PROTOCOL TITLE:

Optimization of Closed-loop Transcutaneous Auricular Vagus Nerve Stimulation (taVNS) as a Neurorehabilitation Tool

PRINCIPAL INVESTIGATOR:

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1.0 Objectives / Specific Aims

Invasive cervical vagus nerve stimulation (invasive cVNS) is a surgically invasive neuromodulation technique developed in the 1980's to treat intractable epilepsy and chronic refractory major depression[1, 2]. Invasive cVNS involves wrapping a cuff electrode around the left cervical bundle of the vagus nerve and has seen a reemergence in the past decade following several promising animal model findings demonstrating the ability to induce neuroplasticity in a target-dependent manner[3, 4]. In animal models, the temporal pairing of cVNS bursts paired with motor rehabilitation can restore pathologically insufficient neural activity post-stroke [3, 5-7] or correct maladaptive activity in tinnitus[8-10]. This intricate pairing of VNS and restorative behavioral intervention is known as "targeted plasticity" and is a promising approach to treatment of neuropsychiatric interventions, with potentially transformative potential in post stroke rehabilitation. Recently, a noninvasive alternative known as transcutaneous auricular vagus nerve stimulation (taVNS) has emerged as a promising alternative to conventionally implanted cVNS[11-16]. taVNS, however, targets the auricular branch of the vagus nerve, which innervates the human ear and activates the afferent and efferent vagal networks, allowing for a noninvasive, simple, and rapid translation of cervically implanted VNS findings.

Although paired taVNS rehab is promising, there is much work to do before translating this into the clinic. We to refine and develop closed loop taVNS, establish activity with key biomarkers, and show initial feasibility in a small clinical trial. For paired taVNS to succeed as a clinical treatment, it is critical to develop and refine a closed-loop taVNS platform that delivers stimulation concurrently during specific movements of the motor rehabilitation training. Aim 1 develops this novel motion-gated closed-loop system that delivers taVNS in synchrony with specific upper limb motor activation. Aim 2 will combine the development of the closed-loop system with our mechanistic understanding to explore an open-label pilot trail using closed-loop taVNS paired with task-specific training to determine the feasibility, safety, and potential effect size of this novel combination therapy. This work would provide the needed information to move closed loop taVNS into the realm of early phase clinical trials in rehabilitation.

In Aim 2 we propose combining taVNS with protocol-based task-specific training (TST), which is a form or motor rehabilitation therapy that increases the functional use and control of a paretic extremity [17-20]. TST improves functional outcomes and also induces neurophysiological changes in the ipsilesional motor cortex measured by functional neuroimaging and cortical excitability. Combining taVNS with the already established TST may rapidly accelerate and enhance the benefits of TST alone in a targeted neuroplasticity approach.

AIM 1: Establish a motion-activated, closed-loop taVNS platform for post-stroke motor recovery. Hypothesis: Individualized EMG recording during upper limb rehabilitation is feasible and will generate a robust, specific (<80%), and sensitive (>80%) motion signal to drive taVNS in a safe and automated fashion (n=5).

AIM 2: Determine target engagement and optimal taVNS dosing and effect size of taVNS-paired TST to improve upper limb motor recovery. Hypothesis: taVNS delivered to the left ear of chronic stroke patients will activate the vagal afferent network and will induce greater motor cortex BOLD activity in a paired motor task compared to unpaired and placebo stimulation. Pairing closed-loop taVNS with TST induces a targeted cortical neuroplasticity that facilitates the enhancement of TST effects in upper limb hemiparesis in chronic stroke patients. This open label exploratory study will determine safety, feasibility, and effect size to power future controlled trials (n=20).

2.0 Background

Nearly 800,000 people in the United States have a stroke annually, with motor impairments being the most common long-term functional disability. 85% of stroke cases result in reductions in upper limb function, and this has a severe negative impact on daily living[21, 22] Motor recovery is achieved most commonly through professionally assisted motor rehabilitation training, and experimentally through various robotic, virtual reality, and brain stimulation techniques. Many of these rehabilitation techniques attempt to restore pathologically insufficient neural activity post-stroke to regain function, as the stroke induces damage to brain tissue and subsequent reorganization of cortical motor representations in surrounding undamaged tissue [23, 24]. Vagus nerve stimulation (VNS) can enhance motor rehabilitation by increasing neuroplasticity and accelerating the restoration of neural activity and function when paired in a temporally synchronized manner. <u>This proposal aims to develop noninvasive taVNS as an enhancer of neuroplasticity and accelerate motor restoration in post-stroke rehabilitation</u>.

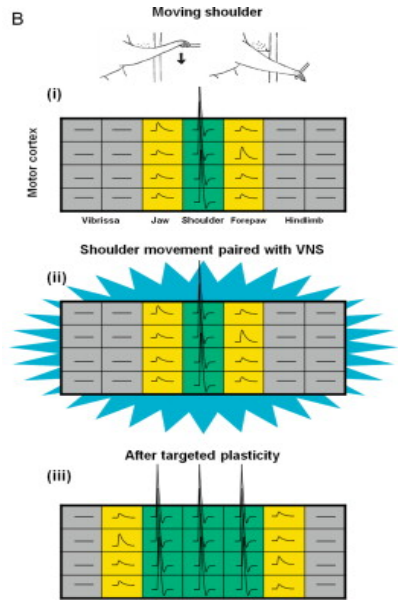


Figure 1. VNS-paired shoulder training facilitates cortical plasticity. Adapted from Hays et al, 2013

Vagus Nerve Stimulation (VNS) Facilitates Neuroplasticity:

Underlying basic fundamentals of neuroplasticity is the release of neuromodulators that influence this dynamic neural process. Plasticity may be endogenously influenced (via repeated behavioral training) or exogenously influenced (via noninvasive brain stimulation). One of these exogenous influencers of plasticity is vagus nerve stimulation (VNS), which delivers electricity to the vagus nerve, sending an afferent signal to the brainstem, activating the brain's primary source of norepinephrine (locus coeruleus) which results in broad downstream neuroplastic cellular effects. Lesions to locus coeruleus inhibit this noradrenergic-driven neuroplasticity[25, 26]. VNS is an influencer of neural plasticity and has been paired with various forms of training in animal models to restore deficient or aberrant neural activity[6, 7, 27, 28]. Most notably, rats with implanted VNS electrodes were concurrently stimulated while receiving rehabilitative training post-ischemic stroke induction. 100% of the rats receiving the paired intervention fully recovered forelimb function compared to 22% of rats in the control condition[6, 7]. Cortical reorganization in the VNS group restored pathologically inactive circuits (**Figure 1**). These findings have led to large scale clinical trials (Microtransponder, 2012) exploring the use of VNS-paired rehabilitation to restore upper limb function and are early positive indications of using paired-therapy for rehabilitation. The ongoing invasive cervical clinical trial will report out results in early 2020.

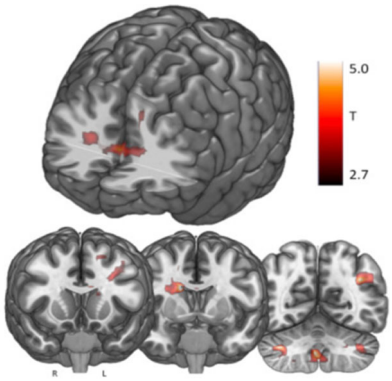


Figure 2. taVNS activates the vagal afferent network when compared to sham auricular stimulation. Badran et al. 2018

Transcutaneous Auricular Vagus Nerve Stimulation (taVNS) is a Noninvasive Alternative: The auricular branch of the vagus nerve (ABVN) spans from the main bundle of the vagus nerve and innervates the human ear and is the target of a novel form of noninvasive vagus nerve stimulation known as transcutaneous auricular vagus nerve stimulation (taVNS). Modern taVNS was perhaps first described by Ventureyra in 2000[11], with several more recent studies using this novel form of neuromodulation[14, 16, 29-34]. Dr. Badran (PI) and the team at the MUSC Brain Stimulation Lab have pioneered several of the early studies demonstrating that taVNS activates the afferent (parasympathetic) vagal system by using cardiac effects as a reliable biomarker to determine optimal stimulation parameters. Following the optimization of the parameter space, Dr. Badran conducted a novel concurrent taVNS/fMRI study exploring the direct brain effects of taVNS which revealed activation of the vagal afferent network induced by stimulation of the ABVN[13] (**Figure 2**).

These data confirm other groups' fMRI findings that taVNS elicits a similar neurophysiological signature as does conventional, implanted VNS. This has led to an emerging new form of brain stimulation – accessed via stimulation of the ABVN, which might enable a non-invasive translation of already FDA-approved invasive treatments for conventional VNS (epilepsy, major depression). This proposal will use taVNS as an inducer of cortical neuroplasticity, pairing taVNS with an already established motor rehabilitation paradigm, and ultimately accelerating the restoration of upper limb function in post-stroke patients. This proposal furthers the already established taVNS paradigm developed by Dr. Badran (PI) and allows for the investigation of target engagement of taVNS in a chronic stroke population; refining the method, testing short term biomarkers using fMRI to determine afferent cortical and subcortical activation induced by taVNS, and then showing feasibility in an open label small clinical trial.

3.0 Intervention to be studied

We will be using a closed-loop taVNS system that will deliver taVNS with upper-limb rehabilitation training in a chronic stroke population. The rational and overview is described below:

Motion-Activated, Closed-Loop taVNS May Enhance Upper Extremity Task-Specific Training (TST)

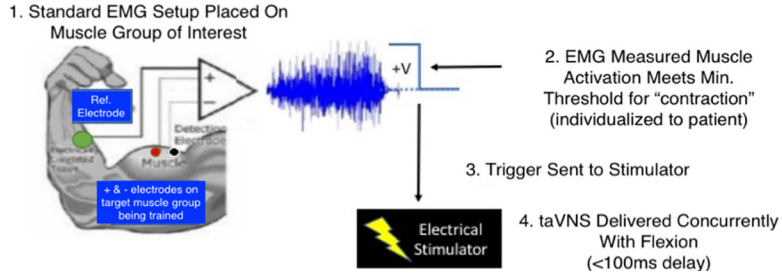


Figure 3. Closed-loop taVNS that is delivered via EMG electrodes placed on the target rehabilitation muscle group. This system would be individually calibrated to meet the patient's individual EMG levels and be computer driven, monitored by the therapist.

Rat models of VNS-paired therapy have demonstrated that timing is critical to the neuroplastic effects of VNS. Rehabilitation training without VNS takes a long time, and VNS alone fails to restore the behavior without the paired training. Delivering VNS immediately following training fails to enhance the training. Therefore, it is important to synchronize stimulation precisely with the paired training.

The second and third aims of our proposal pairs taVNS with upper extremity task-specific training (TST). TST involves a protocol-based in-person intensive practice of the paretic upper limb[17-20, 35]. This post-stroke rehabilitation training vigorously trains the paretic limb in activities relevant to daily life of

the patient. This training recruits muscle groups from the paretic limb (biceps, triceps, flexor carpi radialis, anterior & middle deltoid, etc), which can be used to trigger stimulation in a closed loop fashion. General motor activity can be sensed using large EMG recording array electrodes, rectified, integrated, and thresholded. This signal can then be used to trigger taVNS and deliver concurrent, closed-loop taVNS during motor rehabilitation training.

Aim 1 of this proposal will determine optimal upper limb targets for EMG array placement, sensitivity and specificity values for the system, and ultimately create a platform for taVNS-paired rehabilitation. The PI will use this system to establish brain effects of the system. Finally, we will then use this newly established system to conduct a small study exploring the use of the paired taVNS on motor recovery. We aim to optimize dosing (number of paired stimulation session per day, length of intervention) as well as determine potential effect size of the combined intervention and the neurophysiological effects associated with recovery. Aim 2 will use this system in a 2-arm open label clinical trial.

4.0 Study Endpoints

This table describes the data collected and the endpoints that will be utilized. The endpoints revolve around clinical assessments (scales administered by a therapist) at weekly increments and at follow-up (2 and 8 week follow-up). These outcomes include Fugl-Meyer Upper Extremity (FM-UE) assessment, Wolf Motor Function Test, Action Research Arm Test, and Motor Activity Log.

	Treatment Day					F/U Week	
	0	3	6	9	12	2	8
MRI	X				X		
Clinical Assessment	X	X	X	X	X	X	X
Kinematics	X				X		
Take-Home Actigraphy	Utilized During Treatment Phase					X	X

There will also be baseline and final kinematic assessments that will be used to track outcomes, however these are secondary measures.

More details about endpoints are described in the study design section.

5.0 Inclusion and Exclusion Criteria/ Study Population

Inclusion Criteria: Subjects must meet the following criteria to participate in this study: 18-80 years old with an ischemic or hemorrhagic stroke that occurred at least 6 months prior; completed conventional rehabilitation therapy at least one month prior; Unilateral limb weakness with Fugl Meyer-Upper Extremity Scale score less than or equal to 58 (out of 66).

All participants must have active wrist flexion/extension and active abduction/extension of thumb.

Exclusion Criteria: Subject who meets any of the following criteria will be excluded from the study: Primary intracerebral hematoma, or subarachnoid hemorrhage; ; Other concomitant neurological disorders affecting upper extremity motor function; Documented history of dementia before or after stroke; Documented history of uncontrolled depression or psychiatric disorder either before or after stroke which could affect their ability to participate in the experiment; Uncontrolled hypertension despite treatment, specifically SBP/DBP>=180/100mmHg at baseline; Contraindicated for MRI scanning.

Chronic stroke patients of all ethnic and racial categories will be accepted into this study protocol. No preference will be given based on race, gender or ethnicity. Pregnant females and children under the age of 18 will be excluded for safety reasons. No vulnerable populations or special classes of subjects will be considered for participation.

6.0 Number of Subjects

Specific Aim 1:

Total Planned Enrollment: 5

Specific Aim 2:

Total Planned Enrollment: 20

Chronic stroke patients of all ethnic and racial categories will be accepted into this study protocol. No preference will be given based on race, gender or ethnicity. Pregnant females and children under the age of 18 will be excluded for safety reasons. No vulnerable populations or special classes of subjects will be considered for participation.

7.0 Setting

Aim 1 of the study will be conducted at the MUSC College of Health Professions Building C Rehabilitation Laboratories.

Aim 2 of this study will be conducted both at MUSC 30 Bee St (for imaging) and at MUSC College of Health Professions Building C Rehabilitation Laboratories for rehabilitation training.

8.0 Recruitment Methods

Recruitment will be conducted by the study team utilizing the MUSC RESTORE database and MUSC's patient databases via a TriNetX export list to identify and contact prospective chronic stroke patients. A chart review will be conducted for research purposes. Potentially eligible patients will be identified. The potentially eligible patients in the PIs practice will be informed about the study as the PI feels is appropriate. Then potential patients who have agreed to be contacted for future research by logging their MUSC Research Permissions preferences in MyChart will be contacted by phone and invited to participate. All other patients will be contacted through their providers to be informed of the study if the provider feels it is appropriate. Participants will be contacted by any of the IRB approved study personnel and phone screened for eligibility.

9.0 Consent Process

Written informed consent will be obtained from the participant by the PI or designated study team personnel. Consent process will occur remotely via Doxy.me or at the MUSC Center for Health Professions building C (QBAR CORE). Only individuals who pass an initial phone eligibility screening will be eligible to consent remotely or in person.

eConsent Process: During an agreed upon time, the ICF/HIPAA form will be reviewed, and all questions will be answered with the potential participant via doxy.me teleconferencing. Once the consent form has been reviewed, the consent is signed in real time by research personnel and the participant. A copy of the signed ICF/HIPAA will be emailed or mailed to the participant.

10.0 Study Design / Methods

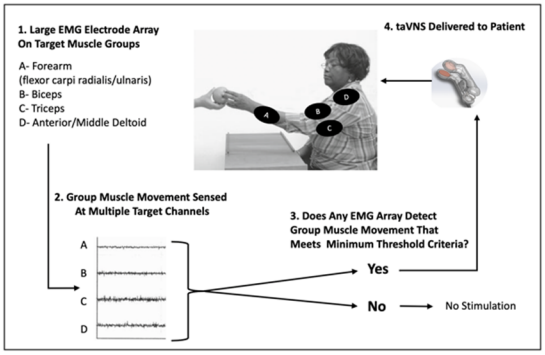
Specific Aim 1 – Establish a motion-activated, closed-loop taVNS platform for post-stroke motor recovery (n=5).

Prospective participants will be individuals who had a stroke > 6months prior to contact and must meet the following criteria to participate in this study: 18-80 years old with an ischemic or hemorrhagic stroke that occurred at least 6 months prior; completed conventional rehabilitation therapy at least one month prior; Unilateral limb weakness with Fugl Meyer-Upper Extremity Scale score less than or equal to 58 (out of 66).

For more information on tVNS screening please see the uploaded tVNS screening form. Written informed consent will be obtained from all participants prior to participation in the experimental paradigm.

Study Design

Participants will chronic stroke having unilateral limb will be enrolled first into the technology development Aim 1 study (n=5) in which participants will attend a single "mock-TST session". This session will serve as a closed-loop device optimization study, during which the closed-loop taVNS system will be tested.



Participants will attend one experimental visit (< 1hour) in which we will quantify General Motor Movement of their affected upper limb. We will use large EMG electrodes (Natus Medical, Inc, USA) affixed to 4 major upper limb areas (forearm, bicep, triceps, anterior/mid deltoid), with ground lead attached to ankle. Real-time EMG recording will be conducted using a 4 channel EMG recording system (Motion Lab Systems, Inc) and MATLAB. All EMGS will be event-locked to movement. Retrospective analysis of EMG size will be calculated and used to determine mean and minimum EMG size required for ANY motor movement time-locked to a known movement. The mean minimum EMG size will be used as a prospective threshold level for the motor-gated closed-loop platform used in Aim 2.

EMG Recording

Subjects will be connected to the following devices to measure their muscle activation:

EMG adhesive electrodes (made by Natus Medical) will be adhered to their upper limb in 4 target areas

(forearm, bicep, tricep, and deltoid). The ground electrode will be attached to the ankle. Electrodes will be connected to a National Instruments DAQ and data recorded and analyzed on MATLAB.

taVNS

Participants will receive taVNS stimulation to their left ear via electrodes attached to the anterior wall of the outer ear canal (25Hz, 500us pulse width, 200% PT). This will not be a therapeutic dose of stimulation, but rather a test of fit, comfort, and closed-loop functionality of the system in development.

We will analyze the EMG data and use it to determine optimal EMG thresholding levels for an automated sensing algorithm to be applied in Aim 2.

<u>This experiment in not intended to provide any benefit to the participant. This serves for a system development and refinement study. All participants in this aim will be able to enroll into Aim 2</u>

Specific Aim 2 – Determine target engagement and optimal taVNS dosing and effect size of taVNS-paired TST to improve upper limb motor recovery (n=20)

Screening

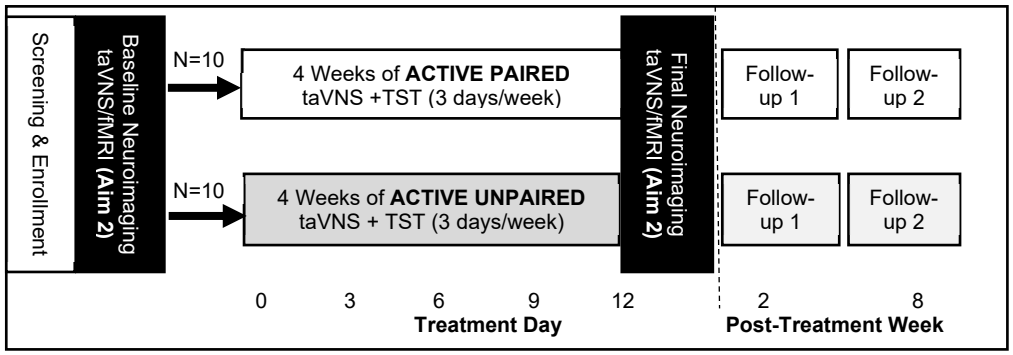
Prospective participants will be individuals who had a stroke > 6months prior to contact and must meet the following criteria to participate in this study: 18-80 years old with an ischemic or hemorrhagic stroke that occurred at least 6 months prior; completed conventional rehabilitation therapy at least one month prior; Unilateral limb weakness with Fugl Meyer-Upper Extremity Scale score less than or equal to 58 (out of 66)

For more information on tVNS screening please see the uploaded tVNS screening form. Written informed consent will be obtained from all participants prior to participation in the experimental paradigm.

Study Design

After successful validation of the closed-loop taVNS system is established, Aim 1 participants will be offered the ability to enroll into the Aim 2 & 3 studies of this proposal (n=20), an open-label pilot trial exploring the use of taVNS-paired vs unpaired motor rehabilitation. The primary goal is to determine safety, feasibility, and effect size of this paired therapy.

Participants enrolled in this study will receive upper limb motor rehabilitation (3days/week for 4 weeks. 1 hour/session) by a trained occupational therapist who is part of the college of health



professions rehabilitation CORE.

Neuroimaging (baseline and final)

All scanning will be performed on a 3T Siemens TIM Trio (Siemens, Erlangen, Germany). High-resolution T_1-weighted anatomical images will be acquired for each participant. Diffusion kurtosis imaging (DKI) or diffusion tensor imaging (DTI) scans will also be acquired for each participant.

Multiple Interleaved taNS/fMRI scan will be carried out each visit using a gradient echo-based echo-planar imaging (EPI) scan sequence. Biphasic electrical pulses (Current = 200% perceptual threshold; Pulse width = 500µs; Frequency = 25Hz) generated by a Digitimer DS7AH (Digitimer Ltd, Hertfordshire, England) located just outside of the scanner room will be delivered to a special non-ferromagnetic Ag/AgCl clip electrode via a 7-m shielded copper cable. The cable will enter the scanner room through a wave guard. A 100kΩ resister will be placed in series to the stimulating electrode to prevent artificially induced currents from the MRI scanner interacting with the cable and the ear electrodes.

Interleaved taVNS/fMRI pulses will be delivered to the same left tragus or earlobe location as conducted in Aim 1. E-Prime 2 software (Psychological Software Tools, Inc.; Sharpsburg, PA) will be used to trigger a series of tVNS pulses in a block design every 10th TR for exactly 10 TRs (e.g. stimulate during TRs 10-20, 30-40, 50-60, 70-80, 90-100, 110-120) in the scan sequence. A total of 6 interleaved stimulation blocks will be delivered over 8-10 runs. The entire scanning sequence should last less than an hour.

Task-specific training (motor rehabilitation)

All participants enrolled in the pilot dosing trial will receive TST by a trained therapist in the **QBAR CORE**. TST will be administered 3 times per week for 4 weeks (12 total sessions). Each session will last 1 hour, and participants will be connected to EMG electrode arrays that will sense movements while the affected limb is trained in functional tasks like reaching forward to hold a glass and drinking from it, picking up and using objects, fine motor task and dexterity training. During TST, taVNS will be delivered in a movement-gated fashion.

Collected Measures :

MRI: Participants will attend 2, 1-hour fMRI scans (baseline & post-treatment 18) at the MUSC Center of Biomedical Imaging 30 Bee St (**Part of the NI Core**). During these neuroimaging sessions, we will collect high resolution T1 structural scans, fMRI BOLD during sequential finger-tapping task (thumb to fifth finger) both paretic and nonparetic hands, followed by the concurrent taVNS/fMRI paradigm (lesioned and contralesional ear targets) developed by Dr. Badran here at MUSC.

Clinical Assessments: A therapist from the **QBAR CORE** will conduct weekly clinical assessments on all participants enrolled in this study, including at follow-up visits (2 & 8 weeks post-treatment). Fugl-Meyer Upper Extremity (FM-UE) assessment, Wolf Motor Function Test, Action Research Arm Test, and Motor Activity Log. Assessments will be video recorded and rated by the study's Treatment Occupational Therapist .

Overground Walking: Participants will walk indoors on and off a pressure-sensitive walkway at multiple

speeds, but we will not ask them to run or go faster than they feel comfortable and safe. The walkway measures the speed of walking and the size of steps. A physical therapist or trained assistant will walk beside participants across the walkway, and when possible, the participant will be attached to a mobile, ceiling-mounted safety support. They will be asked to walk several times across the walkway.

Treadmill Walking: We will test walking adaptability with a series of tasks. Participants will be asked to walk on a split-belt treadmill at varying speeds, but we will not ask them to run or go faster than they feel comfortable and safe. During all treadmill walking, participants will wear a harness attached to a device attached to the ceiling. This device can move with participants and will catch them and prevent a fall in case of a stumble or misstep. A therapist or trained assistant will stay along to provide any physical assistance participants may need.

Kinematics: Using resources in the **QBAR CORE**, a technician will perform brief kinematic assessments at baseline and immediately following the acute treatment phase of the rehabilitation training on both the affected and unaffected limb. Kinematics of hand and arm will be recorded using a motion capture system with 36 active markers and 10 cameras (PhaseSpace Inc., San Leandro, CA). We will capture movement during three specific tasks (reach, grasp, single joint movement) and quantify functional improvements comparing post-treatment kinematics to baseline as well as between affected and unaffected limbs.

Daily Life Activity Improvement Using Actigraphy: At-home upper limb motor activity of both the paretic and nonparetic limb will be collected using 2 independent actigraphy monitors (ActiGraph, Pensacola, FL), one on each arm, during the treatment phase of this trial. We will assess overall activity of upper limbs, as well as the ratio of activity of the paretic vs non-paretic arms.

Estimated Difficulties, Limitations and Time Frames

Estimated Difficulties. Many of the potential problems will manifest in the development of determining optimal EMG Quantification thresholding in chronic stroke patients in AIM 1.Although our lab is very experienced and we have resolved this type of scientific problem in the past with the administration of closed-loop TMS in the Brain Stimulation Lab.

	Treatment Day					F/U Week	
	0	3	6	9	12	2	8
MRI	X				X		
Clinical Assessment	X	X	X	X	X	X	X
Kinematics	X				X		
Take-Home Actigraphy	Utilized During Treatment Phase					X	X

Limitations. There are two potential limitations with these studies. The first is tolerability of electrical nerve stimulation of the tragus and earlobe. Although we do not anticipate any issues, drop-out may be an issue due to discomfort of the stimulation. If discomfort occurs we will decrease stimulating current amperage.

Estimated Time Frames. We plan on completing Aim 1 within 3 months. Specific Aim 2 will take approximately 18 months to complete (20 individuals attending rehabilitation training programs, with about 3 participants recruited every 3 months).

12.0 Data Management

Limited demographic and personal health history data will be collected during the phone screen in order to screen out participants with conditions that might confound the research or put them at risk for an adverse event. All screening data will be kept in a binder in the locked office in the Brain Stimulation Lab

as per the requirements of the IRB. Screening data collected from participants who do not qualify for the study will be securely destroyed.

Most of the data collected in this study are imaging data. These data will be collected from the 3T MRI scanner at 30 Bee Street. All imaging data will be automatically transferred to and stored on a password-protected, encrypted secure server that limits data access to personnel directly involved with the study. The data will be analyzed using standard imaging analysis software packages such as FSL and SPM. Quantitative outcome data will be stored and locked in individual subject folders and kept in the Brain Stimulation Lab and stored on a secured digital redcap platform.

Power Calculation: Assuming a threshold of of 0.05, 12 subjects are required to achieve 80% power at the single voxel level for typical fMRI BOLD activations in a tactile stimulation fMRI paradigm(42). Our primary fMRI findings in 17 individuals suggest that a n=20 within-subject design is appropriate to show taVNS functional changes in chronic stroke. In a recent review of published TST trials, it is suggested to provide 80% statistical power with a medium to large effect size (cohens d=0.8), changes in activity level would require at least 20 subjects in the treatment group and 20 subjects in the control group(43). With 20 participants in the treatment group, (10 per cell), we will be able to determine effect size in an open label pilot design. One of the primary goals of this project is to determine effect sizes for the planned follow-up grant proposal.

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

There are three areas in which safeguards to protect subjects from undue risk require discussion. These Include: (1) procedures used to obtain informed consent, (2) procedures used to ensure confidentiality of the subject data, and (3) procedures used to minimize possible risks associated with the laboratory procedures. Regarding informed consent, participants 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 participation in the study without prejudice, their right to terminate participation at any moment without prejudice, and the name and telephone number of the principal investigator. All subjects will be required to have capacity to consent. Regarding confidentiality, subjects are informed that the information they provide will be kept strictly confidential, with access limited to the research staff. Participation in the study will be treated as confidential, as will all records. The identity of subjects will be protected with alphanumeric codes. All data will be kept in locked file cabinets file cabinets or on secure servers designed for use and access by Brain Stimulation Lab members only.

An independent Data Safety Monitoring Board (DSMB) will be formed to advise the study investigators. The DSMB 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. Wayne Feng, MUSC neurologist with expertise in transcranial direct current stimulation (tDCS); Dr. Jeff Borckardt, MUSC associate professor and assistant provost with extensive VNS, TMS, and tDCS experience; Dr. Jon Halford, MUSC neurologist with expertise in VNS for epilepsy.

The DSMB will review all the data from AIM 1 and we will not proceed to AIM 2 without their approval. They will provide an interim safety analysis during AIM 2 (n=10 of the planned 20).

We do not anticipate any adverse events to occur in this study, however the experienced research team has a long-standing record of recording and reporting unanticipated adverse events to the IRB. We will report any adverse event within 48 hours to the IRB and to the DSMB.

15.0 Risks to Subjects

taVNS: taVNS is nothing more than transcutaneous electrical nerve stimulation (TENS) 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, 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. Skin irritation, redness, or inflammation may occur under the stimulating electrodes if TENS current is delivered for a prolonged period of time.

Implantable cervical VNS is FDA approved for the treatment of intractable epilepsy and treatment resistant depression. Cervical VNS has risks associated with the procedure of implanting the nerve, and the surgery. None of those apply here. taVNS does has some minimal risks that are due to the actual stimulation of the nerve within the neck such as skin irritation. taVNS 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 taVNS. They are the following: reduction of heart rate, blood pressure, and vasovagal syncope.

Given the minimal risk both of these already FDA approved methods introduce, we suspect taVNS will be a very safe procedure. taVNS is not intended to be a therapy for currently existing conditions and all subjects will be healthy controls with no previous history of neurological disorders or trauma.

MRI:

MRI tests are non-invasive and painless. There are no known risks or side effects associated with conventional MRI procedures except to those people who have electrically, magnetically or mechanically activated implants (such as cardiac pacemakers) or to those who have clips on blood vessels in their brain. There are no known additional risks for interleaved tVNS-fMRI procedures. However, an MRI may cause you to feel claustrophobic (uncomfortable in a small space) or anxious from the noises made by the machine.

This MRI scan will be used to answer research questions, not to examine your brain medically. This MRI scan is not a substitute for one a doctor would order. It may not show problems that would be picked up by a medical MRI scan. Nevertheless, a clinical neurologist or neuroradiologist will read your scan. If we find an abnormality we will let you know and will advise you to follow this up with your doctors. If you wish a copy of your MRI scan we can provide it to you on a CD. The MRI scans will be stored on research

computers for 7 years and then they will be destroyed. It is not possible to access them after you complete the study so please get a copy of your MRI on a CD if you think you might want it in the future. Despite efforts to maintain subjects' anonymity and confidentiality, there is always some minimal risk of people other than the study investigators gaining access to your health information. Every effort will be made to ensure that your health information will be collected and stored in a manner that ensures the highest level of protection of confidentiality.

Task-Specific Training:
TST is a form of peripheral occupational therapy with proven efficacy by multi-center clinical trial. It has been widely used in the clinical practice by occupational therapist. it is a more intensive therapy than conventional occupational therapy. It may cause muscle fatigue or mild soreness as a result of exercise, it usually resolves within 12-24 hours.

Motor assessments:
The series of motor evaluation test takes about 60-75 minutes to be completed. Most of them are part of daily clinical assessment. It occasionally causes fatigue and but patient has the option to adjust his/her pace to complete the test

Overground and Treadmill Walking:
The physical activity involved with this study may contribute to temporary muscle soreness or fatigue. These are normal responses to exercise and generally disappear within 1-2 days. Rest periods will be incorporated into the testing and training procedures, but participants will also be allowed additional rest at any time they wish during testing. Assistance will be provided during activities of walking or transferring from one surface to another, including the use of a support harness during treadmill walking. Despite these safety measures, as with any walking activity, there is a risk that participants may lose balance, stumble, fall, and experience an injury. Heart rate and blood pressure will be monitored during testing. Vital signs will be monitored routinely (at least every five minutes of activity) to make sure participants do not have a poor response to activity.

16.0 Potential Benefits to Subjects or Others

1) The benefit to individual maybe two weeks of Task-Specific Training Therapy at no cost
2) The benefit to the society maybe a better understanding of the mechanism of taVNS in stroke recovery, and it could potentially lead to a novel therapy to stroke patients.

17.0 Data Sharing

If the subject agrees, the data collected and generated from this study will be shared and stored within the Registry for Stroke Recovery (RESTORE-Pro#00037803) by the subject's registry ID. Additionally, deidentified data will be shared and stored within the "Rex" research database. Data collected will be released to RESTORE and Rex once the study has completed. Sharing data from this study with the registry & research database will allow for more targeted recruitment efforts in the future and allow researchers at MUSC to have a more complete registry with key stroke recovery elements including common data and

physical function characteristics that are applicable to multiple studies. MUSC researchers and collaborating facilities will be able to query data sets to learn more about recovery of subjects after their stroke through institutionally managed secure servers that will assure HIPAA privacy and security compliance.

Data collected and results will not be disclosed to participants in the study, however will be released for public dissemination in published manuscripts and conference presentations.

18.0 Drugs or Devices

The closed-loop taVNS stimulation system will be stored at the MUSC Center for Health Professions research lab office and be "dispensed" by the PI, study staff, or occupational therapist during study visits.

References

1. Ben-Menachem, E., et al., *Vagus nerve stimulation for treatment of partial seizures: 1. A controlled study of effect on seizures.* Epilepsia, 1994. **35**(3): p. 616-626.
2. Handforth, A., et al., *Vagus nerve stimulation therapy for partial-onset seizures A randomized active-control trial.* Neurology, 1998. **51**(1): p. 48-55.
3. Engineer, N.D., et al., *Reversing pathological neural activity using targeted plasticity.* Nature, 2011. **470**(7332): p. 101-104.
4. Kilgard, M.P., *Harnessing plasticity to understand learning and treat disease.* Trends in neurosciences, 2012. **35**(12): p. 715-722.
5. Dawson, J., et al., *Safety, feasibility, and efficacy of vagus nerve stimulation paired with upper-limb rehabilitation after ischemic stroke.* Stroke, 2016. **47**(1): p. 143-150.
6. Khodaparast, N., et al., *Vagus nerve stimulation delivered during motor rehabilitation improves recovery in a rat model of stroke.* Neurorehabilitation and neural repair, 2014: p. 1545968314521006.
7. Khodaparast, N., et al., *Vagus nerve stimulation during rehabilitative training improves forelimb strength following ischemic stroke.* Neurobiology of disease, 2013. **60**: p. 80-88.
8. De Ridder, D., et al., *Placebo-controlled vagus nerve stimulation paired with tones in a patient with refractory tinnitus: a case report.* Otology & Neurotology, 2015. **36**(4): p. 575-580.
9. De Ridder, D., et al., *Safety and efficacy of vagus nerve stimulation paired with tones for the treatment of tinnitus: a case series.* Neuromodulation: Technology at the Neural Interface, 2014. **17**(2): p. 170-179.
10. Engineer, N.D., A.R. Møller, and M.P. Kilgard, *Directing neural plasticity to understand and treat tinnitus.* Hearing research, 2013. **295**: p. 58-66.
11. Ventureyra, E.C., *Transcutaneous vagus nerve stimulation for partial onset seizure therapy.* Child's Nervous System, 2000. **16**(2): p. 101-102.
12. Badran, B.W., et al., *Tragus or cymba conchae? Investigating the anatomical foundation of transcutaneous auricular vagus nerve stimulation (taVNS).* Brain Stimul, 2018. **11**(4): p. 947-948.
13. Badran, B.W., et al., *Neurophysiologic effects of transcutaneous auricular vagus nerve stimulation (taVNS) via electrical stimulation of the tragus: A concurrent taVNS/fMRI study and review.* Brain Stimul, 2018. **11**(3): p. 492-500.
14. Badran, B.W., et al., *Short trains of transcutaneous auricular vagus nerve stimulation (taVNS) have parameter-specific effects on heart rate.* Brain Stimul, 2018. **11**(4): p. 699-708.
15. Kong, J., et al., *Treating Depression with Transcutaneous Auricular Vagus Nerve Stimulation: State of the*

Art and Future Perspectives. Front Psychiatry, 2018. **9**: p. 20.
16. Rong, P., et al., *Effect of transcutaneous auricular vagus nerve stimulation on major depressive disorder: A nonrandomized controlled pilot study.* J Affect Disord, 2016. **195**: p. 172-9.
17. Waddell, K.J., et al., *Does Task-Specific Training Improve Upper Limb Performance in Daily Life Poststroke?* Neurorehabil Neural Repair, 2017. **31**(3): p. 290-300.
18. Lang, C.E., et al., *Dose response of task-specific upper limb training in people at least 6 months poststroke: A phase II, single-blind, randomized, controlled trial.* Ann Neurol, 2016. **80**(3): p. 342-54.
19. Waddell, K.J., et al., *Feasibility of high-repetition, task-specific training for individuals with upper-extremity paresis.* Am J Occup Ther, 2014. **68**(4): p. 444-53.
20. Birkenmeier, R.L., E.M. Prager, and C.E. Lang, *Translating animal doses of task-specific training to people with chronic stroke in 1-hour therapy sessions: a proof-of-concept study.* Neurorehabil Neural Repair, 2010. **24**(7): p. 620-35.
21. Dobkin, B.H., *Strategies for stroke rehabilitation.* Lancet Neurol, 2004. **3**(9): p. 528-36.
22. Roger, V.L., et al., *Heart disease and stroke statistics--2012 update: a report from the American Heart Association.* Circulation, 2012. **125**(1): p. e2-e220.
23. Calautti, C. and J.C. Baron, *Functional neuroimaging studies of motor recovery after stroke in adults: a review.* Stroke, 2003. **34**(6): p. 1553-66.
24. Nudo, R.J. and K.M. Friel, *Cortical plasticity after stroke: implications for rehabilitation.* Rev Neurol (Paris), 1999. **155**(9): p. 713-7.
25. Meintzschel, F. and U. Ziemann, *Modification of practice-dependent plasticity in human motor cortex by neuromodulators.* Cereb Cortex, 2006. **16**(8): p. 1106-15.
26. Sawaki, L., et al., *Effect of an alpha(1)-adrenergic blocker on plasticity elicited by motor training.* Exp Brain Res, 2003. **148**(4): p. 504-8.
27. Porter, B.A., et al., *Repeatedly pairing vagus nerve stimulation with a movement reorganizes primary motor cortex.* Cerebral Cortex, 2012. **22**(10): p. 2365-2374.
28. Shetake, J.A., et al., *Pairing tone trains with vagus nerve stimulation induces temporal plasticity in auditory cortex.* Experimental neurology, 2012. **233**(1): p. 342-349.
29. Lehtimaki, J., et al., *Transcutaneous vagus nerve stimulation in tinnitus: a pilot study.* Acta Otolaryngol, 2013. **133**(4): p. 378-82.
30. Magis, D., P. Gérard, and J. Schoenen, *Transcutaneous Vagus Nerve Stimulation (tVNS) for headache prophylaxis: initial experience.* The journal of headache and pain, 2013. **14**(S1): p. P198.
31. Kreuzer, P.M., et al., *Feasibility, safety and efficacy of transcutaneous vagus nerve stimulation in chronic tinnitus: an open pilot study.* Brain Stimul, 2014. **7**(5): p. 740-7.
32. Clancy, J.A., et al., *Non-invasive vagus nerve stimulation in healthy humans reduces sympathetic nerve activity.* Brain Stimul, 2014. **7**(6): p. 871-7.
33. Trevizol, A.P., et al., *Transcutaneous vagus nerve stimulation (tVNS) protocol for the treatment of major depressive disorder: A case study assessing the auricular branch of the vagus nerve.* Epilepsy & Behavior, 2015. **53**: p. 166-167.
34. Bauer, S., et al., *Transcutaneous vagus nerve stimulation (tVNS) for treatment of drug-resistant epilepsy: a randomized, double-blind clinical trial (cMPsE02).* Brain stimulation, 2016. **9**(3): p. 356-363.
35. Schaefer, S.Y., C.B. Patterson, and C.E. Lang, *Transfer of training between distinct motor tasks after stroke: implications for task-specific approaches to upper-extremity neurorehabilitation.* Neurorehabil Neural Repair, 2013. **27**(7): p. 602-12.