Study Protocol and Statistical Analysis Plan (SAP)

Official Title:	Noninvasive Vagus Nerve Stimulation (VNS) for Neuromotor Adaptations
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Study Protocol

Noninvasive Vagus Nerve Stimulation (VNS) for Neuromotor Adaptations

Aims

Motor function is compromised with advanced age, and motor impairment is involved in various neuromotor injuries and disorders including stroke, spinal cord injury, and amputation. Development of effective interventions for facilitating neuromotor adaptation for motor skills and strength is essential for accelerating or augmenting rehabilitation outcomes in the voluntary control of impaired limbs or attached assistive devices. Our ultimate goal is to find non-pharmacological, noninvasive, and affordable neuromodulating interventions for enhancing the rehabilitation outcome. Our idea stems from animal studies in which invasive (implanted) afferent vagus nerve stimulation (VNS, i.e. stimulation of cranial nerve X) paired with motor training enhanced neuromotor plasticity and motor recovery, most likely through increased releases of central neuromodulators from the brainstem (e.g. norepinephrine). In a rat model of stroke, implanted VNS paired with rehabilitative training facilitated and enhanced motor recovery and retention in motor function substantially (full recovery with implanted VNS vs. half recovery without implanted VNS). Not only in the injured model, but also in non-injured rats, implanted VNS paired with motor training facilitated neural plasticity in the motor cortex. Applicability of these facilitating effects of VNS in injured/non-injured rats to human neuromotor adaptation is unknown. Additionally, implanted VNS in humans would involve potential risks, higher costs, and inconvenience for rehabilitation. Hence, it is important to investigate the efficacy of adding noninvasive VNS to motor training for neuromotor adaptation in motor skills and strength in humans. As a noninvasive form of VNS for humans, transcutaneous VNS (tVNS) at the outer ear has been developed for studying its potential effect on several neurological conditions including epilepsy, depression, tinnitus, and pain, but not on motor impairment. tVNS can be applied by electrically stimulating the afferent auricular branch of the vagus nerve located medial of the tragus at the entry of the ear canal. In healthy humans, tVNS activates the brainstem including locus coeruleus, where norepinephrine (i.e. neuromodulator) is synthesized. It is unknown whether the noninvasive VNS (i.e. tVNS) actually leads to the facilitation of neuromotor adaptation in humans when combined with motor training in healthy or clinical populations. With potential applicability of this novel intervention to various clinical populations (e.g. stroke survivors, spinal cord injury, amputees, and older adults) in future vision, it is essential to start with the basic understanding about the effect of paired tVNS on the training-induced neuromotor adaptations in non-disabled humans. We have preliminary observations that would suggest the facilitating effect of tVNS on neuromotor adaptations in non-disabled humans. The overarching hypothesis is that an addition of paired tVNS to motor training increases central noradrenaline and facilitates training-induced neuromotor adaptations in human adults. In line with the concept of an R03 mechanism, the scope of this proposal is to produce pilot data demonstrating the proof of concept that would allow us to further investigate this promising approach in humans. To demonstrate the proof of concept of the overarching hypothesis, the specific aim of the study is to examine the effects of paired tVNS during motor training on central norepinephrine and neuromotor adaptations in humans.

Project design

Population. Non-disabled young adults (18-39 years old, men and women)

Intervention. Subjects will be randomly assigned to the tVNS or Sham group. There will be 5 experimental days, and the tasks on each day are summarized as below.

Day 1 (Baseline): Visuomotor Test → TMS (preparation & Test) → Visuomotor Training → TMS (Test)
Day 2: Visuomotor Test → Visuomotor Training
Day 3: Visuomotor Test → Visuomotor Training and Alpha-Amylase Activity Measurement
Day 4: Visuomotor Test → Visuomotor Training
Day 5 (Post): Visuomotor Test → TMS (preparation & Test) → Visuomotor Training → TMS (Test)

Visuomotor tasks with the index finger will be performed for the test and training. The first day is primarily the test day for assessing the baseline skill and neural excitability before training. Subjects will perform training after the initial tests on the first day and subsequent days. The last day is the final test day to assess the effects of training with tVNS or Sham on neuromotor adaptations. On the first and last days, the TMS test will be performed at the beginning and after the visuomotor tasks in both groups. In between these days, paired tVNS and Sham will be applied during training execution in the tVNS and Sham groups, respectively. The

changes in visuomotor skill and corticospinal excitability will be compared between tVNS and Sham groups. On Day 3, salivary alpha-amylase activity will be determined as a biomarker of central norepinephrine.

Protocol. All subjects performed visuomotor task as training and test. TMS test and central noradrenaline test were also performed.

Visuomotor tasks. Visuomotor tasks will be performed to induce and examine the adaptations in the visuomotor skill and neural excitability due to training. Visuomotor tasks include 1) visuomotor *test* on the first and last days and 2) visuomotor *training* on all days. The setup is the same across tasks. Subjects will receive tVNS or Sham during the training but not during the tests. A sensitive force transducer will be attached to the lateral aspect of the index finger and the little finger. The metacarpophalangeal joint will be in the neutral position. Subjects will adjust the abduction force of the index finger and the little finger to track the corresponding target force trajectory as accurately as possible with visual feedback. The target force trajectory for the test and training will be the same complex trajectory composed of multiple sinusoidal waves of various frequencies (<5 Hz). Every trial will use the same waves. The target force level will range from 0-10% of their maximal voluntary contraction force. All subjects will perform 50 trials (10 trials × 5 sets) with the same target trajectory across days. During training, subjects in the tVNS and Sham groups will receive paired tVNS and Sham during the execution phase of these training trials, respectively. All subjects will perform the visuomotor test without tVNS or Sham.

Paired tVNS and Sham during training. In the tVNS group, tVNS will be applied electrically to the afferent auricular branch of the vagus nerve located medial of the tragus at the entry of the left ear canal. One small surface electrode will be attached to the inner surface and another to the outer surface of the tragus. In the Sham group, the same stimulation will be applied to the ear lobe, where the vagus nerve is absent. The electrodes will be connected to an isolated electrical stimulator. In both groups, electrodes will be attached to the tragus and the earlobe. Subjects will receive stimulation in one of the electrode locations. Stimulation will be delivered only during the execution of the motor task.

TMS test. TMS test will be performed immediately before and after the visuomotor training on the first and last days to examine corticospinal excitability. Subjects will not receive tVNS or Sham during the TMS test. Basic procedures with TMS will follow those in our previous studies. The head of the subjects will be secured with a vacuum cushion molded for their neck and head. Surface EMG electrodes will be attached over the first dorsal interosseous muscle in the hand in a belly-tendon configuration. Baseline EMG and motor evoked potential (MEP) will be obtained from surface EMG using a high-gain EMG preamplifier. A figure-of-eight TMS coil will be placed over the primary motor cortex. A TMS coil navigation system and a coil holder will be used to maintain the coil position in 3-dimensional space relative to the head. After identifying the hot spot for evoking MEP in the first dorsal interosseous muscle, the resting motor threshold will be determined. To examine corticospinal excitability in the resting muscle, TMS will be applied at 8 TMS intensities ranging from 90%-160% of the resting motor threshold in random order. At each TMS intensity,13 TMS will be applied with 5-7 s randomized intervals, and the first response in each intensity will be discarded. Subjects will maintain relaxed muscles during the TMS test, and the relaxation will be ensured by monitoring and quantifying their background EMG. To obtain the reference value for normalizing MEP amplitude across days, compound muscle action potentials (M-waves) of the first dorsal interosseous muscle will be obtained just before the TMS test. Stimulation electrodes will be attached to the skin overlying the ulnar nerve of the forearm just proximal to the wrist. Using a constant-current isolated electrical stimulator, single supramaximal electrical stimuli will be applied to the nerve to obtain the maximal peak-to-peak amplitude of the M-wave (M_{max}).

Central noradrenaline. Central noradrenaline will be assessed indirectly with the salivary measure of alpha-amylase activity as a biomarker. Saliva will be sampled via salivette strips before and after the training on Day 3. Three saliva samples will be analyzed for each set and averaged.

Statistical Analysis Plan

Noninvasive Vagus Nerve Stimulation (VNS) for Neuromotor Adaptations

Aim
The aim of the study is to examine the effects of paired tVNS during motor training on central norepinephrine and neuromotor adaptations in humans.

Sample size
24 participants

Outcomes and analyses (all primary outcomes)
For assessing the visuomotor skill, the mean squared error of the produced force trajectory in reference to the target will be determined. For assessing corticospinal excitability, peak-to-peak amplitude of MEP will be averaged across responses. MEP amplitude will be normalized to the corresponding M_{max}. As the dependent variable for visuomotor skill, mean squared error, normalized to the baseline value, will be compared for group and day using a two-way ANOVA with repeated measures. As the dependent variable for corticospinal excitability, MEP amplitude will be compared for group, time, and day using a three-way ANOVA with repeated measures. As the dependent variable for central norepinephrine activity, alpha-amylase activity will be compared for group and time using a two-way ANOVA with repeated measures. An alpha value of 0.05 will be used for statistical significance. Post-hoc comparison will be performed when appropriate.