D E P A R T M E N T O F

Biomedical Engineering

College of Engineering University of Wisconsin-Madison

Cranial Nerve Noninvasive Neuromodulation Using the PoNS™ for Treatment of Symptoms Due to Mild or Moderate Traumatic Brain Injury (AKA TBI Study)

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Key Personnel

- Samuel Acuna, Graduate Student, will be responsible for performing the EMGs under the supervision of Prof. Darryl Thelen, PhD.
- Kristin Caldera, M.D., Co-Investigator, Clinical Advisor, will provide medical consultation for subject eligibility and assist with recruitment and identification of potential subjects.
- Michelle Chiang, Graduate Student, will perform clinical testing, including cognitive assessments.
- Georgia Corner, DPT, will be primarily responsible for subject training and case report forms.
- Yuri Danilov, Ph.D., Co-Investigator, will direct the development of the conceptual framework of the CN-NINM training program. He will also be chiefly responsible for interpretation and reporting of experimental results.
- Kurt Kaczmarek, Ph.D., has primary responsibility for instrumentation development, statistical analysis of experimental data and reporting of experimental results.
- Danielle McIntosh, B.S., Research Intern, will be responsible for coordinating subject testing and training and performing testing.
- Kelly Miller, B.S. Clinical Monitor, will be responsible for randomization of subjects. She will also be responsible for both monitoring regulatory compliance and reporting unanticipated problems and adverse events.
- Ann Pahnke, B.A., will be responsible for randomization of subjects.
- Dafna Paltin, B.S., Research Intern, will perform the cognitive assessments.

- Vivek Prabhakaran, M.D., Ph.D. Co-Investigator, Medical Advisor, will review subject neuroradiologic reports, medications, clinical history, monitor for significant adverse events, and assist with the recruitment of the targeted patient population.
- Amy Remm, B.S., Clinical Monitor, will be responsible for regulatory reporting with the US Army Clinical Research Monitor (USAMMDA) assigned to the study.
- Janet Ruhland, M.A., PT, will be primarily responsible for subject training and case report forms and will perform subject screening.
- Kim Skinner, M.S.P.T., will be responsible for performing blinded analysis of the videos for Dynamic Gait Index testing. She will also assist in interpretation of experimental results.
- Darryl Thelen, PhD, M.E., will supervise and serve as consultant for the EMG testing.
- Mitchell Tyler, M.S., P.E. (Principal Investigator) will direct the overall project, supervise the deployment of CN-NINM training and clinical testing, collaborate on development of the Control device, assist in interpretation and reporting of experimental results, and report both unanticipated problems and adverse events.
- Yakov Verbny, Ph.D., will be responsible for conducting all physiologic testing and assist in interpretation of experimental results.

Summary

Based on the results of our pilot study in moderate traumatic brain injury (mTBI), we will conduct a randomized, controlled study of cranial nerve non-invasive neuromodulation (CN-NINM) in individuals with chronic symptoms of mild to moderate traumatic brain injury (mTBI), post-concussive sequelae, (PCS) and post-traumatic stress (PTS). The study will involve training of both balance and gait, with assessments using standardized and relevant metrics to monitor changes in these indications, as well as cognitive function, sleep, headache, anxiety, mood, and eye-movement control. The training regimen involves using a neurostimulation intervention that addresses primary and secondary symptoms associated with mTBI, PCS, and PTS. This randomized double blind controlled study will enroll a total of 44 subjects (M & F) in 2 equal subgroups: 22 with an Active PoNS™, and 22 with a Control (non-zero, minimally perceivable stimulation) device. Subjects will participate in a 3-phase intervention beginning with a 2-week in-lab training program (ITP) (2 in-lab training sessions and 1 home training session daily), followed by 12 weeks of training at home (HTP) (3 home training sessions daily), and a 12-week withdrawal period (no training). Subjects will return to the clinic weekly during the at-home phase for a single session of retraining and progression, and participate in periodic retesting. All training and testing will be performed uniformly across all subjects in both groups. Multiple assessment metrics will capture data at the beginning and end of the 2-week in-lab CN-NINM intervention period and at 3-week intervals. After completion of the formal training period, subjects will stop using the device (withdrawal stage) and will be tested every 3 weeks over an additional 12 weeks to monitor and assess changes due to withdrawal of the PoNS. This will yield a total of 10 data points for each subject. If successful, this study would indicate that CN-NINM may improve rehabilitation outcomes and reduce time required to improve function.

Background

The Centers for Disease Control and Prevention reports that 5.3 million people live with chronic disabilities related to brain injury, costing Americans $76.5 Billion/yr. in medical, rehabilitation costs and loss of income. The majority (75-85%) of TBI's are considered mild or mTBI's and

concussion. Current epidemiological reports find that under conditions of similar risk exposure, females may have a 2-fold elevated frequency of concussion [West, 2013; Kontos, 2011].

At present, the primary approach for treating TBI is through physical medicine. A systematic and unified approach is not universally established, and does not include cognitive therapy. Recent studies, however, have demonstrated that neuroplasticity and functional benefits are stimulated through specific forms of motor-behavioral interventions [Nudo, 2001; Weiller, 1993], while others have determined that early, intensive, targeted cognitive skill training after injury, even in older adults, are clinically effective [Rohling, 2009; Cicerone, 2000]. Recent studies have identified the potential for noninvasive electrical stimulation of the brain to improve patient ability to learn and retain a motor task [Reis, 2009; Vines, 2010]. Additionally, there has been an emerging call for an integrative approach to developing clinical applications, drawing on the latest understanding of the interactive factors affecting the potential for functional physical, cognitive and psychological recovery from TBI [Bach-y-Rita, 1990, 2000; Bédard, 2003; Taub, 2002].

Traumatic brain injury, and particularly concussive polytrauma due to explosive blast, is a signature injury of the Iraq and Afghanistan wars. From 2000 to 2012 the US military reported a total of approximately 255,000 military personnel from the OEF/OIF theaters have screened positive for TBI, roughly half have been treated for reported indications, and nearly 66,000 (25%) are identified as having significant and chronic symptoms [Kontos, 2013]. Personnel in this latter group present with problems not only with control of balance, posture, gait, and manual dexterity, but also more subtle and insidious difficulties with vertigo, short-term memory, reasoning, attention, sensory processing, psychosocial expression and understanding, depression, anxiety, aggression, personality changes and social inappropriateness. Faced with little prospect of recovery, coupled with the social impact of their functional inability to perform normal activities of daily living, chronic symptoms of mTBI frequently lead to social isolation and substance abuse, resulting in encounters with law enforcement and social services. [Fabing, 1947]

CN-NINM uses sequenced patterns of electrical stimulation on the tongue. Our hypothesis is that CN-NINM induces neuroplasticity by noninvasive stimulation of two major cranial nerves: trigeminal, CN-V, and facial, CN-VII. This stimulation excites a natural flow of neural impulses to the brainstem (pons varolli and medulla), and cerebellum via the lingual branch of the cranial nerve (CN-Vc), and chorda tympani branch of CN-VII, to effect changes in the function of these targeted brain structures, extending to corresponding nuclei of the brainstem – at least in the sensory and spinal nuclei of trigeminal nuclei complex and the caudal part of the nucleus tractus solitarius. We postulate that the intensive activation of these structures initiates a sequential cascade of changes in neighboring and/or connected nuclei by direct collateral connections, brainstem interneuron circuitry and/or passive transmission of biochemical compounds in the intercellular space. By combining neurostimulation with a specific set of physical, cognitive and/or mental exercises, we can further focus brain rehabilitation and target our effort on recovery of selected functional damage.

Pilot Study 1: CN-NINM Application to Balance Disorders

Five subjects (3 males, 2 females, mean age 45.4 yrs.) with a variety of etiologies (1 traumatic brain injury, 1 spinocerebellar ataxia, 3 vestibular dysfunction) and moderate balance, gait, and visual tracking deficits participated in this study. Each had participated in a variety of unsuccessful treatments for problems with balance, gait, vertigo, and nausea. Assessments of balance, gait, posture and eye movement were quantified using a number of mechanisms to measure the effects of the CN-NINM training regimen.

• Standardized self-assessment surveys of perceived dizziness and loss of stability via the Dizziness Handicap Inventory (DHI) and Activity-specific Balance Confidence scale (ABC).

• Digital head-based postural stabilography (HPS - similar to standardized force platform posturography) to assess changes in posture.

• Functional magnetic resonance imaging (fMRI) to observe potential changes in the cerebral cortex, cerebellum, pons varolli and brainstem.

After each subject completed baseline testing, they received CN-NINM stimulation while simultaneously performing progressively challenging postural control training. Subjects also received physical exercise training to develop improved motor coordination and mobility as part of the CN-NINM training. Subjects participated in CN-NINM training for 5 consecutive days and were retested.

The results of these tests are shown in Table 1. All subjects exhibited improved scores on the self-assessment measures, indicating that they perceived themselves as being more stable and less 'dizzy' after CN-NINM training. Stabilography scores indicate that subjects were more stable. The results show that while individual differences varied widely, and are dependent on both the subjects' initial condition and unique symptoms, all 5 subjects improved their postural control.

Table 1. Summary of Results from Various Tests of Stability and Gait on 5 Subjects. Changes greater than 5% are considered clinically significant.

	Subject #						
Calculated % Improvement After CN-NINM Training	01	02	03	04	05	Mean	SD
Activity-Specific Balance Confidence (ABC)	3	12	49	10	16	**18.0**	16.1
Dizziness Handicap Index (DHI)	130	154	81	41	71	**95.4**	41.0
Head - Postural Stabilogram (HPS) [RMS displacement]	58.1	33.8	22.9	45.4	57.1	**43.5**	15.2

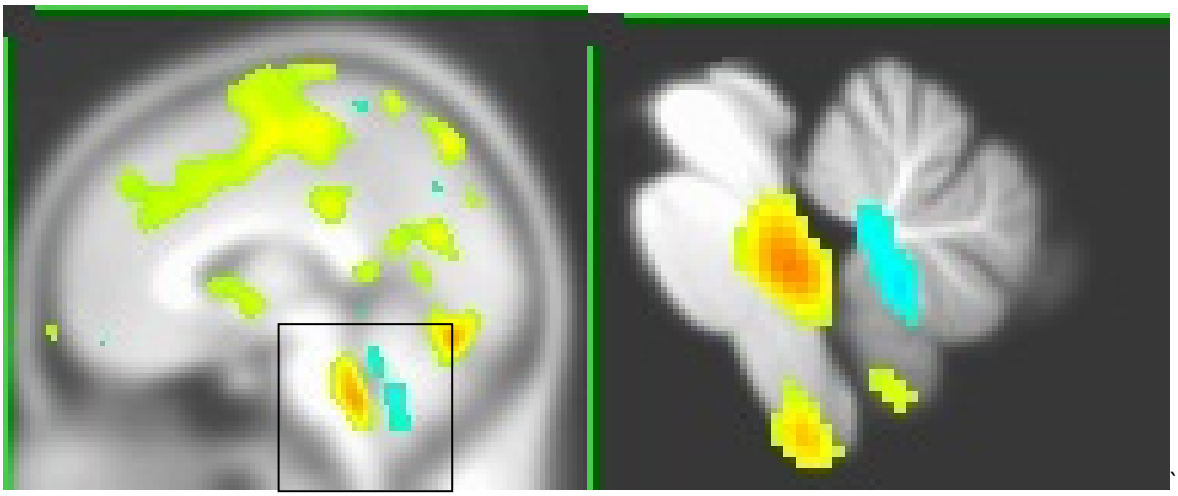
Additionally, three of the subjects reported chronic hypersensitivity to light, visual flow, and/or changes in self-motion that severely interfered with activities of daily living such as driving a car, grocery shopping, and reading. These individuals reported reduced hypersensitivity to visual stimuli and improved image stability after CN-NINM training. Additionally, in subjects with reported or observed oscillopsia or nystagmus (3 of 5), we also noted improved eye-motion control performance during a simple visual object-tracking task after the 2nd day of CN-NINM training. The subject with cerebellar ataxia had the most substantial improvement in the smoothness of self-motion (weight transfer, ankle, knee, and hip flexion/extension), and exhibited a noticeable improvement in symptoms of dysphonia (speech articulation and volume).

Functional MRI was performed before the first intervention ('pre') and then 4-6 hours after the last training session ('post') of the 5-day CN-NINM intervention, i.e. the subjects *did not* receive tongue stimulation during imaging. A color relief map of the difference in neural activation response to dynamic visual stimuli (stereo B/W checkerboards progressing/receding, and rotating in pseudorandom motion) is shown in Figure 1. The results shown are composites of the changes in cortical and brainstem activation from the 5 subjects (using digital subtraction of post-pre) after anatomical correction for region of interest registration.

The images show increased activity in the visual associative area, both the sensory and motor cortices, and especially in both the primary visual area of the occipital lobe and the posterior brainstem. The greatest changes (yellow and red) appear in the dorsal pons varolli, medulla

oblongata, and ventral cerebellum (Figure 1.B). Changes in metabolic activity in these areas are of particular interest because these are major sensory integration and control centers for both body and eye movement, and therefore primary targets for neuromodulation stimulation. [3-5]

An apparent *decrease* in neural activity in the cerebellum following CN-NINM (shown in teal) may explain the reductions in frequency and magnitude of oscillopsia observed by all 5 subjects, and reported increases in both coordination and continuity of motion that was especially apparent in a subject that presented with ataxia due to brainstem microstroke, and vocal modulation improvement in a subject with spinocerebellar ataxia.



A – Whole-brain Activation Map **B** – Enlarged region of interest from A

Figures 1 – A & B. Diagrams of ensemble differential neural activity [Post-CN-NINM training minus Pre-training] from 5 subjects while viewing dynamic visual stimuli. **A** - Saggital view of whole brain with head facing to left. **B** - Enlarged region of interest showing detailed activation of the brainstem (medulla, pons, midbrain), and cerebellum. Color map indicates relative difference in the magnitude of activation after CN-NINM ($p<0.05$). Red represents the highest increase; teal indicates decreased metabolic activity following CN-NINM intervention. [Wildenberg, et. al, 2010, 2011a,b]

Pilot Study 2: CN-NINM Application to Traumatic Brain Injury (TBI)

The results presented below represent the changes in 4 clinical metrics over an initial 5-day period of Cranial Nerve Non-Invasive Neuromodulation (CN-NINM) intervention in subjects that had suffered moderate traumatic brain injury (TBI). Four female subjects (mean age: 48.3) presented with chronic and significant balance and gait deficits due to moderate closed-head, non-penetrating, Type-2 or 3 concussive injury (9-13 on Glasgow Coma Scale) at initial diagnosis. All were approximately 5 years post injury and had previously completed rehabilitative therapy programs at their respective primary care facilities.

Functional Testing. The results from the gait testing for 4 subjects that participated in the pilot study are summarized in Figure 2. The Dynamic Gait Index (DGI) is a clinician-scored index of 8 facets of gait. Scores range from 0 (worst) to 24 (normal). A score change of 3.0 is generally considered clinically significant. The DGI scores indicate significant improvements in stability and gait that are retained for as much as 6 hours after completion of the second intervention session in the day.

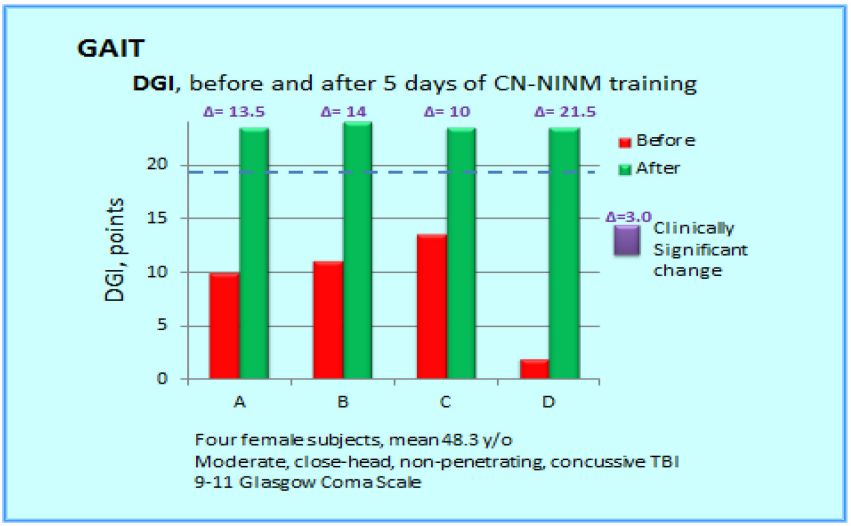


Figure 2. Summary of Results from Dynamic Gait Index for 4 subjects with non-penetrating TBI. Changes greater than 3 points are considered clinically significant improvement in performance.

The 4 subjects were tested on the NeuroCom® Computerized Dynamic Posturography (CDP) Sensory Organization Test (SOT) before and after the week of twice-daily interventions. The SOT is an objective, automated measure of sensory-motor integration that evaluates the functional contribution of the somatosensory, visual, and vestibular components of balance. A composite score is calculated and compared with a database normalized for age and height. Sample pre-post scores are shown in Figure 3 for the latter two subjects in this cohort. The composite scores for all 4 subjects are summarized in Figure 4.

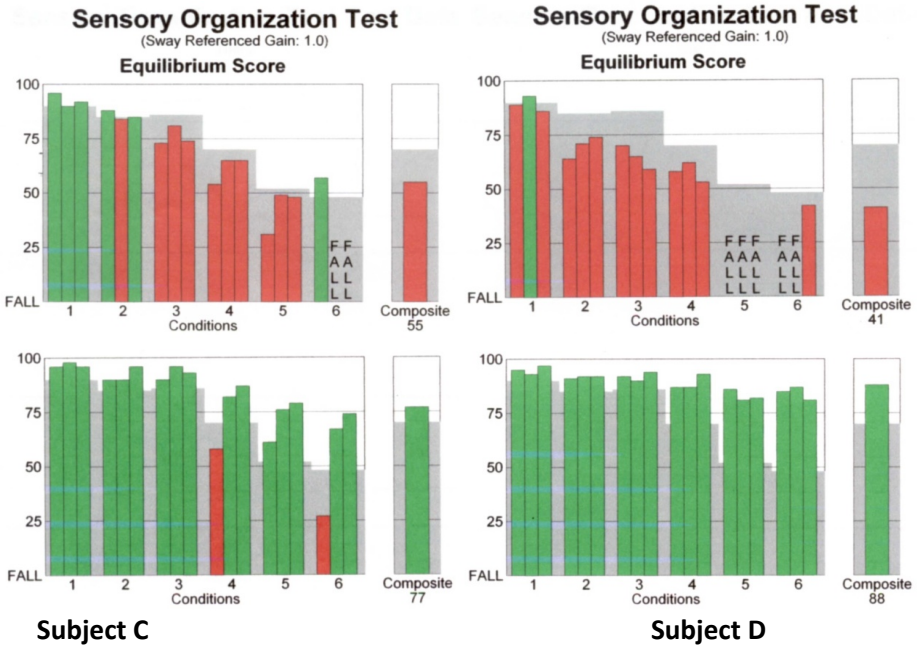


Figure 3. SOT scores before (upper) and after (lower) for two TBI subjects. Note that the greatest functional improvement occurred in the most dynamic and challenging tasks: Condition 5 – Eyes *closed* with platform-induced anterior/posterior sway, and Condition 6 – Eyes open with platform and visually-induced anterior/posterior sway.

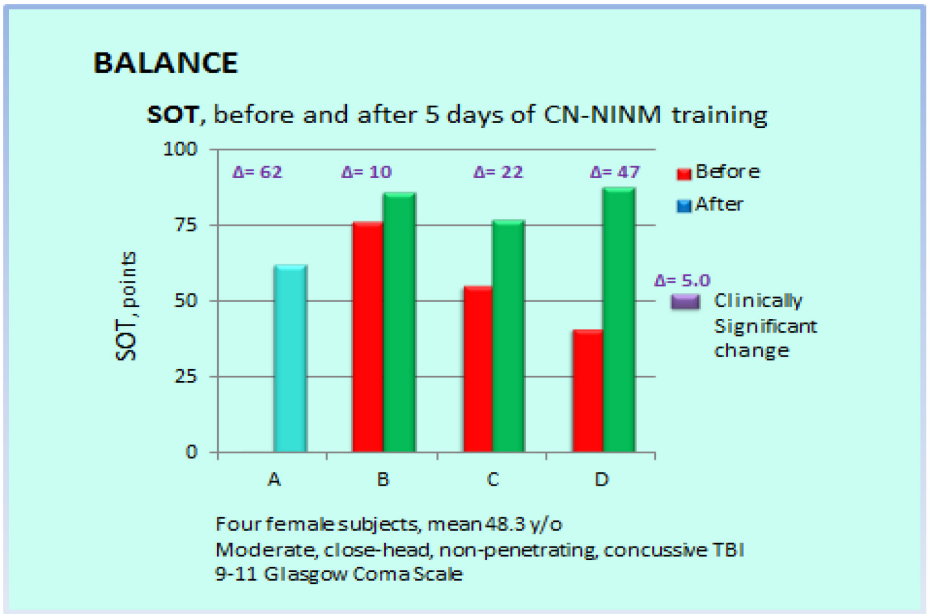


Figure 4. Summary of Results from Computerized Dynamic Posturography Sensory Organization Test (SOT). Changes greater than 5 points are considered clinically significant improvement.

We performed electromyogram recording on the legs of one subject. Activation timing of the left VL and SOL were abnormal before treatment, and normal after treatment. Note that after 5-days of training the muscle activation patterns are more symmetric, agonist-antagonist pairs are coordinated, and show significantly less co-contraction.

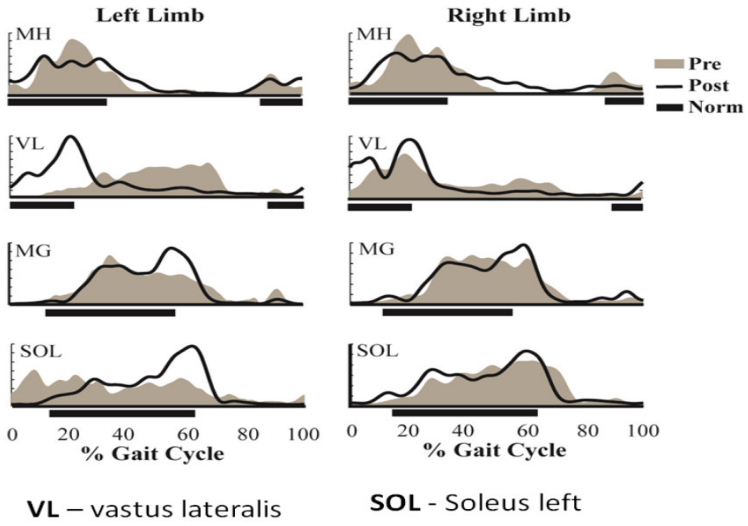


Figure 5. Electromyogram recorded before (Pre) and after (Post) training. (MG – medial gastrocnemius, MH – medial hamstrings).

Additionally, TBI Subjects C & D were tested for changes in cognitive function, memory, attention and mood both before the 5-day intervention began, and within 24 hours of completing the training. Their primary indications and scores on the Brief Repeatable Battery of Neuropsychological Tests (BRBNT) are summarized in Tables 2 & 3, and the attendant lists below each indicate their symptoms and changes.

Subject 'C' - Symptoms: Pre-CN-NINM Intervention

- Difficulties with concentration, emotions, and physical fatigue.
- Slow and stuttered speech (significant dysarthria & expressive aphasia).
- Diplopia & blurred vision, 17˚ visual field deviation requiring corrective lenses
- Depressed balance & gait test scores. Frequent vertigo and loss of balance
- Problems with attention span, sustained and divided attention (low scores on word span on Symbol-Digit and Paced Audio Serial Addition Tests)
- Depression & emotional lability due to chronic disability

Table 2: Subject C

Test Cycle	Word Span (12)	Word Storage (11 min)	Spatial Recall (10/36)	Symbol- Digit (90 sec)	PASAT (3 sec)	PASAT (2 sec)	Word Generation (60 sec)	Depression Scale
Baseline	6	7	6.5	18	34	28	27	4 (mild)
2 weeks	9	9	8	42	34	35	32	2
1 month	10	11	9	53	52	36	33	2

Results: Post-training

- Showed statistically and clinically relevant improvement on most of the tests after 1 month
- Visual field deviation disappeared & vision satisfactory for both day & night driving.
- Verbal and visual were slow but retrieval intact.
- Able to go to appointments, shopping, cook meals and participate family conversations.

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Subject 'D' - Symptoms: Pre-Intervention

- Hypersensitivity to sounds, light, motion, or to objects or people moving in environment.
- Depressed balance & gait test scores; aberrant & asymmetric gait pattern,
- Chronic fatigue & muscle ache due to co-contraction
- Showed significant attention impairment (low numbers on all tests)
- Moderate depression on depression scale, frequent crying
- Both receptive & expressive aphasia - difficulty participating in conversations
- Dysarthria - difficulty pronouncing even single-syllable words, stutters.

Table 3: Subject D

Test Cycle	Word Span (12)	Word Storage (11 min)	Spatial Recall (10/36)	Symbol-Digit (90 sec)	PASAT (3 sec)	PASAT (2 sec)	Word Generation (60 sec)	Depression Scale
Baseline	5	7	9	30	29	23	27	12 (mod)
2 weeks	7	9	9	46	50	31	38	5 (mild)
1 month	11	11	10	59	58	42	38	2

Results: Post-training

- Regained normal balance, and able to jog, walk, and stand with eyes closed.

- Showed significant improvement on all cognitive tests; no signs of depression.
- Working again part-time, re-engaged in activities of daily living.

We are continuing to monitor the changes in performance in these latter 2 subjects through with their referring neurologist, Dr. Charles Davies (Carle Clinic at the University of Illinois). We have received report of progressive improvements in eye movement, short-term memory, executive function, mood elevation, and progressive reductions in both expressive aphasia and social anxiety.

RESEARCH PLAN

Introduction

CN-NINM is fundamentally an integrative therapeutic intervention, combining cranial nerve electrical stimulation with multiple symptom-specific exercises. Our pilot TBI results suggest that, in the absence of identifiable tissue damage, a combination of the neurostimulation and targeted, challenging rehabilitation will effect neuroplastic changes, reduce symptoms, and begin normalizing function. These changes include rehabilitation and re-establishment of movement control (balance, posture, gait, muscular tonus, eyes), cognition (memory, attention, planning, decision-making) and potentially mood (depression, aggression, anxiety).

Study Objectives

The primary objective of the study is to demonstrate that CN-NINM training using the PoNS device with normal stimulation is more efficacious than training using a very low but perceivable stimulation PoNS device in subjects with symptoms due to a mild to moderate TBI.

In this experimental investigation we will gather data to describe the mean changes in balance and gait behavior from baseline for two treatment groups. The results will inform the design and power of subsequent, incremental studies investigating the clinical efficacy of this intervention. Standardized clinical assessment mechanisms will measure changes in balance, gait, cognition, emotional function, headache, eye movement, and sleep in individuals with post-concussive and post-traumatic stress complaints in a 14-week rehabilitation program and after a 12-week withdrawal period.

Study Design

The randomized double blind study will enroll up to 44 subjects (males and females) with identified balance deficits that will be equally distributed into 2 subgroups: an “Active” CN-NINM device (PoNS) group, and a “Control” (very low stimulation) device group. Subjects will be randomly assigned to one of the two treatment groups by the Clinical Monitor as they are recruited using a rolling recruitment method. Cohort assignment will be made by serially selecting from a list of 44 randomly generated three-digit subject identification numbers, with 22 assigned to both the Active and Control group.

The study will be a total of 26 weeks, consisting of three stages. The first stage consists of twice-daily in-lab training (ITP) for 2-weeks (with at-home training during the intervening weekend). The intensive CN-NINM training program will focus on balance and gait. This is followed by the second stage, which consists of subjects performing the same training for 12 weeks during Stage 2 – the Home Training Program (HTP). During this second stage, subjects will return to the lab 1

time per week for a training session. After completion of the second stage, subjects continue in the third stage, the withdrawal stage, where they no longer use the PoNS device.

Multiple assessment metrics will capture data at important points during the study: at the beginning and end of the 2-week in-lab CN-NINM intervention period (ITP), every 3 weeks of the test/retraining intervals during the home training phase (HTP) and every 3 weeks of the withdrawal phase. This will yield a total of 10 data points for each subject on the SOT and gait metrics. All training and testing will be performed in a consistent and identical manner across all subjects in both groups.

To preserve the controlled nature of this experiment, each subject will work with three experimenters. After completion of screening and enrollment, the subject will be assigned to either the active or control group by the Clinical Monitor (CM). The CM will use the randomization list provided by the Biostatistics group and the UW Institute for Clinical and Translational Research (ICTR) for this study, and will issue the appropriate device (Control or Active). The CM is the only person in the study who will know which group to which each subject is assigned. The PI will then instruct each subject in the features and use of the PoNS and follow a standardized stimulus intensity level setting procedure with the subject. The second investigator, specifically the Study Coordinator, will conduct and oversee all testing. A third investigator (Physical Therapist) will perform the actual intervention. Subjects will be instructed that they must not tell the therapist what they feel on their tongue, or ask questions about the stimulation since the PT will not be able to answer these questions. If the subject has questions or feels the need to report anything about the device or stimulation, they are to address these only to the PI, in confidence. This will prevent treatment and testing bias by ensuring that the therapists and Study Coordinator are blinded to the subjects' treatment group identity.

To help preserve the controlled nature of this experiment, subjects will also be instructed that they must not discuss the stimulation, the group they think they are in, or discuss details of the intervention with any other participants in the study or with family members. They may interact with other participants as long as their discussions are not related to the study. Subjects will be discouraged from congregating or socializing outside of the study in order to maintain blinding.

Lab study visits will take place at the Tactile Communication Neurorehabilitation Lab (TCNL) at 455 Science Drive in Madison. Study duration is expected to be 3 years, from time of IRB approval to the completion of data analysis.

An independent, optional sub-study, Characterizing Functional and Structural Brain Changes Following Cranial Nerve Noninvasive Neuromodulation (AKA MRI Sub-study), will enroll 26 subjects (13 subjects in the Active PoNS™ group; 13 subjects in the Control group) who are enrolled in the main TBI Study (see Addendum – MRI Sub-study). MRI scans will be performed at important points during the study: at the beginning and end of the 2-week in-lab CN-NINM intervention period (ITP), at the end of the 12-week home training phase (HTP) and at the end of the 12-week withdrawal phase. This will yield a total of 4 data points for each subject. The sub-study MRI scans will be performed at the Wisconsin Institutes for Medical Research (WIMR), 1111 Highland Ave., Madison, WI 53705.

Methods

Subject Population & Selection.

All subjects will be recruited on a voluntary basis. The study will enroll 44 adults, age 18-65, presenting with a chronic balance deficit due to mTBI. Subjects who also present with other

symptoms, such as gait disturbance, mild to moderate recurrent headaches, sleep, memory, attention, or cognitive deficits, will be considered for participation. All candidates will have had a closed-head, non-penetrating, blunt, whiplash, or explosive blast-induced brain injury. Subjects will be required to provide their most recent neuroradiologic report and evidence of having completed a prescribed therapeutic regiment for their symptoms.

Study Inclusion and Exclusion Criteria.

Inclusion Criteria:

- All candidates will have a balance disorder as a result of a traumatic brain injury (TBI).
- All candidates will have a NeuroCom® Sensory Organization Test (SOT) composite score at least 8 points below normal after adjustment for age and height [based on normative data].
- All candidates will be between the ages of 18 and 65 (at the time of screening).
- If female of childbearing potential, the candidate agrees to use adequate contraception throughout participation in the study (from enrollment to completion).
- All candidates must have access to a treadmill.
- All candidates will be at least 1 year post-injury.
- All candidates will have a neuroradiologic scan and report after their most recent TBI.
- All candidates will be ambulatory and able to walk independently for 20 minutes.
- All candidates, if on medications, will not have had any major changes in type or dosage within 3 months of enrollment.
- All candidates will have participated in a focused physical rehabilitation program for their TBI and feel that they have reached a plateau.
- All candidates will be able to understand and willing to give informed consent.

Exclusion criteria:

- All candidates that have oral health problems (e.g. gum disease, active cankers, cold sores, piercings, oral surgery within the previous 3 months).
- All candidates with non-removable metal orthodontic devices (e.g., braces) or oral cavity piercings that could interfere with PoNS™ use.
- All candidates who have chronic infectious diseases (e.g. hepatitis, HIV, TB).
- All candidates with unmanaged hypertension.
- All candidates with unmanaged diabetes, or complications due to diabetes (e.g. retinopathy, neuropathy, renal disease).
- All candidates with neurological disorders other than those attributed to their primary diagnosis (e.g. MS, PD, ALS, AD or other dementia, uncontrolled pain).
- All candidates with a history of oral cancer.
- All candidates who have been treated for any type of cancer other than basal cell carcinoma within the past year.
- All candidates who have had a penetrating injury, craniotomy (with the exception of a burr hole (trephination) for resolution of acute subdural hematoma), or refractory subdural hematoma.
- Exceptions for other abnormalities identified in neuroradiologic scan reports that are asymptomatic and not expected to change may be made on a case by case basis by the Medical Advisor.
- All candidates with chronic use of psychoactive or psychostimulant medications that, in the opinion of the investigators, would compromise the subject's ability to comprehend and perform the study activities.

- All candidates who have a pacemaker, or are identified as at-risk for cardiovascular events.
- All candidates who are pregnant or lactating.
- All candidates with a lower extremity biomechanical prosthetic.
- All candidates with a history of seizures (except those in the acute or post-acute phases, and are controlled).
- All candidates who experienced a loss of consciousness greater than 24 hours as a result of their TBI.
- All candidates with a "severe" score in any of the Attention, Memory, or Executive Functions categories on the Cognitive Linguistic Quick Test (CLQT).
- All candidates who, in the opinion of the investigators, are unable to feel the stimulation and successfully complete the device level setting procedure.

Recruitment Process.

Candidates reporting, suspected of having, or presenting with persistent deficits in balance as a result of mTBI are eligible for recruitment for this study. Candidates will be drawn from clinician and self-referrals generated from study announcements to clinical colleagues & collaborators, posting on clinicaltrials.gov, UW-Madison Division of Information Technology (DoIT) mass email (faculty, staff and students), ads in local media such as the Wisconsin State Journal (print and online). The recruitment fliers, a list of inclusion/exclusion criteria and/or postings similar to the ClinicalTrails.gov listing will be electronically posted on our website, and the websites of collaborating institutions, such as the Brain Injury Alliance Additionally, we will provide a summary of this research, fliers and postcards to providers that treat this patient population, e.g., neurologists and medical physicists at UW Hospitals & Clinics. These providers will help identify potentially eligible subjects and will introduce this research to them and may provide a copy of the study flyer and/or postcard. Patients interested in participating will be referred to a study team member who will provide more information about participation and will assess initial eligibility.

Potential study candidates who have contacted the TCNL by telephone or email and indicated an interest in participation will be scheduled for a phone interview. The study will be described to the candidate on the phone. If interested, the candidate will be asked to provide oral consent to answer questions related to study eligibility. Persons who meet the eligibility requirements will be asked to mail us their MRI/CT report, PT discharge summary, medication list) to help determine eligibility. These documents will be reviewed by the study Medical Advisor. If the candidate is determined to be ineligible, the documents will be destroyed. Subjects who are eligible will be asked to come to the lab, where we will obtain written informed consent and perform the CLQT, SOT, and 20-minute walk on treadmill to see if the candidates meet the remaining entrance criteria. The MRI/CT report, PT discharge summary and medication list will be filed in a locked filing cabinet in a locked room in the TCNL Lab to maintain subject privacy per HIPAA regulations. All subject recruitment, interventions, and testing are performed at the Tactile Communication Neurorehabilitation Lab, 455 Science Drive, Madison, WI 53711.

Consent Process.

Candidates who have passed the phone screening will schedule a meeting with the Study Coordinator at the TCNL. The Study Coordinator will provide informed consent for the main TBI Study, answer questions and obtain informed written consent. Candidates may ask questions before signing the informed consent form. After completion of the TBI Study consent, the Study Coordinator will also provide informed consent for the MRI Sub-study and explain that participation in the sub-study is optional. All candidates will be informed that declining

participation in the MRI Sub-study will not affect their participation in the TBI Study. The PIs for both studies will be available to answer candidates' questions. All candidates are free to decline participation in either study without question if they so choose. Subjects will be given a copy of the signed consent form(s).

Subject Screening Procedure.

Once written informed consent has been obtained, the candidate will complete the Demographic Survey, the Subject Health Questionnaire, the CLQT, the SOT, and walk on the treadmill for 20 minutes. The results will be used to determine if the subject meets the study remaining entrance criteria. For those candidates that qualify for study, the SOT performed at screening will be recorded as the subject's baseline score.

Candidates who meet all screening criteria will be enrolled in the study and assigned a unique study identifier. Candidates that fail any portion of the screening will be excused from participation. All candidates who choose to enroll in the TBI Study will also be offered the opportunity to enroll in the MRI Sub-study.

Confidentiality of Screening Materials.
For persons who do not meet the entrance criteria, all personally identifiable records used for screening will be destroyed.

Compensation for Participation.

Subjects will be paid for participation at the end of each phase of the study [$800 after Stage 1 (2 weeks In-Lab), $600 after Stage 2 (12 weeks in HTP), and $600 after Stage 3 (12 weeks in withdrawal and 26 weeks total) for a total of up to $2,000]. Subjects will be reimbursed for vehicle transportation costs from their residence to the lab for all visits (except screening), and for housing during Stage 1 if they live more than 30 miles from the UW-Madison campus. Reimbursement will be based on rates published by the University of WI for research subjects during the period of participation in the study. If a subject resides greater than 4 hours away from the lab, we will reimburse for mileage, if driving, or, if he/she chooses to travel by air, $250 + 25% of the cost of round trip airfare for all visits (except screening). Reimbursement for airfare will be based up the requirements of the UW Air Travel Policy.

PoNS^{TM} Device

Technical Description

The portable neuromodulation stimulator (PoNS^{TM}) device is held lightly in place by the lips and teeth around the neck of the tab that goes into the mouth and rests on the anterior, superior part of the tongue. The paddle-shaped tab of the system has a hexagonally patterned array of 143 gold-plated circular electrodes (1.50 mm diam., on 2.34 mm centers) created by a photolithographic process used to make printed circuit boards (Figure 6, lower right). The system has operational limits of 19 V (max) on the tongue (a nominal 5–7 k-ohm load). The biphasic waveform is specifically designed to ensure zero net dc current to minimize the potential for tissue irritation.

The PoNS 2.5 device has 2 user-controlled power buttons: "On" and "Off". The stimulus intensity level for both the Active and Control PoNS v2.5 devices will be set by the PI in cooperation with the subject at the first training meeting and cannot subsequently be altered by anyone other than the PI. It has stimulation level, time, and date logging capabilities to monitor protocol compliance when off site. This feature allows for assessment of stimulus dose response. The system delivers

triplets of 0.4 - 60μs wide pulses at 5 ms intervals (i.e. 200 Hz) every 20 ms (50 Hz) that has been designed to balance stimulus dynamic range and sensation quality [Bach-y-Rita, 1998; Kaczmarek, 2000 (a,b), 2012; Tyler, 2009]. At any instant in time, one of the electrodes in each of the 16 sectors on the array is delivering stimulation while the remaining electrodes serve as the current return path to ground. The voltage and pulse timing to each electrode is programmed in the device and cannot be altered.

The PCB is an industry-standard polyimide composite (P95, Isola Group, Chandler, AZ) that is USP Class VI compliant and meets ISO 10993 biocompatibility standards. The edges and top of the array tab are subsequently coated with a rugged USP Class VI biocompatible epoxy (Epotech 302-3M, Epoxy Technology, Billerica, MA). Therefore, the only materials that contact oral tissues are the gold electrodes and the biocompatible polymers. The remainder of the PCB and all electronic components, including battery, are in a sealed Delrin (USP Class VI compliant) enclosure.

For regulatory purposes the bottom of the device is labeled as follows:

"CAUTION - Investigational Device. Limited by Federal (or US) law to investigational use".
“University of Wisconsin-Madison
http://tcnl.bme.wisc.edu
Model number (TBD)
Version 2.5 S/N 30xx"

The enclosure measures 6.8 cm (w) x 4.4 cm (d) x 2.3 cm (h), and has total mass of 63.6 grams that remains outside of the mouth. This configuration produces a downward moment of only 0.0170 Nm about the front teeth. The paddle shape of the array allows the device to fit behind the front teeth of the upper palate and pivot on the lower incisors so that it may be held it in place with the lips and virtually no bite force is required. It allows subject with all but the most severe problems of bruxism or bite control modulation to hold the device in their mouth without difficulty during the training. The device also has a neck strap with a breakaway clasp to prevent it from falling from the user’s mouth while not presenting a strangulation hazard. The device should be stored and used at temperature between 5 and 50˚C (both in the lab at home by the user), and will be disinfected, inspected and tested after completion of use by each subject. A User Instructions document will be provided with each device for directions on operation and cleaning.

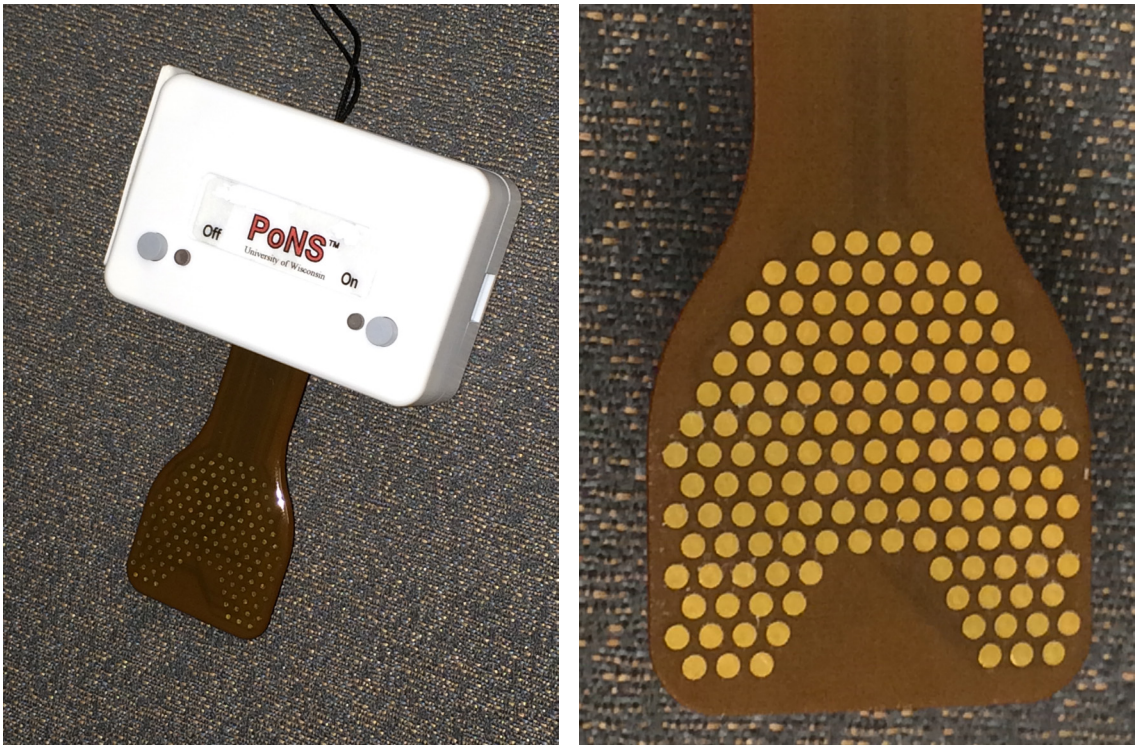

Figure 6. Image of the PoNS 2.5 device. The tongue array is on the right.

Device Deployment

For each CN-NINM training session, participants will place the tab of the PoNS™ a maximum of 45 mm into their mouth so that it contacts the anterior, superior surface of the tongue. At the outset of CN-NINM training, subjects with the Active device will be instructed to adjust the stimulus intensity, to reach their minimum sensation threshold, S_t, and then the maximum level, S_m, without discomfort. The experimental stimulation intensity, $S_{e(A)}$, is calculated and pre-set to a value of 75% of the stimulus dynamic range, $S_m - S_t$, so that $S_{e(A)} = S_t + 0.75(S_m - S_t)$. Sensory adaptation occurs after 1-3 minutes of stimulation, requiring a small increase in the absolute intensity level. To accommodate for this adaptation and to avoid startle due to sudden onset of stimulation at S_e, the intensity will ramp up from S_t to S_e over a period of 30 seconds.

The Control device appears physically identical, but uses a modified stimulus waveform parameter set designed to elicit a mild tactile sensation while minimizing the net energy delivered, thereby minimizing any neuromodulatory effect. This is accomplished by using a pulse frequency that is slowed to approx. $1/2400^{th}$ that of the Active device. The Control group will be identically instructed to determine S_t and S_m, with the Control experimental stimulus, $S_{e(C)}$ preset [$S_{e(C)} = S_t + 0.75(S_m - S_t)$], to encourage compliance with the protocol by assuring that the subject can feel the stimulation.

Study Procedures & Research Interventions.

General Testing Plan: Upon enrollment in this research study the following testing will be conducted at baseline. These tests will take approximately 3 hours. The primary and secondary measures will be used to assess the effect of the intervention on the symptoms of balance and gait deficit. The observational measures will provide essential data for potential future studies investigating CN-NINM intervention specifically targeted to a particular function deficit. These same metrics will be applied during the course of the study according to the schedule in Table 6.

- Primary outcome - NeuroCom CDP Sensory Organization Test (SOT) - Measures standing dynamic balance.
- 6-Minute Walk Test - over ground (6MWT) - Measures walking speed.
- Dynamic Gait Index (DGI) – Assesses walking, walking with head turns, over and around obstacles, and stairs.
- Physiological Stress Test - Resting & post exercise, heart rate, respiration, skin conductance, core temperature.
- Neurobehavioral Symptom Inventory (NSI) - Subjective inventory of TBI symptoms.
- California Verbal Learning Test (CVLT) – Assesses short- and long-term verbal memory.
- Brief Symptom Inventory 18 (BSI 18) – Assesses anxiety symptoms.
- Wechsler Adult Intelligence Scale – Symbol Search and Coding (WAIS-IV) – Assesses visual spatial abilities.
- Pittsburgh Sleep Quality Index (PSQI) - Subjective inventory of sleep habits, duration and quality.
- Headache Disability Index (HDI) – Assesses frequency & severity of headaches
- Quantitative Gait Assessment and EMG – Measures stride length and width, and muscle activation patterns during gait.
- Computerized video nystagmography (VNG) - Measures eye movement control.

There are items on the BSI 18 designed to identify depression and one item that asks specifically about suicide ideation. If the participant endorses this item at the level of "a little bit" or above, we will give him/her information about local (or national, if appropriate) suicide prevention resources. If the participant scores in the clinical range overall or on the Depression and/or Anxiety Scales, we will give him/her information about local or national mental health resources. To minimize risks posed by responding to items on the BSI 18, we will score the inventory while the participant is still on site. If the participant has a Global Severity Index T score of 63 or higher (according to the test norms), we will present him/her with information about local counseling and mental health resources. We will also use the BSI 18 norms to determine if the Depression and/or Anxiety scores are in the clinical range, and if so, will present the participant with information about resources.

CN-NINM Intervention

The goal of clinical training is to maximize the subject's participation in order to optimize the potential effects of CN-NINM. Subjects will complete intervention sessions three times per day for fourteen weeks (stages 1 and 2). Each 1 to 1.5 hour session may include: a) therapeutic exercise for isolated movement control, b) maximal challenge balance training, c) gait training, and d) breathing and awareness training. The intervention intensity in each modality is progressed according to each subject's particular initial symptoms while also being consistent and standardized for all subjects.

In stage 1, subjects will complete the twice-daily training sessions in the lab under the supervision of a Physical Therapist. In stage 2, subjects will complete the twice-daily training sessions at home for 6 days per week. They will return to the lab once each week for a training session to correct and progress their program. Every three weeks they will be re-tested on the primary and two secondary metrics. In stage 3, subjects will no longer use the PoNS device. They will return to the lab for testing every three weeks.

Table 4, below, represents the approximate timeline of events that will occur for each subject

during the study. A typical daily training schedule for the 14-week period of the study for each subject is shown in Table 5. Details of how subjects will be trained and the progression of the exercises are discussed in each subsection.

A Remote Audio Visual (RAV) training session may be performed in place of the in-lab weekly training session for weeks 3, 4, 6, 7, 9, 10, 12 & 13 in the event that the subject does not live locally, defined as a distance greater than a 4-hour drive from the lab, or for any instances, made on a case-by-case basis by the PI, when extenuating circumstances prevent a subject from attending an in-lab training session. In order for this to occur, subjects are required to have computerized video communication capability (e.g., Skype, FaceTime), and 2 assistants (one for safety and one to handle the video interface).

Table 4 – Event timeline for each subject in the study

	Initial Visit	**Stage 1 (2 weeks)**		**Stage 2 (12 weeks)**			**Stage 3 (12 weeks)**
	Prior to beginning intervention	In lab 2x/day	Last P.M. of 2nd week	At home 2x/day	In lab 1x/day per week	Every 3-weeks	Every 3 weeks
Screening, Health History	X						
Demographics	X						
Informed Consent	X						
Randomization	X						
Testing	X		X			X	X
CN-NINM Training		X		X	X		
Adverse events		X	X	X	X	X	X

Table 5 - Daily Training Program

Morning:	Movement Training (warm up)	10 minutes
	Balance Training with PoNS	20 minutes
	Gait Training with PoNS	20 minutes
	BAT with PoNS	20 minutes
	- 3 to 4 Hour Break -	
Afternoon:	Balance Training with PoNS™	20 minutes
	Movement Control Training	20 minutes
	Gait Training with PoNS™	20 minutes
Evening:	BAT with PoNS	20 minutes

Movement Training (warm up)

Movement training has 2 components. The first component is warm up exercises. Subjects perform these exercises to prepare the body for work. These include chin retractions, shoulder circles, scapular circles, pelvic tilts, pelvic figure 8's, and single leg figure 8's. All subjects will be trained in these exercises.

Movement Control Training

The second component of Movement Training is exercises for motor control. Subjects with neurological disorders typically develop compensations resulting in abnormal movement patterns. The exercises for this study are targeted to each subject's gait abnormalities. This targeted training helps the subject develop the proper control patterns and synergy needed for correct movement. This is accomplished by reinforcing correct vectors of control (speed, force, direction, continuity, and range of motion). Emphasis is on quality of movements performed with accurate control.

Exercises to improve gait may include lower extremity isolation, core strengthening, or upper extremity movement to improve arm swing. An example of a lower extremity exercise is a heel rocker. The subject takes a step, holds the feet in place, and rocks forward and backward. This promotes toe lift during heel strike, and ankle range of motion and strength during push off. The PT's will train the subjects in the exercises and monitor their progression. Subjects will be progressed to more challenging exercises as they improve.

Subjects may present with a variety of gait abnormalities, although based on prior experience, we anticipate that many of them will be similar. We will develop a catalog of movement exercises that are most likely to be effective with this population for use in subsequent studies. In the case where a subject exhibits normal gait, we will not assign any movement exercises to that subject. Regardless of their gait presentation, all subjects will perform gait training for this intervention.

Balance Training (with PoNS)

Balance training with CN-NINM focuses on developing stable balance during upright standing posture, and on establishing a sense of confidence in the subjects' posture and stability. The goal of balance training is to create body awareness, correct postural alignment, and improve balance by recalibrating proprioceptive, tactile and vestibular inputs. The balance training begins at whatever initial condition the subject can sustain for 2 minutes, e.g. feet at shoulder width on a hard surface, eyes open and touching a training table with one or two fingers for tactile-spatial orientation. Training will progress from their initial state with increasingly challenging stance postures, up to feet together on a soft base with eyes closed and without tactile support. During this period, subjects will also receive verbal and physical cues to maintain correct postural orientation in order to retrain their nervous system to accept a new postural set point. This is a crucial progressive postural awareness and retraining process that will help the subject to confidently maintain their center of mass within their base of stability without visual or tactile aids.

Initial balance training requires that the subject perform three progressively challenging 2-minute balance trials while using the PoNS™ device to determine their current functional capacity. The challenge levels are on a continuum from "able to perform easily" to "able to perform with difficulty" in that task. This initial assessment is followed by a 20-minute session in a comfortable but challenging position with the PoNS™ device. After the initial training, all subsequent balance training consists of a 20-minute session with the PoNS™ in progressively challenging conditions, e.g. standing with feet closer together on a stable surface, then progressing to a compliant surface (e.g. AirEx foam pad). Foam pads will be supplied to the subject for use during the HTP. Trainers will use the Balance Training Flow Chart for balance progression.

Subjects will use headphones to listen to audio programs with ambient sounds during balance training. This will help mask out external environmental noise that may distract the subject from

their balance task. These audio tracks are installed as MP3 files on TCNL's iPod. The audio files will be supplied to the subject for use on the MP3 or other audio device of their choice during the HTP.

Gait Training (with PoNS)

Deficits in gait speed and spatiotemporal symmetry are common in people with neurological disorders. The goal of gait training is to achieve normal gait. During gait training subjects will walk on a treadmill and over ground at progressive speeds and challenges to re-establish appropriate dynamic balance and gait patterns. Particular attention is given to symmetry of the gait pattern, including dynamic weight transfer, stride kinematics of hip, knee, and ankle flexion/extension, and of the stance and swing phases. In-lab gait training will be performed on the treadmill for the first week, and on the treadmill and over ground the second week. It is recommended that subjects use a treadmill for at least 50% of the HTP to control and monitor their training.

Treadmill Training

The physical therapist will work with the subject to determine an appropriate starting point. As in balance training, the appropriate challenge on the treadmill is assigned relative to the subject's baseline.

On the treadmill, the first 5 minutes are done at a comfortable pace. Every 5 minutes, the challenge should be increased. This is done by changing a variable. Variables include:

- Speed: Increasing the speed increases the challenge.
- Grade: An incline increases effort and affects the work of ankle dorsiflexors, knee and hip flexors.
- Handrails: Using the handrails allows greater stability. Increase the challenge by decreasing hand contact time and force on the rails, with the goal of achieving arm swing commensurate with normal gait.
- Head movement: Turning the head while walking increases the challenge.

For the last 5 minutes, the subject should work at a challenging but comfortable pace. They should be able to maintain good posture and quality of movement. It is expected that each session of gait training will start at a higher level than the previous session.

Over Ground Training

Subjects will work with the trainer on walking over ground to transfer skills learned on the treadmill to regular gait. Trainers will use verbal and tactile cues as needed to correct posture and abnormal movement patterns during gait.

Breathing and Awareness Training (BAT) (with PoNS)

The goal of breathing and awareness training is for the subject to develop relaxed and mindful breathing and awareness. A BAT session requires 20 minutes of continuous PoNS™ use with relaxed attention, breathing, and concentration. Visualization and breathing are the focus points. BAT, also referred to as relaxation training, can have a significant impact on training progress. A 20-minute BAT session should be performed in a supported body position, uninterrupted with eyes closed, and with no distracting sounds. Headphones and MP3s with ambient sounds are sometimes used for BAT sessions.

Data Collection

The scheduled assessments and frequency in which they will be performed is listed in Table 6. If any anomalies or unforeseen circumstances occur (e.g., an unexpected delay occurs between baseline testing and start of treatment, a significant difference is observed in the assessment results from one test point to the next, etc.) additional assessments may be performed. The decision to perform additional assessments will be made on a case-by-case basis at the discretion of the PI. The following exceptions may also be made on a case-by-case basis at the discretion of the PI: 1.) test points 1, 2, 6 and 10 may be performed on more than one day, 2.) test point 2 may not occur before the completion of stage 1, but may be performed within 2 days after the end of stage 1, and 3.) test points 6 and 10 may be performed within 2 days before or after the end of stages 2 and/or 3.

Prior to collecting any of the data scheduled in Table 6, all subjects will have been consented into the study and completed all of the screening stages. All data to be collected are study-related. This investigation will not access any health records of subjects. The health information requested in our screening questionnaire is only that which is necessary to correctly ascertain subject eligibility for participation in the study. Health information is reviewed with the subject in private by the investigators. Subjects are fully clothed at all times.

Subjects may be videotaped during evaluation or during treatment sessions for use in presentations at professional conferences to demonstrate changes in symptoms due to the intervention. Subjects will be asked for their permission to be videotaped and their acceptance or rejection of permission to do this is obtained during the written informed consent procedure. Subjects will be given the option to be recorded but not allow the video to be kept indefinitely. If subjects make this choice we will destroy the videotape when study analyses are complete. Subject faces may be identifiable, but the subjects will not be identified by name or any other personal identifier in these videotapes.

We are not utilizing computer-generated questionnaires. All data collection materials are coded with unique subject identification numbers and the master log of the subject identifiers is stored separately from the health information and informed consent documents. Only members of the core research team have access to the list that pairs subject names with numbers. Subject data folders are maintained in a secure file cabinet where only the core research team has access. Video files are stored on encrypted external hard drives. The external hard drives are encrypted with password protection and access is limited to the TCNL physical therapy staff.

Table 6. Study data to be gathered about each subject:

Assessment	Data	Frequency
General Health Questionnaire	History of brain injury, communicable diseases, diabetes, oral health, seizures, surgeries, medications, rehab, major co-morbidities; concussion history; ability to walk 20 minutes.	• Screening

Assessment	Data	Frequency
Demographic Survey	Name, address, phone, email, date of birth, ethnic background (optional), education, gender	• Screening
Cognitive Learning Quick Test (CLQT)	Attention, memory (working, procedural, semantic, episodic), and executive function	• Screening
Sensory Organization Test (SOT)	Balance	• Screening • End of stage 1 • Every 3 weeks during stages 2 & 3
6-Minute Walk Test (6MWT)	Gait	• Screening • End of stage 1 • Every 3 weeks during stages 2 & 3
Dynamic Gait Index (DGI)	Gait	• Screening • End of stage 1 • Every 3 weeks during stages 2 & 3
Quantitative Gait Assessment*	Gait	• Baseline • End of stage 1 • End of stage 2 • End of stage 3
Physiologic Stress Test	Resting & post exercise: HR, respiration, skin conductance, & core temperature	• Baseline • End of stage 1 • End of stage 2 • End of stage 3
Neurobehavioral Symptom Inventory (NSI)	TBI symptoms	• Baseline • End of stage 1 • End of stage 2 • End of stage 3
California Verbal Learning Test (CVLT)	Short and long term verbal memory	• Baseline • End of stage 1 • End of stage 2 • End of stage 3
Brief Symptom Inventory 18 (BSI 18)	Anxiety symptoms	• Baseline • End of stage 1 • End of stage 2 • End of stage 3

Assessment	Data	Frequency
Wechsler Adult Intelligence Scale – Symbol Search and Coding (WAIS-IV)	Visual spatial abilities	• Baseline • End of stage 1 • End of stage 2 • End of stage 3
Pittsburgh Sleep Quality Index (PSQI)	Sleep habits, duration and quality - Subjective inventory	• Baseline • End of stage 1 • End of stage 2 • End of stage 3
Headache Disability Index (HDI)	Headache frequency & severity	• Baseline • End of stage 1 • End of stage 2 • End of stage 3
Video Nystagmography**	Dynamic oculomotor control behavior	• Baseline • 1st day of stage 1 (pre-treatment) • End of stage 1 • End of stage 2 • End of stage 3

* Quantitative Gait Analysis: Muscle activation patterns for two 60-second treadmill-walking trials and one 60-second period of over-ground walking will be collected using electromyographic (EMG) records of bilateral muscle activity on the lower and upper leg. At the same time, accelerometers placed at the waist and the heels will be used to index and assess the gait cycle. Study personnel will provide stand-by assist to guard for loss of balance.

** Video Nystagmography: Six standardized VNG tests (3 static: Horizontal fixation, Vertical fixation, Spontaneous nystagmus; and 3 dynamic: Random saccades, Smooth pursuit, Optokinetic) will be used to test and score subject eye fixation stability and movement control. VisualEyes™ is the standard for video nystagmographic observation, recording, and playback of ocular movements. Because the visual system is a primary contributor to motor, social, cognitive, and emotive functions, and oculomotor deficits require a significant, if unconscious, amount of attention in persons with TBI. Consequently, oculomotor monitoring methods that can observe this demand on the injured brain can be used to quantify any effects of neurorehabilitation due to the CN-NINM intervention.

Data Safety and Monitoring Plan

All study records will be structured and maintained as required under 21 CFR 812.140, and the investigators will make reports as required under 21 CFR 812.150.

Unanticipated adverse device effects

1. The PI and the Medical Advisor will immediately conduct an evaluation of any unanticipated adverse device effect.

2. If the investigators determine that an unanticipated adverse device effect presents an unreasonable risk to subjects, they shall terminate all investigations or parts of investigations presenting that risk as soon as possible. Termination shall occur no later than 5 working days after the investigators make this determination and no later than10 working days after the investigators first received notice of the effect.
3. If the investigation was terminated due to unanticipated adverse device effects, the investigation may not resume without IRB approval.

Missing Data

We do not plan to recruit additional subjects to replace dropouts because we have increased the sample size to account for potential dropouts. We will make efforts to obtain follow-up data on subjects who withdraw from treatment. Due to the pilot nature of the study, we do not plan to do imputation for missing data points. Instead we will do a complete case analysis as well as a sensitivity analysis to examine the effect of the missing outcome data.

Compliance

We will respect the principle of Intention to Treat and will not remove subjects for protocol non-compliance. We have established the following *a priori* definition of compliance:

> Subjects who miss more than 20% during any one-week period will be considered noncompliant with the training protocol.

Data Access and Management

The TCNL will work with ICTR to develop a database in OnCore® that will capture test scores (both written and electronic), and associate the unique identifier to the file for each subject. The PI, Study Coordinator, Clinical Monitor, PTs and designated study staff will have access to the database during the course of the study. It will be the responsibility of the PI to ensure data are entered into the OnCore database in a timely and consistent manner. Data are recorded on data collection forms, transcribed onto case report forms (CRFs) and entered into the OnCore database by study staff. An independent staff member reviews the data collection forms, CRFs and verifies that data were entered correctly. Treatment group and personal identifying information will not be entered into the OnCore. These records will be maintained in a separate secure file location to maintain control of the study and avoid potential experimenter bias.

Data captured on all source documents from the study (demographics, Health Questionnaire, CRF's, ADE's, SAE's, assessments) will be entered into an OnCore database managed by the Data Monitoring Committee (DMC) of the Institute for Clinical and Translational Research (ICTR), an NIH-funded program comprising UW faculty not associated with the funding source or TCNL and knowledgeable in the Neurorehabilitation field. A US Army Medical Department Medical Research and Materiel Command (USAMRMC) Monitor has been assigned to protocol 2014-0002 and will perform site visits and review study data. The USAMRMC Monitor will compare data on data collection forms and case report forms (CRFs) with data entered in OnCore, including but not limited to accrual, study data, adverse events, serious adverse events and protocol deviations. Any discrepancies will be issued and resolved using the querying functionality in OnCore. Results of the monitoring performed by the USAMRMC Monitor are available for DMC review. Additionally, the study will be monitored annually by the DMC. The DMC members will review protocol-specific reports created by statisticians using data pulled from the ICTR OnCore clinical

research management system. The ICTR DMC will meet with the Investigators to review data, analysis, subject accrual including rate and origin of subject referrals, and any adverse events. An interim analysis of study results may be performed and source documents may be reviewed to allow the DMC to independently judge whether the overall integrity and conduct of the protocol remain acceptable based on data provided and reported by the Principal Investigator. The DMC will make recommendations to the Principal Investigator that could include actions of continuation, modification, or termination. The ICTR DMC will review all manuscripts prior to submission for publication. Written minutes of the ICTR DMC meeting in which this study is reviewed will be prepared and provided to the investigators and the SMPH Office of Research and Graduate Studies."

Confidentiality

The PI will keep records of the subjects' participation in the research. To protect subject privacy, all research-related records will be labeled with a coded identifier that is unique to that research participant and will not include the subject's name, service, or social security number. The PI will keep a separate file that creates the only link between participant identifier and research records in a locked file in a locked records room. Video files are stored on encrypted external hard drives. The external hard drives are encrypted with password protection and access is limited to the TCNL study team. We use VeraCrypt® and the lab's unique secure key code. All TCNL evaluation and/or treatment related records used for the research study or any of research-related records necessary for evaluation will be secured in HIPAA compliant medical records in the TCNL.

Details regarding identities of the participants will be protected per HIPAA regulations regarding Protected Individual Information. Physical files fill be stored in a separate locked file in the locked records room for 7 years. Video files are stored on encrypted external hard drives.

To protect subject privacy during RAV training sessions, Skype and FaceTime call are only made using a TCNL account and physically secure TCNL computer. Both the accounts and the computer are password-protected and accessible only by study PTs, the Study Coordinator and PI. Contact information (e.g., subject name and phone number) may be required to perform RAV training sessions. The PT only creates user accounts for the subjects participating in RAV training and provides the information necessary to access calls from the TCNL. Security certificates are issued when the call is made from the TCNL and can only be received and accessed by an authenticated user (the study subject). The call stream is made using a secure protocol and encrypted by both Skype and FaceTime.

Study staff may use e-mail to communicate with research subjects. Prior to any email exchanges, a study staff member will review the Guidelines for Using Email and the subject must agree to use email communication by signing a Provider-Patient Email Information and Consent form. The information contained in emails will be limited to training program information, such as a summary any changes to training program, answers to any questions that were asked regarding training during a training session and/or a copy of the subject's home exercise program. The subject home exercise training program contains the exercises/physical therapy training to be performed by the subject during the at home training stage and may include a description and/or photographs of the subject performing the exercises/physical therapy training. Images used in the training programs will not contain full-face photographs. All emails to subjects will be sent from UW/wisc.edu accounts; personal or home email accounts will not be used.

When the results of the research are published or discussed in conferences, no information will be included that would reveal the subjects' identity to others.

Authorized representatives of the following groups may need to review this research as part of their responsibilities to protect research participants:

- U.S. Army Medical Research & Materiel Command (MRMC) Institutional Review Board
- U.S. Army Human Research Protections Office and other DOD offices charged with oversight of human research
- U.S. Army Medical Materiel Development Activity
- U.S. Department of Health & Human Services Office for Human Research Protections
- University of Wisconsin – Madison Health Sciences Human Subjects IRB.
- University of Wisconsin –Institute for Clinical and Translational Research – Data Monitoring Committee.

Coded data (results only) will be shared with the MRMC via secure email for purposes of informing future studies.

Statistical Considerations

Sample Size Estimation

This study will be an experimental investigation and will employ descriptive statistics to discover the mean changes from baseline for Control and Active, and then calculate true effect sizes and corresponding variances both within and between the two treatment groups. It is a first step in an iterative, incremental process to generate enough data to make reasonable power calculations in subsequent controlled studies. We believe this approach is justified because we have a very small data set from which to draw estimates of effect size, and none regarding a control group response. Our estimates are based on large changes in SOT scores in the previous pilot study involving 4 civilian subjects with mild to moderate concussive TBI using an Active device.

For this study we have assumed that the Control group response will be half that of the active group. Using a two-sample *t*-test to estimate the sample sizes of the experimental group and the control group, and assuming equal variances in the two treatment groups with $\sigma = 0.5$; a 2-tailed significance level, $\alpha = 0.05$; the true difference of the means = 0.5, and setting the power at 0.80, we calculate that a cohort of 17 subjects in each arm is sufficient. Based on our previous experience in two controlled pilot studies, we estimate a drop-out rate of not more than 25%. In using this conservative estimate, we believe the study is sufficiently powered at 22 subjects per treatment group (44 subjects total) to generate the data necessary to calculate effect sizes and cohorts in the future.

Primary Endpoints and Observational Measures

The primary outcome measure is the Composite SOT score. Observational measures are the Neurobehavioral Symptom Inventory, California Verbal Learning Test, Brief Symptom Inventory, Wechsler Adult Intelligence Scale – Symbol Search and Coding, Pittsburgh Sleep Quality Index, Headache Disability Index, Physiologic Stress Response, Quantitative Gait Assessment using EMG, 6 minute walk test, Dynamic Gait Index, and Computerized Video Nystagmography. The observational measures will provide essential data for potential future studies investigating CN-NINM effects on a particular functional deficit.

Data Analysis

An interim analysis will be performed when 22 subjects have completed the second phase (At-Home Training), and a full investigation at the completion of the study.

• Interim Analysis: In compliance with the request from the funding agency (US Army Medical Research and Materiéls Command), a single independent interim analysis will be conducted under a Statistical Analysis Plan (SAP) developed for this study and reviewed by the Biostatistics Group at ICTR. The primary independent measure: Active vs. Control PoNS. The interim analysis will be performed after 22 subjects have completed the 6th data point (at 14 weeks - the completion of the At-Home phase of the study)

The primary analysis will be conducted under the intention-to-treat principal, and all data will be included. We will not conduct a missing data imputation analysis. Every attempt will be made to obtain follow-up data on subjects who withdraw from treatment.

The interim analysis will be performed using the primary efficacy endpoint for the data of first 22 subjects that have completed the Week 2 and Week 14 visits. The two treatment groups will be listed only as "Group 1" and "Group 2" to maintain the blinding of the treatments.

The number of subjects (most likely 11), and both the Mean and standard deviation (SD) for each group will be calculated by the study statistician. Student's *t*-test will be used to compare the two means. A *p*-value will be generated but no decision will be made at the interim analysis time, and the trial will continue to completion unless there are serious safety concerns.

The study statistician will present the interim analysis results (*n*, Mean, and SD) by treatment group in unblinded fashion including the *p*-value to the DSMC.

Also, the statistician will present these results to the sponsor of the study but in the blinded fashion by identifying the treatment groups only as Group 1 and Group 2.

• Final Analysis: A repeated-measures ANOVA will be performed on the data derived from the 10 outcome measures gathered for each subject. Both individual (n=1) and cohort data set (n=22) analysis will be conducted to identify both specific and generalizable effects of the intervention methods. Sub-analysis by gender and age in both treatment groups will also be performed to assess the relative response of females and males.

Evidence of efficacy will be determined by significant differences in outcome measures both within and between groups after both the two-week intensive phase and at conclusion of the 14 weeks. Changes of 8 points in the SOT are considered clinically significant.

Data logs for each individual will be entered into the OnCore Data Management System used by ICTR for independent analysis and experimental verification.

Risks/Benefits Assessment

Potential Risks

1. Electrotactile Stimulation. The sensation could become uncomfortable at high intensity levels, which could be distressing to the subject. Some subjects have reported a mild tingling sensation that lasts from several minutes up to an hour after the stimulus is turned off. Although this sensation may feel unusual, it is not reported to be annoying and does not interfere with normal activities.

2. Excess Salivation. Subjects may experience excess salivation due to the presence of the PoNS in the mouth.
3. Disease Transmission. Because more than one subject may use the same device, there is a potential for disease transmission.
4. Falls. Subjects will be performing balance and gait tasks that may cause them to lose their balance or fall.
5. Decreased Performance or No Improvement in Symptoms. There is a risk that when subjects stop using the device, they will not retain any improvements they may have gained while doing CN-NINM training and their function will return to its prior level. There is also the possibility that CN-NINM training will not improve their symptoms.
6. Confidentialty. There is a risk that someone who is not involved in performing or monitoring this study could view study information.

Minimizing Risk

1. PoNS Stimulation. The experimental apparatus cannot deliver a dangerous level of electric current. Furthermore, subjects will pre-set the intensity level to within the preferred stimulus intensity range, between sensation threshold and maximum level without discomfort. Subjects can remove it from their mouth if it does not feel comfortable.

   Although no data are available on the effects of long-term electrotactile stimulation in the mouth, we have never observed, nor had report of any tissue irritation following tongue stimulation on over 300 subjects and conducted under UW-Madison HS-IRB Protocols H2000-0119, H2000-0527, H2001-364, H2004-375, H2005-0192, H2007-0251, H2008-0057, H2010-0405, H2011-0335, and 2012-0482. We do not anticipate any problems here.

2. Excess Salivation. Salivation is a natural response to the presence of anything in the mouth. Subjects typically learn to regulate saliva by learning to swallow with the device in their mouth. This is less a risk than a side effect.

3. Disease Transmission. This risk is reduced to an extremely small level by two mechanisms. First, subjects reporting transmittable diseases, or having apparent oral lesions or inflammation will not be allowed to participate in the study. Secondly, the electrode array will also be cold sterilized according to the protocol developed & approved by UWHC Infection Control Office.

4. Falls. Subjects will be protected from falls wherever possible. During in-lab balance training the subject is surrounded on 3 sides by a custom laboratory bench designed for this purpose, while the treadmill has a handrail that surrounds the participant on 3 sides. Additionally, subjects may be required to wear a safety belt during gait tests and training to prevent falls.

   For RAV training sessions, two additional assistants must be present. The first assistant protects the subject from injury during performance of the various tasks (balance, gait, exercise). The second assistant handles the computer or video interface, and directs the camera angle at different positions in order for the physical therapist (PT) to observe the training as it occurs. The training session is interactive and the PT instructs the first assistant how and when to support the subject. The interaction that occurs in the RAV session with the PT, the assistant, and the subject will minimize the risk of falls in a manner equivalent to that of an in-lab training session.

During in-lab training, the PT will provide stand by assist during testing and training. Subjects must demonstrate safe, independent performance of all at-home training tasks before they are assigned these activities to be performed at home. The at-home training program is designed for each individual study subject, specifically to reduce the risk of falls

5. Decreased Performance or No Improvement in Symptoms. Subjects will be educated in regards to awareness of symptoms and the potential for a return to their prior level of symptoms once they stop using the device.

6. Confidentiality. The PI will keep records of the subjects' participation in the research. To protect subject privacy, all research-related records will be labeled with a coded identifier that is unique to that research participant and will not include the subject's name, service, or social security number. The Clinical Monitor will keep a separate file that creates the only link between participant identifier and research records in a locked file in a locked records room. Video files are stored on encrypted external hard drives. The external hard drives are encrypted with password protection and access is limited to the TCNL study team. We use VeraCrypt® and the lab's unique secure key code. All TCNL evaluation and/or treatment related records used for the research study or any of research-related records necessary for evaluation will be secured in HIPAA compliant medical records in the TCNL.

   Details regarding identities of the participants will be protected per HIPAA regulations regarding Protected Individual Information. Physical files fill be stored in a separate locked file in the locked records room for 7 years. Video files are stored on encrypted external hard drives.

   E-mail communication with research subjects will be performed in compliance with UW Policy and Procedure 8.6 E-mail Communications Involving Protected Health Information. All emails will be sent from UW/wisc.edu accounts; personal or home email accounts will not be used. The information contained in emails will be limited to training program information, such as a summary any changes to training program, answers to any questions that were asked regarding training during a training session and/or a copy of the subject's home exercise program. The subject home exercise training program contains the exercises/physical therapy training to be performed by the subject during the at home training stage and may include a description and/or photographs of the subject performing the exercises/physical therapy training. Images used in the training programs will not contain full-face photographs.

   When the results of the research are published or discussed in conferences, no information will be included that would reveal the subjects' identity to others.

In the event that a subject is injured while participating in the study, the investigators will follow the established standard UW HS-IRB protocol for handling and reporting accidents and injuries. The PT will be 'hands-on' in all aspects of the training, and will review both daily and weekly progress of each patient for the two weeks of study training. The PI will also review both daily and weekly progress of each patient for the 14 weeks of the study. If at any time there are indications that the patient is in distress, the training session will be stopped, and both the circumstances and the plans for progression will be evaluated and discussed with the patient. Because the primary interest is in maximizing beneficial outcome for all subjects, we will take whatever actions necessary to ensure their safety and wellbeing.

Potential Benefits

Based on our preliminary results, we anticipate that some subjects may derive some improvement in symptoms from participation. If successful this treatment could reduce rehabilitation time, improve return toward normal health status, and enhance retention through reintegration.

Risk/Benefit Ratio

The anticipated benefits from this intervention include symptom improvement that will affect the subject's quality of life and functional capacities. These benefits far outweigh the risks associated with the study. With proper instruction and monitoring, these risks are managed and minimized.

Benefits to Society: If successful, this treatment could improve the rehabilitation outcomes of people with chronic symptoms due to a TBI. Recovery from symptoms and improvement in function will allow this population to return to normal social interaction, participate in activities, and contribute to society.

The scientific benefit is that the results obtained will likely far exceed those already obtained, and will lend greater credibility to future applications of the intervention.

Adverse Events, Unanticipated Problems

Adverse Events

The most likely adverse events are detailed in the section "Risks/Benefits Assessment," above. All possible adverse events are identified and characterized in the appended PoNS Investigators Brochure and the PoNS Device Safety Chart.

Reporting Unanticipated Problems Involving Risks to Subjects or Others, Serious Adverse Events and Deaths.

All unanticipated problems involving risk to subjects or others and all serious adverse events will be promptly reported by the PI and the Medical Advisor according to proscribed procedure: http://my.gradsch.wisc.edu/hrpp/10122.htm

Withdrawal from Study Participation

Subjects are free to withdraw from the study at any time without penalty or explanation. The intervention is relatively rigorous in terms of degree and continuity of effort. Some participants may find it more challenging than expected.

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Addendum – MRI Sub-study

Title: Characterizing Functional and Structural Brain Changes Following Cranial Nerve Noninvasive Neuromodulation (AKA MRI Sub-study).

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22 March 2016

Modified: 12 September 2016
13 October 2016

Key Personnel

- Jenelle Fuller, MRI technologist, will be performing the MRI scans.
- Kelli Hellenbrand, MRI technologist, will be performing the MRI scans.
- Sara John, MRI technologist, will be performing the MRI scans.
- Danielle McIntosh, B.S., Research Intern, will be performing informed consent.
- Kelly Miller, B.S., Clinical Monitor, will be responsible for randomization of subjects. She will also be responsible for both monitoring regulatory compliance and reporting unanticipated problems and adverse events.
- Veena Nair, PhD, will be accompanying participants to the MRI scans and perform statistical analysis of the MRI Sub-study data.
- Vivek Prabhakaran, M.D., Ph.D., Co-PI, will direct the overall project, assist in interpretation and reporting of experimental results, review images for adventitious findings and report both unanticipated problems and adverse events.
- Alexander Remsik, PhD, will be accompanying participants to the MRI scans and perform statistical analysis of the MRI Sub-study data.
- Amy Remm, B.S., Clinical Monitor, will be responsible for regulatory reporting with the US Army Clinical Research Monitor (USAMMDA).
- Kim Skinner, M.S.P.T., Co-Investigator, Study Coordinator, will be responsible for performing screening.
- Mitchell Tyler, M.S., P.E., Co-PI, will direct the overall project, assist in interpretation and reporting of experimental results, and report both unanticipated problems and adverse events.

Study Objectives

The **primary objective** of this sub-study is to investigate the cortical and non-cortical changes induced by CN-NINM training in patients with symptoms due to a mild to moderate TBI. In this sub-study, we will use functional magnetic resonance imaging (fMRI) tasks to identify the anatomical locations of the modulated neural activity induced by the tongue stimulation and how these changes can produce the behavioral and subjective improvements seen in patients with balance disorders after therapy with CN-NINM. A **secondary objective** is to evaluate the relationship between changes in brain and behavioral measures over time. We will evaluate the **hypothesis** that CN-NINM induces greater neuroplasticity in the Active than the Control group in areas involved in balance processing as well as by evaluation of major cranial nerves involving the tongue: trigeminal, CN-V, and facial, CN-VII and CN XII. An optic flow fMRI task will be used to evaluate areas involved in balance processing. A jaw-clenching fMRI task will be used to evaluate CN-V; A Tongue-thrust fMRI task will be used to evaluate CNV, VII, and CN-XII. This stimulation also has a cascading effect on neural activity in the brainstem (pons varolli and medulla), and cerebellum and higher order cortical structures such as the anterior cingulate. Neuroplasticity changes will be measured by task fMRI activation changes.

In addition to traditional task fMRI we will also use **advanced neuroimaging methods** to characterize functional and structural brain connectivity patterns in this population. **Resting state fcMRI** (rs-fcMRI) refers to the temporal coherence between different brain regions indicated by spontaneous blood-oxygen-level dependent fluctuations in the absence of any active task performance, i.e., when the brain is "at rest" [1-3]. Strong coherence has been identified between clinically relevant networks such as the somatomotor, visual, language, memory, and other functional networks and several studies have demonstrated test-retest reliability of rs-fcMRI measures. In a group of TBI patients in the sub-acute to chronic stage, Bonelle and colleagues recently showed that abnormality in default mode network (DMN) function is a sensitive marker of impairment of sustained attention [4].

To identify abnormal structural connectivity patterns in the concussed brain we will collect Diffusion Tensor Imaging (**DTI)** data. DTI evaluates integrity of the white matter (WM) by exploiting the fact that water diffuses faster along the main axis ($\lambda 1$) of fibers compared with diffusion perpendicular to fibers ($\lambda 2$ and $\lambda 3$) [5-8]. Neuroplasticity changes over time from pre- to post-stimulation will be measured by functional connectivity as measured by rs-fcMRI and diffusivity and tractography measures as measured by DTI.

Methods

Sub-study Inclusion and Exclusion Criteria.

In addition to the inclusion and exclusion criteria in the main study, we will also exclude any subjects with contraindications to MR (e.g., claustrophobia, metal implants, etc.).

Subjects must also complete the MRI Screening Form (UWH# SR301436-DT [Rev. 02/17/14]) to determine final MRI Sub-study eligibility. The MRI Screening Form will be reviewed with subjects prior to each scanning session for any changes that could affect eligibility.

Total Subjects.

The sub-study will enroll 26 subjects (13 subjects in the Active PoNS™ group; 13 subjects in the Control group).

Consent Process.

After completion of the TBI Study consent, the Study Coordinator will also provide informed consent for the MRI Sub-study and explain that participation in the sub-study is optional. All candidates will be informed that declining participation in the MRI Sub-study will not affect their participation in the TBI Study. The PIs for both studies will be available to answer candidates' questions. All candidates are free to decline participation in either study without question if they so choose. Subjects will be given a copy of the signed consent form(s).

Compensation for Participation.

Subjects are eligible for the main TBI Study compensation; there is no additional compensation for completion of the MRI Sub-study.

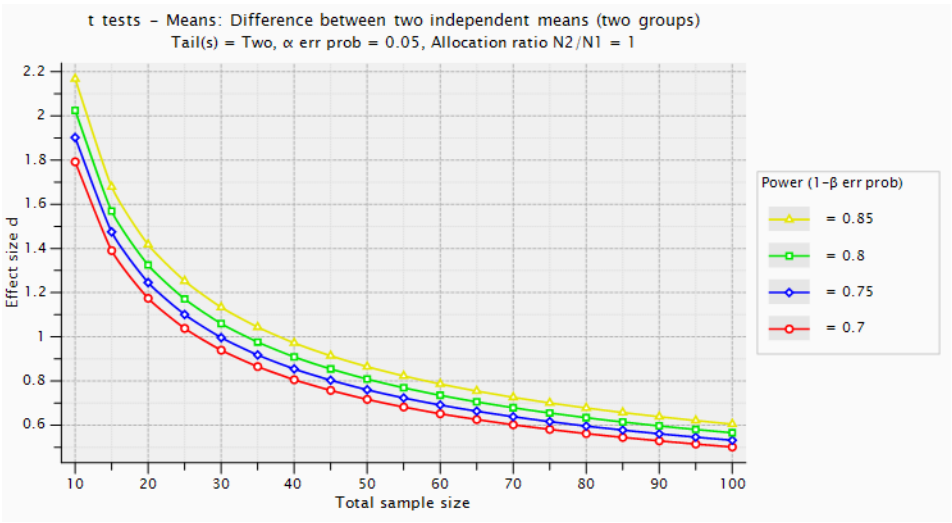
Data Points

The imaging scans performed in this sub-study will coincide with the 4 major data collection points in the main study (Table 1): (1) at baseline (after enrollment but before training has begun); (2) at the end of the 2-week in-lab training period; (3) at the end of the 12-week at-home training period; and (4) at the end of the 12-week withdrawal period. For data consistency, every attempt will be made to schedule MRIs on the exact dates listed in Table 1 or within 2 business days after that date. If any anomalies or unforeseen circumstances occur (e.g., inability to schedule MRI scans due to scheduling conflicts, etc.) MRIs may be performed at the next closest in-lab training session or scheduled test point. The decision to perform MRIs outside of the visit schedule listed in Table 1 will be made on a case-by-case basis at the discretion of the PI.

Table 1 – Event timeline for each subject in the study

	Initial Visit	**Stage 1 (2 weeks)**	**Stage 2 (12 weeks)**	**Stage 3 (12 weeks)**
	Prior to beginning intervention	Last day of 2nd week	Last day of 12th week	Last day of 12th week
Informed Consent	X			
fMRI Sub-study	X	X	X	X
Adverse events		X	X	X

Sample size consideration: Power calculation is based on 2-sample t-test using the standard G*Power 3.1.9.2 package [9, 10]. With n = 13 patients in the Active PoNS group, and n=13 in the healthy control group, at a two-sided significance level of 0.05, we will have 85% power for detecting an effect size of 1.2 standard deviations (see figure below) in our primary outcome measure of signal change in task fMRI. Our data from our preliminary study with N=12 subjects was sufficiently powered to detect a significant effect of the intervention on brain changes as assessed by similar task fMRI [11] as proposed in this study.



Functional MRI Scanning and Analysis

All MRI data will be acquired with the University of Wisconsin-Madison Department of Radiology's 3T MRI scanner (GE Healthcare, Waukesha, WI) using an 8-channel brain coil. The T1-weighted anatomical scans will be acquired with a 3D-SPGR pulse sequence (TR= 10 ms, TE= 3 ms, 94 slices, 0.938 x 0.938 x 1.5 mm resolution). One functional scan will be acquired with a T2*-weighted gradient-echo echo-planer imaging sequence (time repetition = 2000 ms, acquisition time = 1800 ms, echo time = 30 ms, flip angle = 75 degrees) to acquire BOLD signal in a 128x128 matrix over a 12cm^2 FOV and 10 slices giving a resolution of 0.938 x 0.938 x 2.0 mm^3 centered over the brainstem and cerebellum. This increase in resolution will allow for multiple measurements of BOLD signal in each brainstem nuclei, which are on the order of 3x3x3 mm^3, smaller than a single voxel at standard fMRI resolutions [12]. A second functional scan will be acquired with a T2*-weighted gradient-echo echo-planer imaging sequence (time repetition = 2000 ms, acquisition time = 1800 ms, echo time = 30 ms, flip angle = 75 degrees) to acquire BOLD signal over 30 axial slices (3.75 x 3.75 x 4.5 mm resolution) covering the entire brain.

Rs-fcMRI data: A 10 minutes eyes closed, whole brain resting state scan will be collected using gradient-echo echo-planar imaging with the following parameters: TR = 2.6 s, TE = 22 mms, field of view = 22.4 cm, flip angle = 60°, 40 sagittal slices, acquisition matrix = 64 x 64, 3.5 mm isotropic voxel size, 162 time-points. T1-weighted anatomical images will be collected for overlaying the fMRI data using a FSPGR BRAVO sequence (TR = 8.132 ms, TE = 3.18 ms, TI = 450 ms) over a 256 x 256 matrix and 156 slices (flip angle = 12°, FOV = 25.6 cm, slice thickness = 1 mm).

Resting data will be preprocessed as in our earlier studies [13] using FSL and AFNI [14-16]. Data will be motion and slice-time corrected, spatially smoothed using a Gaussian kernel of 6 mm FWHM, and scaled to the grand mean. Time series will be band-pass filtered (0.005 – 0.08 Hz), linear and quadratic trends removed. To control for participant head motion and physiological noise, motion parameters, white matter and CSF signal will be regressed from the time series. The

residual time series will be demeaned, re-sampled to 3 mm^3, registered to MNI152 standard space using FSL's FLIRT program.

Seed-based connectivity analyses: Time courses will be extracted from 160 seed regions of interest (ROIs) previously defined in the literature that were generated from meta-analyses focused on tasks involved in high level cognitive and motor functions [17]. The time series from these spherical seed-regions, each 6 mm radius, will be extracted from the spatially standardized residuals for every EPI scan. The resulting time series for each seed-region per scan per subject will be then imported into Matlab (Mathworks) and correlated against every other seed-region, creating 160x160 roi-to-roi connectivity matrices containing correlation coefficients for each subject. Correlation coefficients will be z-transformed using Fisher's r-to-z transformation to normalize the distribution and will be used as inputs to the NBS [18, 19] toolbox (as in the preliminary study [20]). NBS is a non-parametric approach that controls for family-wise error (FWE) in mass-univariate testing of an N x N ROI-to-ROI connectivity matrix and identifies networks of regions that is significantly different between groups.

DTI data: Diffusion Tensor Imaging (10 minute) scans will be collected using single shot diffusion weighted EPI images, 21 contiguous slices[b=0 sec/mm2 and b=1000sec/mm2], with TR=4500 ms, TE=71.8 ms, FOV = 24 x 24 cm, 3mm slice thickness, amplitude of diffusion gradient = 40 mT/ applied in 56 non-collinear directions uniformly distributed in three-dimensional space.

DTI data will be processed using FSL (v5.0) [21] with the following steps: 1) correction for motion and eddy current distortion; 2) head-motion correction for gradient direction vectors; 3) image registration and brain mask extraction; 4) estimation of tensor diffusion and generating diffusivity measures; 5) registration of standard white-matter atlases (JHU-ICBM-FA-2mm.nii.gz and JHU-ICBM-labels-2mm.nii.gz) to each patient's native space; 6) estimation of diffusivity measures for specific tracts of interest.

Task fMRI: **1)** In order to evaluate regions involved in balance processing, a checkerboard pattern will be used as the basis for a 2-dimensional visual stimuli in an optic flow task. A static checkerboard of alternating black and white squares will be used (CBstat) as the control stimulus. A rotating checkerboard (CBrot) will also be employed, in which the image appears to cyclically approach and recede relative to the observers' viewpoint, as well as rotate about the center of the viewfield [22]. During the scan, subjects will be shown the visual stimuli using a pseudo-randomized block-design paradigm. Each stimulus will be displayed for twelve seconds in blocks of three to six repetitions. Within a block, the stimulus presentations will be separated by six seconds of fixation to reduce the contamination of neural responses from one stimulus presentation into the next [23]. Each block will be separated by six seconds of fixation. The order of stimulus presentation will be the same for all subjects. Task scan will be approximately 7-8 minutes. **2)** In order to evaluate CNV, subjects will perform a jaw-clenching task, in which subjects will alternate between 20 seconds of jaw-clenching and 20 seconds of rest for a total of 6 cycles or 4 minutes. **3)** In order to evaluate CNV, VII, CNXII, subjects will perform a tongue thrust task in which they will alternate between thrusting their tongue to the roof of the mouth for 20 seconds and 20 seconds of rest for a total of 6 cycles or 4 minutes. We will also evaluate relationship of these brain MRI measures with balance and gait measures for the two treatment groups at each of the four time-points.

Each fMRI sub-study scan will take approximately 60 minutes to complete. All MRI scans will be performed at the Wisconsin Institutes for Medical Research (WIMR), 1111 Highland Ave., Madison, WI 53705.

Statistical Analysis

Task MRI Outcomes: Percent signal change will be computed for specific ROIs based on our previous work [11]. A repeated measures ANOVA will be used with Time (pre-intervention, stage 1, 2 3) as factor to examine change in percent signal over time in the patient group. To assess specific changes due to our intervention, we will perform paired t-tests (pre-intervention & stage 1; pre-intervention & stage 2 etc.). 2-sample t-tests will be used to compare differences between the Active PoNS group and control group.

Resting fcMRI Outcomes: The output from the NBS toolbox is a network of brain regions that are significantly impaired between groups (identified using 2-sample t-test) and networks that are significantly influenced by the intervention over time ((identified using paired t-test) [18, 19]. For each significant network, the toolbox provides a list of all pairwise regions and their connection strengths and the t-statistic associated with each connection.

DTI outcomes: DTI analysis will yield whole brain fractional anisotropy (FA), and diffusivity measures (radial, axial, and mean diffusivity). A repeated measures ANOVA will be used with Time (pre-intervention, stage 1, 2 3) as factor to examine change in FA and diffusivity measures over time. 2-sample t-tests will be used to compare differences in these measures between the Active PoNS group and control group.

Brain Behavior correlations: A Pearson correlation or Spearman rank correlation test, as appropriate, will be used for correlation analyses between brain measures (task fMRI derived percent signal change, resting fcMRI derived connectivity measures, DTI FA and diffusivity measures) and behavioral outcome measures

Potential Risks and Benefits

Functional MRI: MRI, a Class II device, is recognized as a Non-Significant Risk (NSR) device by the FDA. All the systems, features, and accessories that will be used in the scanning of subjects under this protocol will be operating outside the limits identified by the FDA as "Criteria for Significant Risk Investigations."

Risks

1. MRI: Aside from the standard risks associated with persons with certain metallic implants discussed above, no known risks of MRI exist. We know of no risks or adverse effects from the magnetic fields or radio waves used in the standard MRI pulse sequences. A small increase in risk may be associated with rapid gradient waveform switching times associated with fast MR imaging. In certain situations, the rapid switching of gradient waveforms has caused peripheral nerve stimulation in subjects. Significant nerve stimulation, however, has not occurred as long as the imaging system has been programmed to stay within certain limitations of gradient strength and switching time (dB/dt). The MR scanners currently being used at the UW stay within these guidelines, and any additional pulse sequences which we program in our

laboratory will be designed to stay within the current guidelines for dB/dt established by the FDA.

Occasionally some individuals experience feelings of claustrophobia during an MR exam.

The following instructions will be given to the subjects: Support will be provided to keep you from becoming uncomfortable, and you will be able to stop at any time. Using a microphone built into the MR scanner, a technologist will be able to hear you if you need assistance at any time. Please inform the technologist of any discomfort you may experience during the MR exams.

2. Pregnancy: Women who are pregnant or breastfeeding are excluded from the study. Although there is no evidence that MR exams can cause harm, there may be risks that are not known at this time. For this reason, this sub-study is not approved to enroll pregnant women or those that are breastfeeding.

Adverse Events, Unanticipated Problems

The most likely adverse events are detailed in the "Risks" section above. All unanticipated problems involving risk to subjects or others and all serious adverse events will be promptly reported by the PIs according to proscribed procedure: http://my.gradsch.wisc.edu/hrpp/10122.htm.

Adventitious Findings

The images collected for all subjects will be reviewed for adventitious findings by Co-PI, Dr. Prabhakaran. Subjects will be informed of all findings of clinical significance. During informed consent, subjects will be provided with the option of whether they will also be informed of findings of uncertain or no known clinical significance and whether any findings should be released to their primary care physician.

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