Approach

Our central hypothesis is that brain anatomy impacts the efficacy of iTBS therapy. The basis for this hypothesis is that anatomical complexities of cortical motor regions and their corresponding fiber tracts determine TMS induced electric fields in the brain, and these electric fields are the mechanistic drivers of TMS-based therapies. The rationale for our proposed research is that elucidating the impact of brain anatomy on the efficacy of iTBS is beneficial for two primary reasons: 1) imaging techniques provide the opportunity to identify the best patients for iTBS therapy based on their brain anatomy, and 2) stimulation therapy could be optimized based on the patient's brain anatomy. Thus, the effect of brain anatomy on iTBS efficacy is important to tailor rehabilitation appropriately.

In order to determine the effect of brain anatomy on the induced electric fields acting on cortical neural fiber tracts projecting to upper limb muscles from single pulse TMS we will create anatomically accurate computational brain models using MR images from ten nonimpaired individuals. The brain models we develop will incorporate anatomical features, including fiber tracts, from magnetic resonance imaging (MRI) data we record from these ten nonimpaired participants. We will record neurophysiologic data from the same ten nonimpaired participants in order to test for correlations between biceps and FDI motor evoked potentials before and following iTBS with modeled induced electric fields acting on their corresponding cortical fiber tracts.

Human Subjects

Ten nonimpaired individuals will be recruited for participation. All subjects will complete and sign a safety questionnaire to verify that they are free of contraindications for MRI and TMS. All subjects will provide informed written consent. We will seek study approval from the VCU Institutional Review Board.

Magnetic Resonance Image Acquisition (Session 2 or 3)

T1-weighted images will be acquired using a 3D MPRAGE sequence (time echo (TE)/time repetition (TR)=3.2/6.8 ms acquired sagittally with a 1.0x1.0x1.2 mm resolution at a flip angle of 9°, echo train length (ETL) = 240, matrix=256x240). T2-weighted images will be acquired using a 3D multishot turbo spin echo sequence (TE/TR=245/2500 ms acquired sagittally with a matching resolution of the T1-W images, two averages, flip angle=90°, ETL=133, matrix=256x256). DTI images will be acquired using a high resolution single-shot spin echo EPI sequence (TE/TR=89/5700 ms acquired axially with a 1.7 mm isotropic resolution at a flip angle of 78°, ETL=141, matrix=140x140, max b-value=2000 s/mm^2, 96 directions).

Computational Brain Modeling and Simulation of TMS

We will use MRI data from ten nonimpaired individuals to create 3D head and brain models of each individual. Using the software Freesurfer, along with high-resolution T1 and T2 weighted images, we will create high-resolution anatomically accurate models with differentiated skin, bones, cerebrospinal fluid, grey matter, white matter, and ventricles within the brain. The Freesurfer software we will use is open-source and developed for analyzing MRI images for development of 3D models. We will input MRI slices and uses contrast information to estimate anatomical boundaries, such as that between the skin and skull, or between grey matter and white matter. A 3D mesh is then created using these estimated boundaries and other geometrical information taken directly from the input MRI data.

We will use high resolution DTI data and extract fiber tracts using a graphical toolbox ExploreDTI.[20] Fiber tracts are constructed using whole brain tractography. Tracts will be drawn from a seed region of interest, such as M1, with seed fractional anisotropy threshold of 0.2, minimum fiber length of 50 mm and angle threshold of 30 degrees. Coordinates of tracts are imported into SolidWorks and

extruded to a solid, 3D line. These 3D fiber models are exported as .STL files for finite element analysis in Sim4Life. Figure 3 shows on the left the fiber tracts originating from M1, and on the right fibers being stimulated by a figure-of-eight TMS coil. The fiber models will be embedded into our 3D head models to calculate the induced electric field during TMS in different regions of the brain.

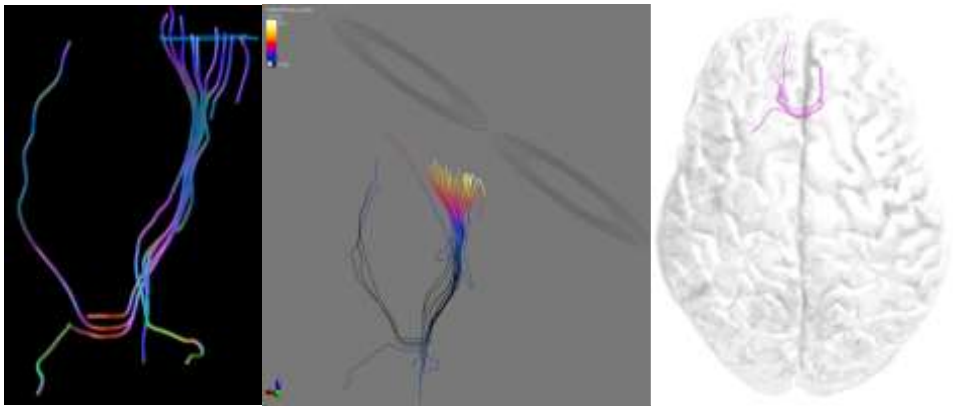

Fig. 3. M1 fibers will be extracted from DTI MRI. Isolated fiber tracts will be exported as 3D objects in order to simulate the induced electric field from TMS on the 3D fibers at regions of interst in the brain.

Using finite element analysis, we will determine the induced electric fields acting on isolated fiber tracts by simulating TMS in Sim4Life software. We will specify material properties (see Table 2), grid details, and the current source, similar to our previous work. Induced electric fields will be calculated using the Slice Viewer and Surface Viewer utilities within Sim4Life. Voxels stimulated above a threshold electric field of 150 V/m will be recorded.

Table 2. Material properties we will specify in Sim4Life simulations of TMS.

Structure	Mass Density (kg/m^3)	Electric Conductivity (S/m)
Skin	1109	0.17
Skull	1908	0.32
CSF/Ventricles	1007	1.7765
Grey Matter	1044.5	0.239149
White Matter	1041	0.26507
Cerebellum	1045	0.659667

Transcranial Magnetic Stimulation in Human Subjects

Subjects who underwent MR imaging, will also participate in two sessions of TMS. Each TMS session will consist of baseline measures of MEPs in response to single pulse TMS, followed by the iTBS protocol. One iTBS session will one target the biceps, and one session will target the FDI. Each session will consist of active iTBS applied to the hotspot of the target muscle, and sham iTBS. Sessions will be separated by at least 3 days to minimize the potential for carry over effects. Before and 10, 20 and 30 minutes after each iTBS session, MEPs will be recorded in order to quantify corticomotor excitability similar to our preliminary work.

Subjects will be instrumented with surface electromyography (EMG) electrodes on their dominant arm after the skin is shaved and cleaned with alcohol wipes. Surface EMG electrodes (disposable Ag-AgCl, Noraxon) will be located over the biceps and the FDI. EMG signals will be amplified (x1000) and bandpass-filtered prior to A/D conversion (CED Micro 1401 MkII, Cambridge Electronic Design). All EMG data will be sampled at 2 kHz using Spike2 software (Cambridge Electronic Design) and stored on a personal computer for offline analysis.

Prior to the iTBS session, the maximal compound muscle action potential (Mmax) will be recorded from the target muscle (biceps or FDI). The amplitude of Mmax is required to normalize MEP amplitudes.

To record Mmax, single pulse electrical stimuli, 0.2 ms pulse width will be delivered to Erb's point via a bipolar stimulating electrode (0.47 cm^2; 2.5 cm inter-electrode distance) connected to a constant current stimulator (DS7AH, Digitimer Ltd.). To determine RMTs and record MEPs, single pulse TMS will be delivered to the motor cortex contralateral to the resting target arm using a Super Rapid^2 Plus^1 stimulator via a 70 mm figure-of-eight coil. The vertex at the intersection of the inion-nasion and inter-aural lines will be marked on a linen cap tied snugly on the subject's head. The coil will be held tangentially on the scalp via a support stand with the coil center rotated to induce a posterior-to-anterior cortical current across the central sulcus. The hotspot for the target muscle will be identified as the location evoking the largest peak-to-peak amplitude MEP using the lowest stimulation intensity. Resting motor threshold (RMT) will be determined as the lowest stimulus intensity that induces MEPs of ≥ 50 μV in at least 5 of 10 consecutive stimuli with the target muscle fully relaxed.[21] The stimulus intensity for ensuing single pulse MEP trials will be set at 120% of RMT. To record MEPs, the stimulator will be triggered to deliver 15 stimuli at a rate of 0.2 Hz. Active motor threshold (AMT) will then be determined during sustained contractions of 20% the subject's maximum effort. With visual feedback provided relative to their maximum EMG root mean squared value, subjects will sustain a 20% maximum effort contraction of the target muscle during which AMT will be determined as the stimulus intensity that elicits a MEP ≥ 200 μV in at least 5 of 10 consecutive stimuli.

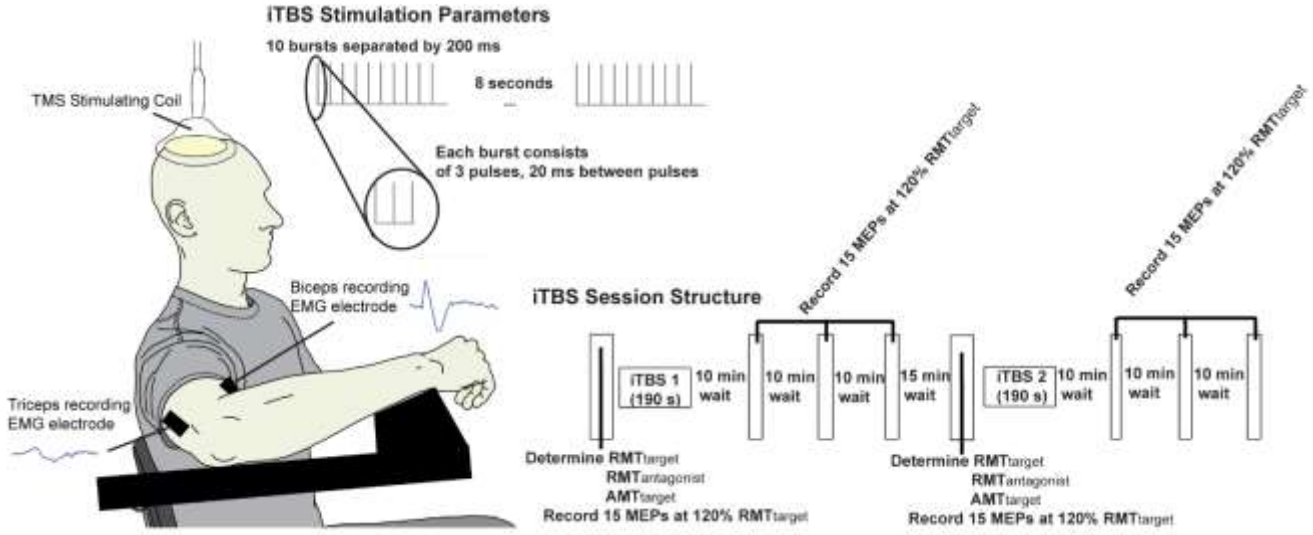

Fig. 4. The biceps and FDI will be targeted for the iTBS protocol in nonimpaired subjects. All subjects will be seated with the arm supported against gravity in the horizontal plane. The intensity of all iTBS pulses will be 80% of the subject's active motor threshold (AMT). Before each iTBS session (iTBS 1 and 2, which are sham and active, respectively), RMTs and motor evoked potentials (MEPs) will be recorded. MEPs will also be recorded at 10 minute intervals following iTBS.

Intermittent TBS stimulation will be applied using a Magstim Super Rapid^2 Plus^1 stimulator and a 70 mm double air film coil that includes a built-in cooling system to maintain operating temperature. iTBS applied to the target muscle will consist of three pulses presented at 50 Hz, repeated every 200 ms for 2 s at an intensity of 80% AMT. Two second bursts will be repeated every 8 s for a total of 600 pulses (Figure 4).[2] For the sham condition, a sham coil (Magstim 70mm double air film sham coil), looking identical to the active coil and making a similar noise but without delivering any active stimulation, will be applied to the hotspot of the target muscle. Throughout each session subjects will wear earplugs, be kept unaware of the type of stimulation, and be presented with nature videos to control engagement.

Data and Statistical Analysis

We will calculate the root mean square (RMS) amplitude of the evoked response, the RMS amplitude of the pre-stimulus background EMG (over a duration of approximately 25 ms matched to the

duration of the corresponding evoked response), and the peak-to-peak amplitude of the evoked response (MEP or M-wave) using purpose-written Matlab code (The MathWorks, Inc.). Stimulus events where the pre-stimulus RMS amplitude is larger than the evoked response, or where voluntary activity is detected, will be discarded to ensure similar levels of background activity across subjects and trials. MEP amplitudes will be normalized by their corresponding Mmax amplitudes. All patient characteristics, including RMTs and AMTs, will be summarized using means and standard deviations. The overall mean and standard deviation of MEPs (normalized to Mmax and unnormalized) will be reported. Linear mixed effect models will be used to determine the overall mean difference between the normalized MEP amplitudes between the active and sham protocols. Separate mixed effect model analyses will be used for each muscle (biceps and FDI), condition (sham and active) and time point following iTBS (in total 12 mixed effect models). Since this is a pilot study, all inference will be performed at the 0.25 level and no adjustment for multiple comparisons will be made. Pearson correlation coefficients will be computed to test for correlations between mean MEPs in response to single pulse TMS (before iTBS protocol) and the maximum induced electric field. A strong correlation between MEPs and induced electric fields would indicate a strong relationship between our experimental and computational outcome measures and provide a foundation for our future work. Pearson correlation coefficients will also be computed to test for correlations between mean MEPs following iTBS and maximum induced electric fields to determine whether greater induced electric fields indicate a participant is more responsive to the iTBS protocol.

Biostatistical Justification

The difference in the corticomotor excitability (as measured by the amplitude of the motor evoked potentials (MEPS)) can be used to determine if there is a difference between the active and sham protocols. Under the conditions that the true difference between the active and sham protocols is 5% of the normalized MEP with a standard deviation of 9%, and that the correlation between the differences within an individual is 0.5, ten nonimpaired subjects will be needed to achieve at least 80% power using a two-sided test of the overall mean using a repeated measures model. The Type-I error rate was set at 0.25 for this analysis.

In our **future research**, we will design neuromodulation techniques that promote neuroplasticity to increase muscle strength and function in individuals with SCI or post-stroke. The goal of our larger project, to be submitted as an R01 application to National Center for Medical Rehabilitation Research (NCMRR) within the Eunice Kennedy Shriver National Institute for Child Health and Human Development (NICHD) in June 2020, is to determine the effect of neuroanatomy and intermittent theta burst stimulation on corticomotor excitability, and clinical scores of function in individuals with tetraplegia. This larger project will include development of a computational framework to model hysteretic effects of iTBS using participant specific MRI in order to more definitely determine the effect of brain anatomy on the efficacy of iTBS. This larger project aligns with two research priorities within NCMRR: 1) to explore multimodal approaches that promote plasticity and sensorimotor function, particularly the combination of physical therapy with stimulation treatments, and 2) to develop objective measures that may predict rehabilitation treatment response, monitor functional progress, and tailor interventions to the individual abilities, needs, and resources of the person with disabilities.

Potential Risks

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique. TMS protocols, including the single pulse and intermittent theta burst stimulation protocols that we propose to use in human subjects, present non-significant risk to the health, safety, or welfare of a human subject. The risks associated with TMS protocols have been investigated and discussed in detail.[3] Risks and side effects associated with TMS include: heating, induced voltages, forces and magnetization, effect on hearing, induced seizure or syncope, headache, local pain or discomfort, and neurophysiological changes.

The risk of heating includes the overheating of the stimulating coil and/or brain tissue. This risk will be minimized by using a Magstim Double 70mm Air Film Coil. The Double 70mm Air Film Coil contains two temperature sensors. When the internal coil temperature approaches 20°C, indicators on the UI illuminate. When the internal temperature reaches 41°C, the stimulator will shut down and the Replace Coil Light will illuminate.

The risks of induced voltages, forces and magnetization will be minimized by screening to exclude individuals with implanted devices. Our protocols will exclude the use of TMS on, or in the vicinity of, patients or users with cardiac demand pacemakers, implanted defibrillators and/or implanted neurostimulators.

The effect on hearing will be minimized by providing ear plugs to all participants.

The risk of induced seizure or syncope will be minimized by screening procedures to exclude individuals taking medications that lower the threshold for inducing seizures. Intake of or withdrawal from certain central nervous system (CNS) active drugs lowers seizure threshold. The actual risk for seizure induction may depend on additional, not yet fully explored, factors such as drug dose, speed of dose increase (or decrease), and combination with other CNS active drugs. The majority of reported TMS-induced seizures have occurred in subjects/patients on drugs with seizure threshold lowering potential. Thus, we will exclude individuals taking medications as listed in section 5.3 of the report by Rossi et al.

Since the introduction of theta burst stimulation (TBS), a review of the literature reveals 49 publications using TBS in normal participants or patients with tinnitus, stroke, movement disorders, or chronic pain. Overall, a total of 741 participants have undergone either continuous or intermittent TBS. A single seizure has occurred in a 33-year old man healthy control without any risk factors for epilepsy and not taking any medications.[4] Physical exam, detailed neurologic exam and mental status exam were normal starting 45 min after the event and remained normal later. Vital signs were stable, and all tests done were unremarkable. It should also be noted that most of the published reports of TBS use an intensity of 80% of Active Motor Threshold while the seizure occurred in a study applying an intensity of 100% of Resting Motor Threshold.

Neurophysiological changes may occur and is the basis of our research. There is evidence that iTBS may increase the excitability of the neural pathway projecting to a muscle.[2] The purpose of our research is to investigate whether iTBS increases excitability of the pathway projecting to proximal upper limb muscles of nonimpaired individuals and individuals with spinal cord injuries, as well as muscle that has been altered my upper limb reconstruction.

In the majority of subjects/patients experiencing local pain during TMS, including toothache, the effect rapidly vanishes. Headache may occasionally persist, however, after TMS application; in this case, a common analgesic administered orally may be helpful. No migraine attacks have been described following repetitive TMS, neither in nonimpaired individuals nor in migraine patients who underwent repetitive TMS applications as treatment [5].

The devices we will use are labeled as investigational with the following statement: "CAUTION--Investigational device. Limited by Federal (or United States) law to investigational use." We will not market or promote the device. We will report unanticipated adverse device effects to the IRB, sponsor, etc.

MRI can cause discomfort or anxiety and participants will be screened and accompanied by a member of the research team, as well as observed during MRI scanning.

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