Customized Cortical Stimulation Therapy in the Rehabilitation of Stroke Patients

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In this project, experiments conducted in healthy subjects and stroke patients are geared towards understanding and enhancing stroke recovery in a stroke patient population. The experiments proposed in this research project correspond to the experiments proposed in the original NIH application for patients; we will also obtain measurements in healthy controls. The experiments proposed in this research project correspond to the experiments proposed in the original NIH application.

1. Abstract

The role of the motor cortex in the hemisphere spared by stroke (contralesional motor cortex) in motor performance during post stroke recovery is still unclear. Here we propose to identify the role of contralesional motor cortex in motor performance and post stroke recovery. We will carry out experiments that first identify the extent of stroke and brain areas involved in a motor task using functional MRI of the brain and TMS. We will then determine the functional role of the contralesional motor cortex by studying the effect of low frequency and high frequency repetitive transcranial magnetic stimulation (rTMS) of primary motor cortex (M1) (Chen et al. 1997) on interhemispheric inhibition (IHI) (resting and active) and motor cortex excitability (short interval intracortical excitability and corticospinal excitability) and behavior. We will thereby also identify rTMS protocols that enhance motor performance in stroke patients.

2. Introduction and Background:

It is well known that the motor area of one hemisphere of the brain (motor cortex) controls the movement of the opposite of the body. However, it is not clear whether as

the movement becomes more complicated, the motor cortex of both hemispheres of the brain are involved. Currently the role of the motor cortex on the same side of the body (referred to as ipsilateral motor cortex) in hand performance remains controversial. We demonstrated previously in healthy subjects that transiently lowering the activity of ipsilateral motor cortex improved the performance of the opposite hand. What is not know are the mechanisms involved in these changes of behavior. Transcranial magnetic stimulation (TMS) is a device that allows the non- invasive stimulation of the brain. When brain is stimulated repetitively at a very low rate and low intensity for about 15 minutes, the stimulated brain area becomes less active. This effect lasts 10 minutes and is called a "transient artificial lesion" as it mimicks the effects of transiently interfering with the function of the stimulated brain area. In the present study we will conduct experiments using repetitive TMS to downregulate the activity of the motor area as in previous experiments and measures its effect on activity of motor cortex of both hemispheres. We will study healthy subjects and this collected data will provide normative values for task related changes in M1s and their interactions – a prerequisite to studying abnormalities in stroke patients during motor recovery. It would be important to understand the effects in more detail for the design of treatment strategies in patients after stroke, which will be a topic of future studies.

In most patients with stroke, only one side of the brain is affected by the stroke (affected hemisphere) resulting in weakness of half of the body opposite to the side of the stroke. Over the recent years, researcher discovered that the side of the brain, that is spared by the stroke (non- affected hemisphere) may support the recovery after stroke. However,

there is also a question whether the non- affected hemisphere may interfere with the process of recovery. It is currently not known what factors influence the activity of non-affected hemisphere to either support or interfere with the recovery of stroke. A better understanding of those events is critical to development of optimal therapeutic strategies. For example, non-invasive stimulation of specific areas of the non- affected hemisphere may help to improve functional recovery following stroke. The objectives in this application are to define the factors that influence the activity in the non- affected hemisphere to either support or interfere with the recovery after stroke. We will study the area of the brain that controls movements called motor cortex of the non- affected hemisphere (non- affected motor cortex) as it relates to motor function post-stroke.

In a longitudinal study of stroke patients, non- affected motor cortex reorganization will be assessed in two Specific Aims. In the first Specific Aim, we will determine the extent of functional and structural non- affected motor cortex reorganization using complementary techniques of transcranial magnetic stimulation (TMS), functional and structural MRI of the brain. In the second Specific Aim, the contribution of non- affected motor cortex reorganization to the recovery of motor function will be studied. Repetitive TMS will be used to transiently disrupt non- affected motor cortex function, thereby determine its role for the motor performance of the paretic limb. RTMS related improvement of motor performance would identify a supportive role of contralesional M1 while deterioration of motor performance would indicate its detrimental role. Different rTMS protocols will be applied to non- affected motor cortex to determine

whether non- affected motor cortex can be primed to enhance restorative therapy.

3. Objectives:

A. Specific Aim #1: ***Define key factors that determine the reorganization of contralesional M1 during post stroke recovery process by comparing M1 excitability of stroke patients to that of healthy subjects.*** Our working hypothesis is that the extent of M1 and/or corticospinal tract (CST) damage, as measured by the electromyographic response to TMS of lesioned M1, stroke lesion volume, increased fractional anisotropy (FA) asymmetry of the CST, and quality of wrist/finger extension movements, will determine the extent of contralesional M1 reorganization. In a subset of patients we will also use electroencephalography and TMS to determine the additional key factor of connectivity between the lesioned and the non- lesioned M1 in the post- stroke reorganizational process of contralesional M1.

B. Specific Aim #2: ***Determine the role of contralesional M1 in motor performance during the post stroke recovery process.*** Our working hypothesis is that, during recovery, contralesional M1 contributes to improved motor performance and recovery of the paretic hand in patients with larger M1 and/ or CST damage. Specifically, the extent of M1 and/or CST damage as measured by the electromyographic response to TMS of lesioned M1, lesion volume, increased FA asymmetry of the CST, and quality of wrist/finger extension movements(Nijland et al. 2010) will determine whether contralesional M1 plays a supportive or detrimental role in post-stroke recovery process.

At the completion of this project, we expect to have identified the extent to which

contralesional M1 reorganization determines motor performance during the different phases of post-stroke recovery and can be targeted by interventions depending on its "state" (role) in the recovery process. This latter knowledge would be expected to have a substantial positive impact on treatment for stroke patients as it will provide crucial evidence to develop evidence based TMS treatment protocols that are tailored to the state of therapeutic target. The clinically important question of whether decreasing contralesional M1 activity in patients post stroke is always helpful or at times is detrimental will be addressed.

4. Study design and Methods:

The measurements and interventions described will be obtained at 2 time points in all patients (1 and 6 months post stroke). The data will be compared to the results of healthy age matched controls to establish abnormalities

4.1. Studies pertaining to Specific Aim #1: *Define key factors that determine the reorganization of contralesional M1 during post stroke recovery process.*

Motor function assessment post-stroke. In all patients, motor functions will be evaluated in 3 categories of measurements: motor function, motor kinematics and overall function in activity of daily living, to include measures that reflect the behavioral impact of CST integrity. In the first category motor function will be determined using (1) Wolf Motor Function Test (WMFT)(Wolf et al. 2001) and (2) Perdue Pegboard test (Buddenberg and Davis 2000; Mathiowetz et al. 1985) (3) Jebsen Test (Jebsen et al. 1969). Patients may be videotaped during performance of these tests so that scores obtained during testing can be verified. In the second category, laboratory-based measures of upper extremity

movement kinematics will be obtained. In a third category, the use of the paretic arm outside the laboratory will be quantitatively and qualitatively evaluated using the Motor Activity Log MAL (Uswatte et al. 2006). Patients will be contacted by phone 3 – 4 times between the time of stroke until the final study visit in order to complete the MAL. The first phone call will be 5 - 10 weeks after their stroke date. The second call will be 9 – 15 weeks after their stroke date. The third phone call will be 14 – 20 weeks after stroke. The fourth phone call will be 19 – 24 weeks after stroke. In all cases, a randomization process will determine the exact week of contact. A one-week window will be allowed for making contact and completing the MAL.

Stroke date.

Window for first call: 5 weeks – 10 weeks post stroke.

Window for second call: 9 weeks – 15 weeks post stroke.

Window for third call: 14 weeks – 20 weeks post stroke.

Window for fourth call: 19 weeks – 24 weeks post stroke.

Sample script:

Script.

- Hello [name of subject], this is [name of coordinator] calling from Dr. Buetefisch's lab, how are you?

- Do you have a few minutes to answer some questions about how you're using your hand and arm? This should take about 15 minutes.

- This questionnaire is 30 questions and each question has two parts. I'll name a task and ask you how often you use your affected side to do that task. You'll give me a number on a scale from 0 to 5 where 0 is "not at all" and 5 is "the same as before my stroke". Then I'll ask you how useful your hand is while completing these tasks and you'll give me a number from 0 to 5.
- Do you have any questions?
- In the past week how often have you used your affected hand to ____? And how useful was your hand while doing ____?
- Complete Motor Activity Log with remaining questions.

4.1.1. Structural and functional MRI, data acquisition: All subjects will be scanned. The MRI will be performed on a Siemens 3T Prisma scanner located in the Biomedical Imaging Center (BITC) at Emory University. High resolution T1 weighted images, Flair images and DTI images of the brain will be done to determine the location and size of the stroke and changes in white matter. Arterial spin labeling (ALS) will be used to quantify the blood flow. Functional magnetic imaging of the brain (fMRI) will be used to determine hand movement related activity in M1 of all subjects. For this purpose standard EPI sequences will be obtained. The experiment will run under the control of Presentation® software. Prior to being placed in the scanner, subjects will be given ample time to practice the experimental motor task with both hands at least one day prior to the fMRI experiment.

4.1.2. EMG recording during the motor task in the scanner: The subject's EMG from the extensor carpi ulnaris muscle ECU of both arms will be recorded with MRI compatible shielded surface electrodes, band pass filtered, amplified (MRI compatible amplifier), digitized, sampled at 1-kHz frequency, and stored for offline analysis (LabVIEW, National Instruments, Texas, USA, BrainVision, Brain Products, USA).

4.1.3. MRI data analysis:

Brain morphometry and CST: From the T1-weighted image, the cross-sectional area of the left and right cerebral peduncles will be determined(Schaechter et al. 2008). We will measure M1 volume and cortical thickness using FreeSurfer (https://surfer.nmr.mgh.harvard.edu). The distance between representations of the white-grey matter boundary and cortical surface will be calculated across each point of the cortex to produce approximations of cortical thickness and then applied to a standard atlas to extract thickness in the pre-specified region of M1(Desikan et al. 2006; Fischl and Dale 2000). The total lesion volume will be computed using MRIcron software, and corrected for differences in head size by dividing it by the total intracranial volume. The CST in each hemisphere will be reconstructed using TRACULA (TRActs Constrained by UnderLying Anatomy;(Yendiki et al. 2011)) and the DTI processing stream as part of the FreeSurfer image analysis package.

Functional MRI: tfMRI and rsfMRI data will be processed using the minimal preprocessing pipeline(Glasser et al. 2013) which includes motion correction, cross-modal registration, and conversion to surface- and volume-based standard space.

Following minimal preprocessing, the task-based fMRI data will be surface-smoothed to 6mm FWHM and entered into a multiple regression analysis with head motion parameters included as regressors of no interest. Temporal derivatives will be added to the model accommodate subject-to-subject variation in hemodynamic response functions. Subjects with right hemisphere lesions will be flipped about the X axis so all lesions are in the same hemisphere. Whole brain and region of interest based analysis will be performed.

4.1.4. TMS stimulation and EMG recording

Subjects will be comfortably seated in a dental chair surrounded by a frame that carries a coil holder to assist with the application of TMS to the brain. The MRI of the brain will be reconstructed for neuro-navigation assisted TMS of the brain using Brainsight (Brainsight, Rogue Research, Montreal, Canada). Surface electromyographic (EMG) (bandpass 1 Hz – 1 kHz) activity will be recorded from the target muscle (right and left extensor carpi ulnaris (ECU) and flexor carpi ulnaris (FCU) muscles and other muscles as needed for control), using surface electrodes (11 mm diameter) in a belly-tendon montage and a data acquisition system (LabVIEW, National Instruments, CA, USA). A picture of the subject's arm will be taken to help ensure consistent electrode placement across days. TMS will be applied through an air-cooled figure of eight-shaped coil (7 cm wing diameter) using the rapid Magstim200^2 (Magstim Company, UK). The coil will be positioned on the scalp over the left M1 at the optimal site (hot spot) for stimulating the right ECU. At the optimal site, the resting motor threshold (MT), defined as the minimum stimulus intensity to evoke an MEP of >50 □V in at least five

of ten trials (Rossini et al. 1994b), will be determined to the nearest 1% of maximum stimulator output (MSO). The position will be marked on a standard MRI of the brain in Brainsight to ensure coil stability during the experiment.

Interhemispheric inhibition : For the measurements of interhemispheric inhibition (IHI) two Magstim 200 stimulators (Magstim Company, UK) will be used. With the subjects at rest, a conditioning pulse (CS) will be applied to the optimal scalp position of the M1 of either hemisphere to stimulate the corresponding contralateral ECU muscle using a figure of eight coil (70 mm diameter) (Ferbert et al. 1992). The intensity of CS will be adjusted to produce a MEP of about 1.5 mV (Ferbert et al. 1992). A test pulse (TS) will be applied to the homotopic area of the opposite hemisphere, defined as optimal scalp position to stimulate the contralateral ECU using a smaller figure of eight coil (50 mm diameter). The intensity of the TS will be adjusted to produce a MEP of about 1.5 mV (Ferbert et al. 1992). Paired pulses at different ISIs will be intermixed with single TS and single CS and applied at random.

Short interval cortical inhibition: TMS will be applied through a figure of eight-shaped coil (7 cm wing diameter) using two Magstim 200 stimulators connected via a Bistim module (Magstim Company, UK). Short interval cortical inhibition (SICI) will be measured using paired pulse TMS at an ISI of 2 ms. The intensity of the CS will be varied between 30 and 80% of MT and will be administered randomly (Butefisch et al. 2003; Butefisch et al. 2008) while the intensity of TS will remain suprathreshold. Paired pulses will be intermixed with single test- and conditioning pulses and administered

randomly to M1 of either left or right hemisphere. The sequence of timing of stimuli will be controlled by customized software.

Active Interhemispheric inhibition: Active interhemispheric inhibition (aIHI): Active IHI will be measured during the pre- movement time period of a pointing task. In this task subjects have to manipulate a joystick in response to a visual target presented on a computer screen. Real-time feedback about the joystick position is provided by a cursor moving on a computer screen. Subjects will be instructed to move the cursor as quickly as possible to the center of a target square immediately upon its appearance on the screen with a preset time of 2s for movement completion. Feedback ("hit" or "miss") will be given depending on their ability to move the cursor is in the center of the tar-get within a pre set time of 2 sec. This task was previously reported in detail by our group but modified to allow its manipulation by patients with more impaired hand function and automatic indication of the movement time. This adjustment eliminates the need for selective finger function. For aIHI measures, CS and TS will be applied at the time of the target presentation, 300, 400 or 450 ms after the target presentation at the same ISIs and intensities described for the rIHI.

Cortical Mapping: TMS will be applied through a figure of eight-shaped coil (7 cm wing diameter) using the rapid Magstim200^2 (Magstim Company, UK). Motor maps will be derived for the right and left ECU and FCU muscles. A rectangular grid consisting of stimulation sites spaced at least 0.5 cm apart will be superimposed on the subject's MRI and centered on the subject's hotspot for a given muscle. EMG activity

and stimulation locations will be recorded as no more than 10 suprathreshold single TMS pulses are applied at each of the stimulation sties. The coordinates of each stimulation site and corresponding EMG activity will be exported for further off-line analysis.

4.2. TMS stimulation and EEG recording

EEG data will be recorded using a 64-channel TMS-compatible electrode cap (Easy Cap). Signals will be collected at 2000Hz (impedance: <5kW, low-pass filter: 0.1-500Hz) during pre- and post-TMS stimulation epochs (-100ms to 200ms). Fifty suprathreshold TMS pulses will be applied to M1 while the subject is seated quietly with eyes open. This procedure will be conducted bilaterally at each assessment timepoint. Peripheral auditory and somatosensory stimulation effects will be minimized to avoid EEG artifact. Single-channel evoked potentials will be defined as the area under the curve obtained between 10 and 150ms poststimulus. These TMS-evoked potentials will be used to index local M1 excitability and connectivity with the contralateral homotopic region. As a secondary exploratory analysis, EEG recording will also be captured during motor performance using a joystick task.

4.3. TMS data analysis:

Peak to peak MEP amplitudes will be measured off-line. Recordings with EMG background activity will be excluded from further analysis. Data analysis SICE, SRC, rIHI, aIHI, and mapping: Peak to peak MEP amplitudes will be measured off-line. Recordings with EMG background activity will be excluded from further analysis. For

the SRC, the mean MEP amplitudes at each stimulus intensity will be calculated. For SICE, rIHI and aIHI, mean MEP amplitudes elicited at different ISIs and CS intensities will be calculated as a percentage of the mean test MEP amplitude evoked by TS alone. CS intensities will be expressed as percentage of the subject's MT. For mapping, the area will be calculated as the number of stimulation sites that produce an MEP in of the trials. The center of gravity (CoG), an estimate of the center of the map, will be calculated for each target muscle (ECU or FCU) based on the weighted MEP amplitudes at each active location. Statistical analysis: We will use linear mixed models to quantify the effects of subject type (cortical stroke patient, subcortical stroke patient, healthy control) along with other, measure-specific factors (outlined below) on outcomes of interest. The analyses will incorporate models of within-subject correlations stemming from multiple measures on each subject. Hypothesis testing will be conducted on mean model parameters at a significance level $\alpha = 0.05$. Post-hoc analysis will be corrected for multiple comparisons. For SICE: A mixed model will assess the effect of subject type and stimulated hemisphere (ipsi- or contralesional for the stroke subjects, zero for the controls) along with repeated measure CS intensity (60%, 80% MT) on relative conditioned MEP amplitude. *For rIHI*: A mixed model will be used to determine the dependence of relative conditioned MEP amplitude on subject type along with the repeated measures inhibition (cl_M1 on il_M1 and il_M1 on cl_M1) and ISI (2ms, 10ms). *For aIHI:* We will perform a mixed model analysis to determine the dependence of relative conditioned MEP amplitude on subject type along with the repeated measures inhibition (as above), time after go signal (0ms, 300ms, 400ms, and 450ms), and target size (small, extra-large). *For SRC:* The effect of the repeated measure stimulus intensity on the average MEP

amplitude, along with subject type and stimulated hemisphere (as above), will be examined. We will evaluate linear and nonlinear trends of MEP amplitude across intensity levels and will consider various parsimonious parametric structures for the variance-covariance matrix. *For MT:* A mixed model will quantify the effect of subject type (as above) and repeated measure stimulated hemisphere (as above) on MT. *For Mapping:* A mixed model will quantify the effect of subject type (as above) and repeated measure stimulated hemisphere (as above) on the area and CoG of the maps for both ECU and FCU. Furthermore, exploratory analyses and advanced statistical techniques will be use to investigate the relationship between the area and CoG of the maps (i.e., muscle representations at rest) and task-based fMRI activation patterns (i.e., muscle representations during movement).

4.4. **Studies pertaining to Specific Aim #2:** ***Determine the role of contralesional M1 in motor performance during the post stroke recovery process***

In two studies (Study 1 and 2) we will test the effect of contralesional M1 transcranial magnetic stimulation on the subject's performance in a manual pointing task (4 different level of complexity, Presentation®) using either the paretic or the non-paretic hand in a counterbalanced randomized order and measures of motor excitability (IHI, SICI, MT, SRC, see above for details). To accomplish the goal of each study, a total of 6 experiments will be conducted in a randomized counterbalanced order. In Study 3 we will implement prediction and classification models to determine the predictive roles of variables measured in specific aim 1 on the supportive/detrimental role of cl_M1 derived

from the effect of M1 downregulation by rTMS (Specific aim 2, study#1).

Study #1: Role of cl_M1 on motor performance. In all subjects, we will test the effect of 1 Hz rTMS and sham cl_M1 stimulation on the subject's performance in a pointing task of increasing difficulty using either the paretic or the non-paretic hand in a counterbalanced randomized order. Specifically, as previously described by our group in detail (Butefisch et al. 2003; Butefisch et al. 2008) rTMS at 90% MT will be used to decrease cl_M1 function using a rapid Magstim (Magstim Company, UK). Briefly, coil position will be monitored online using Brainsight. EMG activity will be recorded from the ECU and other control muscles with the settings already detailed in Specific Aim 1. After determining the MT in the ECU hot spot(Rossini et al. 1994b), rTMS will be applied through the air - cooled figure-of-eight coil at 90% MT and 1 Hz frequency for 15 min(Buetefisch et al. 2009). The effects will be compared to Sham stimulation applied at similar intensity and frequency(Buetefisch et al. 2009). An additional two visits will be conducted for healthy subjects that include a repeat of the study measures described in specific aim 1 but we will stimulate at 80% rTMS to explore the effects compared to 90% rTMS and these visits will take the standard amount of time of 2-3 hours per visit. Preliminary data demonstrated different effects of different intensities and this needs to be confirmed in this population.

Study #2: Effect of transient disruption of cl_M1 on M1 excitability and IHI. In all subjects, we will test the effect of cl_M1 stimulation on the outcomes of MT, SRC, SICE and IHI in both motor cortices one month post stroke. All measurement will be repeated 6

months after infarction. The data will be compared to the results of healthy age matched controls to establish abnormality.

Statistical analysis: The statistical tests performed in this section will mirror those in the specific aim 1 with the addition of repeated measures factors specifying acquisition time (pre- or post-rTMS stimulation) and time post-stroke (1and 6 months). The mixed-models will investigate the effect of cl_M1 stimulation on TMS outcomes MT, SRC, SICE, rIHI over time post-stroke. Additionally, we will use the mixed model to assess the effects of cl_M1 excitability (SICE from SA #1) and cl_M1 activation (number of activated voxels and % signal change within ROI) on rTMS-related changes in TMS measures.

Study #3: Identify biomarkers in the subacute and chronic phase of stroke that predict either the supportive or detrimental role of contralesional M1 as determined by responses to specific intervention protocols. In this study, we will implement prediction and classification models to determine the predictive roles of variables in SA #1 on the supportive/detrimental role of cl_M1 derived from the effect of M1 downregulation by rTMS (SA#2, study#1). Specifically, we will train (and test) a binary classifier to predict subjects' responses (positive or negative) to rTMS (down regulation and sham) at 1 and 6 months post-stroke based on sets of predictors defined by SAs # 1 & 2. The association between the set of predictors and the binary response will be established during an iterative cross-validation process involving training and testing, in which different data are used to establish a predictive model and to evaluate its performance. We will assess performance using standard measures of prediction accuracy such as sensitivity,

specificity, positive predictive value, and negative predictive value. We will employ and compare multiple modeling techniques such as the Elastic Net, logistic classifier, and Bayes classifiers. Developing and optimizing the classifiers will identify subsets of measures that have the greatest predictive power for forecasting the role of cl_M1.

5. Participant Selection:

Stroke patients: For SAs # 1-2, subjects will be adult females and males, ages 40-80 years with (1) one cerebral ischemic infarction < 1 month affecting the primary motor output system of the hand at a cortical (M1) level as defined by MRI of the brain, (2) paresis of the hand for more than 3 days after their cerebral infarction (MRC ≤ 4- of wrist- and finger extension/flexion movements), (3) extension of the wrist (MRC≥ 3, > 10 degree) at the time of the enrollment into the study, (3) no other neurological disorders, (4) no aphasia that prevents subjects from following instructions or their inability to communicate effectively with the study team, (5) no or only mild cognitive impairment but not to the level of dementia (RBANS(Randolph et al. 1998)), (6) no or only mild depression (Hamilton Depression score of <19) (7) no contraindication to TMS or MRI, (8) no intake of CNS active drugs that blocks plasticity (9) the ability to give informed consent. We have chosen this age range because stroke is a disease of elderly people. Only 8.9% of ischemic strokes affect people under the age of 50 years(Fonarow et al. 2010). As plastic changes are likely age-dependent (Sawaki et al. 2003), we would like to exclude "age" as a possible confounding factor by matching our patients post-stroke to the healthy controls (data gathered under R56 NS070879-01) thereby avoiding the possibility that older age may result in a false negative result in our stroke patients.

Healthy Subjects: We will study 30 healthy adult females and males, ages 40- 80 years with no neurological or psychiatric diseases and a normal neurological examination, normal MRI of the brain and normal neuropsychological testing(Randolph et al. 1998), no intake of CNS active drugs, no contraindication to TMS and ability to give informed consent.

6. Statistical analysis

The statistical analysis of the data is outlined in each section of the study plan.

7. Adverse event reporting

During the study, the PI will promptly report to the IRB any serious event, defined as a hospitalization, seizure or death. Since this is a minimal risk study, we see no need to establish a DSMB. The PI will not make any changes in the research without IRB approval, except when necessary to eliminate immediate risks to human subjects. The Investigator will also report to the IRB on the progress of the study and adverse events annually.

8. Data and safety monitoring plan (DSMP)

8.1. Justification of risk

According to the recent guidelines about rTMS, the proposed research falls in Class 3 (indirect benefit, low risk): studies in normal subjects and patients that are expected to yield important data on brain physiology or on safety, but have no immediate relevance to clinical problems.

RTMS at 0.1 Hz is below the frequency of most research settings using single TMS and was reported to have no effect on cortical excitability (Chen et al. 1997). TMS applied at a frequency of 0.9 Hz applied over 15 min resulted in decreased cortical excitability of untrained M1 (Chen et al. 1997). Frequencies used in the proposed research (1 Hz and 0.1 Hz) are similar or lower, and are, therefore well below the risk of inducing a seizure (Wassermann 1998b). As the contralesional M1 is targeted, risk should be comparable to normal healthy subjects.

As mentioned above, the proposed TMS protocols are within the recommended safety guidelines. The introduction these safety guidelines (Wassermannn, 1998) have proven efficacious in preventing seizures, both in normal subjects and in patients with neurological and psychiatric diseases, despite the fact that such guidelines were based on a relatively restricted sample of normal subjects and considered only conventional rTMS (Rossi et al. 2009; Rossini et al. 1994a; Wassermann 1998a). Furthermore, the use of high frequency/high intensity rTMS was unsuccessful as a non-invasive procedure to activate epileptogenic foci (Tassinari et al. 1990). Subjects are screened (see attached TMS screening form) for, and sign a Consent Form stating that they do not have any metallic (potentially magnetic) material in their body, such as aneurysm clips, pacemakers etc., take drugs that decrease the threshold for a seizure, history of any epileptic seizure. Each subject will be screened with the TMS safety questionnaire used by the PI in previous TMS experiments. Each TMS stimulus produces a loud click and all subjects are issued with ear protectors, so as to avoid potential hearing damage. Since the non- affected hemisphere of patients after stroke is stimulated, the risk should be comparable to the risk of healthy subjects (Hummel et al. 2008). In the event the subject

has a device implanted that is not described in any of their medical records we may consider to obtain a plain x- ray. The x- ray will be taken of the body part that is affected by the device and will ensure the safety for MRI.

For devices that are safe in the MRI and TMS environment but require preparation prior to TMS or MRI, we will contact treating physician and proceed as recommended by the physician and device manufacturer.

For subjects with loop recorders, we will contact the treating cardiologist to download the data prior to TMS and MRI to prevent the possibility of MRI and TMS related loss of data.

8.2.

Since this is a minimal risk study, we see no need to establish a DSMB. The PI will not make any changes in the research without IRB approval, except when necessary to eliminate immediate risks to human subjects.

8.3. Stopping rules:

If any subject develops a seizure whether related or unrelated to the TMS, these subjects will be excluded from the participation in the experiments. The research projects involving TMS will be stopped if subjects develop a seizure that is related to the TMS until continuation of experiments is approved by the IRB.

9. If applicable: pharmaceutical, biologic, and device information

For the product manual please refer to the eIRB submission, device information. For

guidelines regarding safety of TMS please refer to the attachments in the eIRB (Rossi et al. 2009; Wassermann 1998b).

10. References and appendices

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