays feeding attempts, the feeding mechanism must be taught through rehabilitation, when it should be a normal reflex.

Neuroplasticity may be harnessed to improve outcomes and perhaps re-open developmental windows by activating neural connections or recruiting neurons as work-arounds for damaged circuits. Importantly, even with significant brain injury, we know that delivering neuromodulation during a task can harness activity- dependent plasticity and lead to improved, near-normal outcomes. In animal studies, pairing VNS with rehabilitative training increases cortical reorganization and functional outcomes after stroke or CNS injury, enhancing this activity-dependent plasticity [5, 19-22]. In April 2020, an FDA pivotal study of cervical-VNS paired with rehabilitation for adult stroke, reported clinically and statistically significant improvement with active paired VNS compared to sham (Microtransponder, MSG on DSMB). *Thus, the data in both animals and adult humans provide substantial evidence in support of the scientific premise of this proposal, that VNS paired with a stimulus improves functioning* [5, 19, 21-25].

Dr. Badran has also shown via fMRI in adults that noninvasive taVNS activates key sensorimotor areas, including thalami and basal ganglia that are also areas of injury in HIE and preterm brain injury[26]. Activating the afferent vagal fibers in the dorsal nucleus and locus coeruleus with an associated stimulus results in secretion of norepinephrine[27], a high-alert signal that helps re-establish normal connections if the vagus nerve is activated concurrently with a stimulus, whether **sensory** (tinnitus [28-30]) or **motor** (upper limb after stroke [5, 31]).

Since suck-swallow is a complex sensorimotor sequence, both central vagus targets may be affected by taVNS during feeding to reinforce motor pattern learning. Furthermore, the non-invasive taVNS approach may be particularly effective in infants, whose developmental and reparative plasticity is greater than in adults.

3.0 Intervention to be studied

Babystrong device: For this proposal we will use two potential prototypes for the BabyStrong system: 1) an FDA cleared taVNS unit, with Neolead® electrode in front of the tragus and

custom ear electrode for infant sized ears at the inner tragus or cymba conchae, wired trigger control of stimulation with a trigger button or direct input in the taVNS unit; 2) an FDA cleared taVNS unit, stimulating on the vagus stimulation channel, with custom electrode at the cymba conchae with return to the mastoid and direct wired control of stimulation vis the taVNS unit.

These two different TENS units are FDA-cleared for clinical research and will serve as the electronic pulse generators, manufactured in compliance with ISO 13485 in a Medical Device Single Audit Certified (MDSAP) certified and FDA registered facilities. They both possess constant impedance readings to indicate loss of electrode contact.

Ear Electrodes: We will use a custom 3-D printed ear electrode with carbon conductive material for the inner tragus electrode, and a standard Micro Neolead® hydrogel return electrode at the outer tragus (used on neonates in the NICU), and wired connection to the EPG, for the inner tragus or cymba conchae to outer tragus group. For the second prototype, the ear electrode with be used with stimulation at the cymba conchae and return to the mastoid.

Operation of the EPG will be performed as follows, using the same protocol as in our pilot trial:

a. To determine the PT, we will press the increase button in 0.1mA increments until we observe for infant detection of the tingling, with shoulder shrug or facial expression change, indicating the perceptual threshold (PT).
b. Once PT is determined, the current will be delivered at 0.1mA less than PT. Parameters can be reset for each session or for infant possibly sensing the tingling, by pressing *pause* button and pushing the increase or decrease button.

FDA designation*:* We recently received Breakthrough Device designation from the FDA for the BabyStrong device. Our infant taVNS feeding data,[16-17] our early FDA breakthrough device development for young infants, and other studies indicating positive results of taVNS paired with motor learning in adults,[26-32] support study of this neuromodulatory technique in infants with significant motor deficits of hemiplegia after perinatally acquired brain injury.

Non-Significant Risk device designation: We have IRB approval for two pilot studies in preterm and term infants using the custom modified devices and taVNS study procedures proposed in this application. The taVNS systems have non-significant risk device designations from MUSC's IRB in our pilot trials.

Proposed Trial of BabyStrong taVNS feeding system

We will conduct a small-scale safety and efficacy study of several versions of the prototype BabyStrong portable taVNS feeding system. We will test the prototypes using a maximum of active (n=10) or sham (n=10) taVNS in infants with twice daily A/ B treatment for 10days, with crossover to B/A treatment if there is no progress with feeds. Care providers, study personnel and parents will be blinded to treatment assignment.

4.0 Study Endpoints

Primary outcome: We will compare the numbers of infants who attain full oral feeds (> or = 130ml/kg/d with weight gain). We do not anticipate having power to compare any outcome with valid statistical measures.

Primary Safety Outcomes: Bradycardia (HR<80bpm for 5 seconds); discomfort measured by Neonatal andInfant Pain scale (NIPS) >3 point change, equivalent to pain with heelstick for blood drawing [34, 35].

5.0 Inclusion and Exclusion Criteria/ Study Population

Inclusion criteria: Infants born at any GA, failing oral feeds after trying to learn feeding for 2 weeks if term, and 4 weeks if preterm, who are deemed safe to po every feed without volume limitations by OT/SLP, and who the clinical team has determined will likely need a G-tube.

Exclusion criteria: Cardiomyopathy, unstable bradycardia, significant respiratory support, or infants of poorly controlled diabetic mothers, defined by obstetrical care providers and HgbA1C>5.6% or ketonuria.

6.0 Number of Subjects

We will enroll up to 20 infants.

7.0 Setting

The neonatal intensive care unit at Shawn Jenkins Children's Hospital, MUSC.

8.0 Recruitment Methods

The clinical team will refer parents for participation if their infants are failing oral feeds and are approaching G-tube placement per unit guidelines. Dr. Jenkins will also screen EPIC (chart review) for infants in the nursery with known brain injury that may have poor feeding, such as perinatal arterial stroke, HIE, severe IVH, PVL or cerebellar infarct, regardless of sex or race.

After screening and consent we will randomize infants to receive either active or sham taVNS. The assignment will be blinded to care providers, investigators and parents. Recruitment of 20 infants in 9 months will require enrolling 2-3 infants/mo. In the pilot study we frequently were unable to accommodate all requests, due to equipment limitations. With several BabyStrong units, we anticipate no problems completing enrollment.

Infants of all ethnicities and racial categories will be accepted into this study protocol. No preference will be given based on race, gender or ethnicity. Young infants who had perinatal complications leading to perinatally acquired brain injury are **vulnerable** populations and are the target of this proposal.

9.0 Consent Process

The clinical team will inform the parents of the potential to participate in the study. Dr. Jenkins or the study coordinator will then explain the study to the parents and will obtain written informed consent in the NICU or E-consent if parents are not from the immediate area, have transportation problems, or are unavailable. They will provide a patient handout used in the pilot trial to help explain the BabyStrong taVNS system and review the consent form with all parents. We will not have a Spanish language consent.

10.0 Study Design / Methods

Phase I Protocol:

MRI scan: We will obtain an unsedated diffusion scan on our clinical Siemens 3T MRI prior to initiating treatment and at the end of the 10day treatment period for all infants (2 scans if no cross over) and again at the end of the 7d alternative treatment if applicable (3 total scans for those infants who receive cross-over treatment).

Active taVNS treatment: The study team will determine the PT daily, then the nurse, therapist or parent will deliver active taVNS at 0.1mA less than the PT, with one of the BabyStrong prototypes during 2 bottle feeds a day. We will record the HR, NIPS scores by nurses before, during taVNS, and whether current is decreased for NIPS.

Sham treatment will use one of the BabyStrong prototypes via on/off switch as for active taVNS. We will test the PT with active stimulation, as care providers expect a brief HR decrease. We will then program a sham setting on the TENS unit to deliver no current after the PT is determined. We will check integrity of sham protocol by de-identified survey of nurses and parents, asking which treatment condition they thought the infant had received.

Cross–over treatment: If progress in daily oral feeding volumes is <4ml/kg/d (based on response rate in pilot trial) after 10 days with either treatment A or B, we will offer cross-over to the alternate treatment. If no further progress is noted after 7 days on alternate treatment, parents and the clinical team will arrange for a G- tube. Any parent can decide to withdraw the baby from the study at any time.

Breast feeding will be allowed, and we can stimulate with breast feeding, if the parent desires. We will offer bottle supplementation ad lib post-BF, and count this as a full volume feed, per standard clinical practice.

Training for safety using a variety of feeders: All individuals, including pediatric speech and languagepathologist (SLP), OT, nurses and parents who feed infants using this device will undergo training on the device operation by study staff. Research staff will supervise all feeders during the taVNS feedings. All feeds will be done in the NICU

The alternative treatment is the usual and customary occupational or speech therapy services which the infant has already been receiving, or to get a G-tube.

Safety measures:

We will monitor for and mitigate following risks are present with this study: Risk of skin irritation, risk of discomfort, risk of bradycardia, and risk of loss of confidentiality.: We will monitor for bradycardia (HR<80bpm), as all infants are on cardiorespiratory monitoring in the NICU; We will monitor for skin irritation at left ear daily.

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 will protect against the risk of discomfort by close monitoring. We will measure infant discomfort using the NICU standard scoring tool, the NIPS (Neonatal Infant Pain Scale) recording at the beginning, middle and end of taVNS session. If we observe a sustained >3 point ΔNIPS, we will decrease current by 0.1mA until discomfort resolves.

Risk of loss of confidentiality: We will check the infant's medical records to gather the following information: gestational age at birth, ventilator support, head ultrasound and MRI results, bottle feeding, infection, neonatal condition during labor and delivery, infant's treatments and conditions, and developmental progress. The neonatal chart by necessity contains some

information about mother during her pregnancy, and labor and delivery. We will collect mother's information if it is pertinent to infant's condition, including chorioamnionitis, abruption, fetal movement, bleeding, preterm labor, infections, medications,

We will assign study numbers to each participant, keep CRF and consent forms 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.

11.0 Specimen Collection and Banking

MRI raw data will not be analyzed and used solely in this trial due to small study numbers. MRI data will be stored for future use and combined with MRI data from other pilot studies for potential statistical analysis. Dr. Jenkins and her team will store this data in a password protected file on the pediatric server, behind MUSC's firewall. Future use will include analysis to describe the effect of taVNS treatment. They will be identified only by code. Only Drs. Jenkins, Badran and study personnel will have access to subject identities. Only coded identifiers will be used to store and linked to MRIs. If parents wish to withdraw the infant at any age prior to 18 years, they may request to do so in writing, and withdraw any future use of the child's MRI. Deidentified MRI raw data may be released to other parties involved in research per Data Sharing Plan.

12.0 Data Management

Power Analysis: We do not anticipate being powered to determine efficacy in feeding response rate, or test for A/B or B/A effects

Keeping Data Confidential: We will assign study numbers to each participant, keep CRF and consent forms 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.

13.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

Data and Safety Monitoring Plan

The participants who receive active/sham taVNS involved with this proposal will be infants 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. Dr. Jenkins 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. Dr. Jenkins or her designee, should she be unavailable, will be responsible for reporting all unanticipated problems or AEs to the IRB. The PI, Co-I or the Research Assistant will be present for patient visits and will report any perceived AE with Dr. Jenkins. Dr. Badran will be responsible for reporting any unanticipated device- related AE's to FRD Accel and the FDA. All screening data will be kept in a binder in Dr. Jenkins' locked 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 the taVNS system. Dr. Jenkins will be immediately available to the RA 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, as in the pilot trial. They also will make recommendations concerning continuation, modification, or termination of any of the taVNS studies. It will be composed of Dr. Steve Kautz PT PhD, NM4R PI, and Dr. Jeff Borckardt, MUSC associate professor psychiatry and assistant provost with extensive VNS, TMS, and tDCS experience. The SMC and IRB will be notified immediately of any and all SAE's.

Drs. Jenkins and study personnel will obtain informed consent for mother and infant to participate, 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.

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 Shawn Jenkins Children's Hospital. The identity of subjects in databases will be protected with alphanumeric codes. All data will be kept in locked office or on secure servers designed for use and access by Brain Stimulation and Neonatology Lab members only.

14.0 Withdrawal of Subjects

Parents may withdraw their infants from the taVNS procedures at any time. If a parent wishes to withdraw from the study entirely, then no further data will be collected on the patient, but safety and treatment data up to that point will be retained under deidentified study number. Infants may be withdrawn without parental consent, if taVNS is poorly tolerated. If either occur, parents will be referred for G-tube with primary clinical team.

15.0 Risks to Subjects

Risks of taVNS

taVNS is transcutaneous electrical stimulation of the auricular branch of the vagus nerve that innervates the ear. Although this novel therapeutic modality is still in the development and optimization process, few risks are expected by the peripheral taVNS in adults and infants. 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 (Soterix) is a custom modified, FDA-cleared electrical stimulator that meets the standards of the FDA for investigational use. Skin irritation, redness, or inflammation may occur under the stimulating electrodes, but these have not been observed with short term use.

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 to 5 mA for 2.5 minutes (± 0.2 sec).

taVNS also has associated risks that may arise from the direct brain effects stimulating the vagus nerve. The transient decrease in heart rate is a well described risks associated with neuromodulation of the parasympathetic nervous system that we have also observed in infants in the administration of noninvasive taVNS. The decrease in heart rate of 20±10bpm from a baseline HR of 160bpm occurs within 30 seconds of onset of stimulation and resolves in 60 seconds.

There have been multiple studies in which taVNS has been used on humans, none of them reporting significant adverse events. A simple PubMed search for "transcutaneous auricular vagus nerve stimulation" shows more than 100 peer-reviewed articles, most of which have been published within the past 5 years. 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, the side effects of cervically implanted VNS (alteration of voice, coughing, pharyngitis, hoarseness, headache, and nausea), have not been reported with taVNS. Cardiac evaluations have been made on hundreds of VNS patients, and more recently taVNS 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).

We have performed taVNS paired with feeding in 34 infants in our pilot study in over 500 treatment sessions without adverse effects and with potential benefit, as detailed in the preliminary data for this proposal ("Non-invasive Brain stimulation to improve oromotor function in neonates", #67997). A summary of potential risks in infants include the risk of skin irritation, discomfort, transiently decreased heart rate, and loss of confidentiality. Bradycardia: Our own safety studies designate a transient measurable change in heart rate (-20 to 10bpm), lasting <60 seconds, which is not clinically significant in infants with baseline HR of 140- 160bpm in our pilot study (Badran BW, et al., Frontiers in Human Neuroscience 2020). We have used the custom Soterix EPG device for several of the latest patients in this study and observed no AEs. The Soterix unit was considerably easier to use than the Digitimer EPG, and the additional safety features should ensure that this version of the TENS device is as safe as the existing ones available over the counter.

Given the minimal risk of taVNS FDA approved/cleared devices, we anticipate that taVNS paired with CIMT will be a very safe procedure in infants. The potential risks will be clearly outlined in the informed consent document, as in our prior infant taVNS study. MUSC's IRB has previously determined our taVNS system to be minimal risk device.

Risk of MRI: MRI does not involve radiation, is safe in newborns, and is routinely obtained in infants after significant brain injury. MRI uses a magnet and radio waves to make diagnostic

medical images of the body. We swaddle infants after a feeding so that they will sleep, then they are placed on FDA-cleared vacuum swaddle device, used for clinical MRIs in infants. Earmuffs will be placed to block out the loud machine-like noise. We do not use sedation for infant MRIs. We will have pediatric neurologists read the MRIs and Dr. Jenkins will share this information the clinical team and with parents if we detect injury that was not already reported.

Risk of loss of confidentiality: We will assign study numbers to each participant, keep CRF and consent forms 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.

This study will involve research on vulnerable subjects: infants with motor delays and feeding problems, largely due to perinatal brain injury.

Unknown Risks: TENS stimulation of peripheral nerves is FDA approved and is considered very safe. Although taVNS is essentially TENS on the ear, it is still an experimental procedure that has not been approved by the FDA to improve outcome of infants with motor problems. Therefore, there may be risks and discomforts that we are not aware of.

16.0 Potential Benefits to Subjects or Others

The infants with perinatally acquired brain injury constitute a vulnerable population, but the procedures in this protocol are largely minimal risk procedures (MRI, developmental tests and motor assessments). taVNS also involves little risk: equivalent TENS units are widely available on the internet for home purchase and use in all ages.The potential benefit to the infant and their families from participating in this study is learning to feed faster or more effectively, but we are not sure taVNS will have any effect on feeding. However, the information gained from the study may help researchers learn about how to better stimulate brain function in infants and also whether and how to use taVNS to help with recovery from preterm birth or brain injury.

17.0 Sharing of Results with Subjects

Results of all motor skills test will be shared with parents as soon as the scoring is completed. Many infants have MRIs as part of work up for brain injury. MRIs will be read by pediatric neurologists and incidental findings discussed with clinical team and parents by Dr. Jenkins, if different than what has been previously known.

18.0 Drugs or Devices

The taVNS devices and electrodes, and hand-held trigger will be stored in the NICU in a locked cart when not in use, and in a cart in the participant's room when in daily use for a participant.

Study team members will be in-serviced on use of the taVNS unit, and the study team, Dr. Jenkins or Dr Badran will be available for questions about use of the device.

The taVNS units are based on taVNS devices that are FDA cleared for investigational use. They have been designated non-significant risk devices in neonates and infants, similar to taVNS used in "Non-invasive brain stimulation to improve oromotor function in neonates" Pro #67997, and "N-acetylycysteine and taVNS for oromotor rehabilitation in infants of diabetic mothers, Pro #103800.

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