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An Investigation of Short-Term Plasticity in Human Motor Cortex

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An Investigation of Short-Term Plasticity in Human Motor Cortex

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Abstract

Transcranial magnetic stimulation (TMS) produces a transient magnetic field that activates underlying cortical tissue by eliciting an electrical discharge of the neurons in the targeted area. Repetitive TMS (rTMS) uses patterns of repetitive TMS pulses and has been reliably shown to produce changes in the state of cortical excitability outlasting the time of stimulation. One such protocol that has demonstrated states of increased excitability is intermittent theta burst stimulation (iTBS). This method applies high-frequency bursts (50Hz) of pulses every 200 ms in trains of ten bursts. The effects of and differences between rTMS protocols have been investigated since gaining popularity in the 1990's, however, there are still many unknowns regarding the neurophysiological changes that accompany this plasticity. Much research on these effects takes place in motor cortex due to reliable and quantifiable measures of cortical excitability observed there. Here, I sought to further investigate the effects of iTBS on inter-hemispheric changes in in motor cortex using EEG simultaneously recorded with TMS pulses. That is, I examined if iTBS conducted over right motor cortex would lead to measureable changes in excitability indices of left motor cortex. I quantified changes in right and left motor cortex excitability with measurements of TMS-evoked potentials (TEPs) and motor-evoked potentials (MEPs) elicited through blocks of single pulse TMS immediately following iTBS and 30 minutes post-iTBS. I compared the effects of the iTBS condition to those found in a control condition where a sham version of iTBS was administered. Results indicate differential modulations of cortical TEPs between hemispheres, including an initial enhancement of the P30 in right hemisphere, coinciding with sustained suppression in left hemisphere, and an enhancement of the P190 during left hemisphere stimulation.

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Introduction

Principles of TMS

Transcranial magnetic stimulation (TMS) is a powerful tool, increasingly used in neuroscience for study and manipulation of cortical plasticity. TMS works on the principles of electromagnetic induction (Faraday, 1832), in which an electrical current is generated in a conductor when a magnetic field is passed through it. When the TMS coil is placed near the scalp, a strong, brief current that is run through the coil produces a magnetic field that passes transiently through the skull (Barker, Jalinous, & Freeston, 1985). The magnetic field produced induces an electrical field that depolarizes the pyramidal cells and interneurons in the underlying cortical tissue and a subsequent cascade of activity to cells with which they have synaptic connections (Ilmoniemi et al., 1997; Komssi et al., 2002). In contrast to other non-invasive brain stimulation techniques that use electricity to stimulate cortex, TMS can focally target and disrupt functioning in specific regions on the cortical surface. For this reason, TMS is often given the colloquial description of producing "virtual lesions". This characteristic of TMS has allowed researchers to make causal assertions as to the functioning of particular brain regions by measuring behavioral changes due to a disruption in its activity. A myriad of advances in our understanding of the brain, and how it relates to psychological functions, have come from patients with accidental or medically necessitated lesions to the brain. The ability to safely and experimentally produce reversible disruptions of activity of the functioning brain has been a boon to the fields of neuroscience and psychology.

Further developments with TMS have led to the discovery that administering pulses in repetitive temporal sequences is capable of modulating cortical excitability, extending beyond the time of stimulation. This technique known as repetitive TMS (rTMS) can be used to increase

cortical excitability when a high-frequency (≥ 5 Hz) rate of pulses is used (Pascual-Leone, Valls-Solé, Wassermann, & Hallett, 1994), or decrease excitability with low-frequencies (≤ 1 Hz) (Chen et al., 1997). Other forms of rTMS based on previous animal research (Larson & Lynch, 1986; Hess & Donaghue, 1996; Huemmeke, Eysel , & Mittmann , 2002) deliver trains of very high frequency (50Hz) bursts of three pulses at a rate of 5Hz (Huang et al., 2005). This method, known as theta burst stimulation (TBS), can lead to increased states of excitability when delivered intermittently (iTBS; two seconds of stimulation, every ten seconds) or decreased excitability when delivered continuously (cTBS; Huang, et al., 2005). Excitatory effects of iTBS have been shown to persist up to twenty minutes post-stimulation, and use shorter stimulation times and lower intensities than other rTMS protocols. The mechanisms by which these effects take place is still not completely understood, however pharmacological studies in humans indicate long-term potentiation (LTP)- or long-term depression (LTD)-like mechanisms may play at least some role. Huang and colleagues demonstrated that the NMDA receptor antagonist memantine can block the effects of plasticity induced both iTBS and cTBS (Huang, Chen, Rothwell, & Wen, 2007). NMDA receptors are known to be critical for the implementation of long-term synaptic plasticity, however, the effects of rTMS on cortical plasticity are a result of changes occurring across large populations of both excitatory and inhibitory neurons, so is unlikely to be completely driven by LTP or LTD alone.

TMS in human motor cortex

Much of the research concerning the plasticity-inducing effects of rTMS has occurred in human motor cortex. Fortunately, due to the architecture of the brain, the motor cortex provides an easily accessible region on the cortical surface to stimulate with the magnetic field of TMS.

When the focus of the magnetic pulse is applied over motor cortex, activation of pyramidal cells with excitatory connections to motor neurons in the spinal cord produce a contralateral peripheral muscle contraction that can be measured using surface electromyography (EMG). Most commonly, this motor stimulation is conducted by administering TMS pulses to a portion of the hand knob area on the precentral gyrus (Yousry, et al., 1997). When stimulating this region at a sufficient intensity, a measurable contraction occurs in the musculature of the contralateral hand. The amplitude of the resultant motor-evoked potential (MEP) is a quantification of cortico-spinal excitability (Barker et al. 1985). Changes in the mean amplitudes of these MEPs reflect modulations of the intrinsic excitability of motor cortex at that time (Bütefisch, Netz, Weßling, Seitz, & Hömberg, 2003; Ziemann, 2004). In this way, single-pulse TMS can be used as a probe to gauge the current state of excitability of the cortex.

As motor cortex gives a readily available measure of the state of cortical excitability, the excitatory and inhibitory effects of rTMS on motor cortex are generally examined with regard their effects on MEP amplitudes after stimulation. Administration of 1 Hz rTMS over motor cortex (1500 total pulses) reduced MEP amplitudes for up to 30 minutes (Touge, Gerschlager, Brown, & Rothwell, 2001), while 5 Hz TMS (1800 total pulses) has increased MEP amplitudes for up to 30 minutes (Peinemann et al., 2004). Huang and colleagues (2005) first demonstrated the effects of TBS in human motor cortex, showing enhanced MEPs for up to 20 minutes post-iTBS and suppressed MEPs for 20-60 minutes post-cTBS. Additionally, both forms of TBS did not alter H-reflexes evoked in the arm, but did modulate short-interval intracortical inhibition (SICI). The H-reflex is a reflectory contraction in a peripheral muscle due to direct electrical stimulation of the afferent fiber to the spinal cord (Hoffmann 1918; Magladery & McDougal 1950), while SICI is a widely used paired-pulse TMS paradigm known to assess cortical

inhibition (Kujirai et al., 1993). Taken together, these results have been interpreted as reflecting modulations of excitability in the circuits contained in motor cortex and not having direct effects on the spinal cord. When TMS is administered at intensities sufficient to induce a muscle response, there is direct depolarization of the axons of pyramidal corticospinal cells. Discharge of these neurons is the earliest of the series of waves that have been measured in the corticospinal tract (Di Lazzao, et al., 1998a, 1998b). Following this direct, or D-wave, are a series of indirect, or I-waves. These waves reflect depolarization of interneuron axons oriented perpendicular to pyramidal cells with which they are trans-synaptically connected (Patton & Amassian, 1954). These I-waves occur preferentially at lower TMS intensities and begin later than D-waves with latencies of 1-1.5 ms. There is evidence that cTBS reduces the first volley of I-waves and iTBS affects later I-waves (Hamada, et al., 2012), suggesting that their differential effects on MEP and cortical excitability reflect influencing different interneuron networks. Though the effects of TBS occur at the level of cortex, understanding the neural mechanism of this TMS-induced remain unknown.

TMS and neuroimaging

Advancements in technology in recent years have allowed for concurrent measuring of EEG activity during TMS stimulation. Because of the strong electrical field induced by TMS, recording EEG can be technically challenging, but recently developed TMS-compatible amplifiers and electrodes have now made it possible. With this development, the direct cortical reaction and temporal dynamics of the response can be measured. It has been demonstrated that TMS produces predictable event-related responses in the EEG (Ilmoniemi et al., 1997; Paus, Castro-Alamancos, & Petrides, 2001; Komsi et al., 2002) and we are beginning to understand

how these waveforms relate to the state of the cortex. It is widely reported that TMS-evoked potentials (TEPs) elicited from motor cortex evoke a series of replicable voltage deflections: P30, N45, P55, N100, and P130 (Bonato, Miniussi, & Rossini, 2006; Ilmoniemi & Kicic, 2010; Komssi et al., 2002). Responses earlier than 20-30ms are largely lost due to the TMS artifact. While little is known about the neural generators of these TEP components, some functional differences have been described between them. The P30 component has shown to correlate with the amplitude of MEPs, suggesting that the earliest peaks may provide some information of EPSPs in the corticospinal pyramidal cells (Maki & Ilmoniemi, 2010). The N100 increases in amplitude when the subject tries to actively resist the effect of the TMS pulse (Bonnard, Spieser, Meziane, De Graaf, & Pailhous, 2009) and decreases when subjects prepare to make a movement (Bender et al., 2005). The timing of the N100 also coincides with $GABA_B$-mediated IPSPs (Connors, Malenka, & Silva, 1988; Premoli et al., 2014).

Combining TMS with other neuroimaging techniques such as fMRI, PET, and optical imaging has demonstrated changes in activity in distributed networks connected synaptically with the stimulated region (Ilmoniemi et al., 1997; Komssi et al., 2002). A number of fMRI studies have shown that changes in activity can be detected well beyond the site of initial stimulation (Bohning et al., 1998; Bestmann, Baudewig, Siebner, Rothwell, & Frahm, 2004). Neuroimaging methods have been used to assess functional connectivity between interconnected areas of motor system (Baudweig et al., 2001; Parks et al., 2012), frontal eye fields (Morishima et al., 2009; Paus et al., 1997), and the default mode network (Eldaeif, Halko, Buckner, & Pascual-Leone, 2011).

Inducing changes in functionally connected areas have found utility in the clinical domain as well. Inhibitory rTMS over contralesional M1 in stroke patients decreased the

interhemispheric inhibitory effects this region had over ipsilesional M1 and was correlated with behavioral improvements (Grefkes et al., 2010). Anti-depressant effects of rTMS have been reported to relate to functional connectivity between prefrontal cortex and cingulate cortex (Fox, Buckner, White, Greicius, & Pascual-Leone, 2012) and a number of psychologic disorders have begun using rTMS as a treatment option (Kim, Pesiridou, & O'Reardon, 2009). To this end, much of the current research on the mechanisms of plasticity induced by rTMS can be useful in aiding treatment of psychological and neuropathic disorders.

The current investigation

To date, the effects of rTMS-induced plasticity have been primarily investigated in the local region of cortex stimulated by rTMS and the functional connectivity between regions in which it is interconnected. However, the impact of rTMS-induced plasticity on the excitability of these interconnected cortical areas remains poorly understood. A small number of studies have examined the effects of rTMS in non-primary motor areas has on M1 excitability. The findings in these studies have not been consistent and have only examined a limited number of rTMS protocols (Baumer et al., 2003; Schlaghecken, Münchau, Bloem, Rothwell, & Eimer, 2003; Rizzo et al., 2004). Another study has shown disinhibition in contralateral motor cortex after low frequency rTMS (Plewnia, Castellanos, & Gerloff, 2003), however the direct cortical responses were not tested here and it is unclear if these results would hold for other rTMS protocols. Here, I describe the results of an experiment designed to elucidate the effects that modulating the state of excitability in one brain region has on the excitability of a region with which it has intrinsic inter-hemispheric connections. Motor cortex provides the most replicated and robust measures of cortical excitability and has well documented results with rTMS protocols. In this study I

administered an iTBS protocol over right motor cortex to examine the extent to which upregulation in excitability will be propagated to left motor cortex. I measured cortical excitability in each hemisphere by eliciting MEPs and TEPs with blocks of single-pulse TMS. I hypothesized that measures of excitability would be significantly increased in the stimulated right cortex and there would be some measure of transfer effects to the contralateral left motor cortex.

Method

Subjects

Twelve right-handed subjects (Oldfield, 1971) were recruited from the University of Arkansas undergraduate and graduate population. Subjects participated in an experimental and control session conducted on separate days, with each session being approximately four hours in duration. All procedures were approved by the Institutional Review Board of the University of Arkansas and subjects provided written consent, were screened extensively for safety with TMS (Rossi et al., 2009; Wassermann, 1998), and compensated $15.00/hour and/or class credit for their time.

Procedure

Participants completed two separate sessions (iTBS and sham control) which each contained blocks of behavioral motor tasks performed on a computer and blocks of single-pulse TMS over each hemisphere to assess cortical excitability. Each subject completed a baseline block of behavioral tasks and single-pulse TMS blocks prior to the iTBS or Sham (control) protocol. The iTBS (or Sham) protocol was followed by two post-stimulation time points of

single-pulse TMS, one immediately following the iTBS or sham stimulation and one occurring approximately 30 minutes after, and one block of behavioral tasks (Figure 1). Each time point of single-pulse TMS contained two blocks of 60 pulses over each left and right hemispheres, alternating between them. As such, each hemisphere was stimulated 120 times per block, with the hemisphere of first stimulation counterbalanced between subjects. The total experiment consisted of 360 single-pulses per hemisphere across two pre-iTBS and four post-iTBS blocks of single-pulse TMS. Throughout the experiment the subjects were seated in a comfortable chair approximately 60 cm from the computer screen. During the stimulation procedures, subjects were asked to relax and maintain fixation on the center of the computer screen while the letters 'T' and 'F' were randomly displayed one at a time. Subjects were instructed to count the number of 'T' letters they saw to direct their attention away from the ongoing stimulation procedure.

Behavioral Tasks	Single-Pulse TMS (MEP/TEP)	Active iTBS or Sham iTBS	Single-Pulse TMS (MEP/TEP)	Behavioral Tasks	Single-Pulse TMS (MEP/TEP)
~20 minutes	~15 minutes	~10 minutes	~15 minutes	~20 minutes	~15 minutes

***Figure 1**: Sequence and approximate timing of an experimental session.*

Transcranial Magnetic Stimulation (TMS)

TMS was administered using a PowerMag 100 stimulator (Brain Products, Munich, Germany) with a figure-of-eight coil. Coil placement on the scalp was determined by mapping right and left M1 hand representations according to a standard grid method. The location that provided the maximal evoked MEP in each hand was marked on the electrode cap to ensure consistent coil placement. A small foam patch, approximately 3 mm thick, was placed over the surrounding electrodes to attenuate any artefactual electrical noise introduced by the coil.

All effects of rTMS-induced plasticity were investigated within right M1 as it is not overrepresented in right-handed individuals and ensures the generalizability of results to other regions of cortex. Stimulation intensities were set to each subject's active (rTMS) and resting (single-pulse) motor thresholds. Resting motor threshold (RMT) was determined in right and left hemispheres as the stimulator intensity required to obtain an MEP with amplitude greater than 200 μV on half of trials with the hand at rest. RMT was measured using a 1-up 1-down staircase (12 reversals). Active motor threshold (AMT) was measured in right hemisphere in the same manner as RMT but with the hand contracted at 10% maximum grip force. A dynamometer was used to give continuous visual feedback of force output during AMT measurement.

Single-pulses used to elicit our dependent measures of cortical excitability, MEPs and TEPs, were delivered at 110% RMT to right and left M1 at a random delay between 5000 and 8000 ms. The iTBS protocol applies bursts of three 50Hz pulses (80% AMT) occurring with a 200ms interval between burst onsets (Figure 2). Trains of 10 such bursts were followed by a 8000 ms rest in accordance with safety recommendations (Huang et al., 2005; Rossi, Hallet, & Rossini, 2009) with the standard protocol for iTBS being 20 trains of 10 bursts (Huang et al., 2005). Sham-iTBS was conducted by orienting the coil 90º from its active position and placing the edge of the coil, like an axe, against the scalp to control for tactile and auditory stimulation.

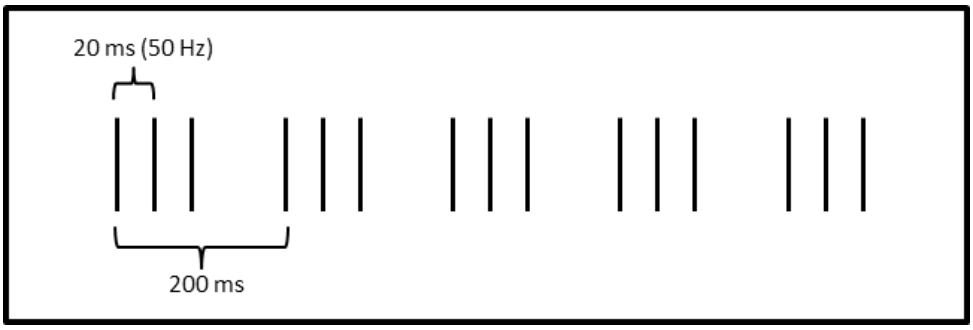

Figure 2*: iTBS protocol administers three pulses given at 50Hz, repeated at 5 Hz. Ten bursts are applied for 2 seconds, followed by an 8 second break. Total stimulation time is approximately 192 seconds.*

EEG and EMG Recording

EEG was recorded with a 64-channel BrainAmp DC (Brain Products GmbH, Munich, Germany), which is designed for use with TMS and is capable of recovering from magnetic artifact within 10 to 20 ms. The electrode cap is fitted with low-profile, notched, Ag/AgCl ring electrodes to ensure the TMS coil is within close proximity to the scalp, and placed according to the modified 10-10 system. Electrodes from which we recorded were: FPz, FP1/2, AF3/4, AF 7/8, Fz, F1/2, F3/4, F5/6, F7/8, FCz, FC1/2, FC3/4, FC5/6, FT7/8, Cz, C1/2, C3/4, C5/6, T7/8, CPz, CP1/2, CP3/4, CP5/6, TP7/8, Pz, P1/2, P3/4, P5/6, P7/8, POz, PO3/4, PO7/8, Oz, O1,2, Iz. Continuous EEG was recorded in reference to the left mastoid and re-referenced to the average of left and right mastoids, offline. Bipolar electrode pairs above and below the right eye and on the outer canthus of each eye recorded the vertical and horizontal electrooculogram (EOG), respectively. Electrode impedance was kept at or below 5 kΩ and data was recorded at 5000 Hz (DC - 1000 Hz). EMG was recorded simultaneously with EEG from the left and right hands using a 16-channel BrainAmp ExG bipolar amplifier (Brain Products GmbH, Munich, Germany). Bipolar Ag/AgCl electrodes were positioned on the first dorsal interosseous muscle

of the left and right hands in a belly-tendon montage with left wrist ground. Electrode impedance was kept at or below 15 kΩ and data recorded at 5000 Hz (DC - 1000 Hz).

Offline, EEG data containing TMS artifact was removed by means of the cubic interpolation function in MATLAB (Mathworks, Natick, MA) (Thut et al., 2011). A conservative time window of 0 - 25ms relative to the TMS pulse was used to ensure no distortion from the magnetic artifact would arise during filtering. All subsequent pre-processing was done using BrainVision Analyzer software 2.0 (Brain Products GmbH, Munich, Germany). After interpolation, the data was filtered at 1.0 – 50 Hz with a 24db/oct rolloff and a 60 Hz notch filter to reduce any line noise artifacts. Blink and eye movement artifacts were removed from the filtered data using independent components analysis (ICA) (Johnson, Hamidi, & Postle, 2010; Veniero, Ponzo, & Koch, 2013; Casula et al., 2014). Independent components were visually inspected and identified for removal by scalp topographies and timing coincident with the EOG channels. The data was epoched in to 300 ms segments with a 50 ms baseline prior to the TMS pulse, baseline corrected, and artifact rejected using ± 200 μV criterion for segment removal. Remaining segments were used for subject and grand averages for subsequent analyses. Based on the literature (Ilmoniemi & Kičić, 2010; Casula et al., 2014) and verified with visual inspection of grand average waveforms I chose the following time windows for analyses of TEPs: P30 (25-35ms); N45 (40-50 ms); P55 (45-65ms); N100 (75-125ms); P190 (150-250ms). Mean amplitudes in each time window were calculated from each subject average and used for statistical analyses.

EMG data was digitally band-pass filtered from 1 to 1000 Hz (24 dB/oct) and data was segmented in to 300 ms epochs, with a 50 ms baseline relative to the TMS pulse to quantify MEPs. Segments were baseline corrected and rejected if they contain EMG exceeding an

absolute voltage of 50 μV in the baseline or an MEP of less than 100 μV in magnitude. Peak-to-peak amplitudes of the positive and negative deflections of the MEP from remaining segments for each subject were used for statistical analysis. Outlier MEP trials were discarded within each block, for each participant, if amplitudes exceeded 3 standard deviations from the mean.

Statistical Analyses

All statistical analyses were conducted using IBM SPSS version 20 (IBM Corp., Armonk, New York). Assumptions of normality were tested prior to analysis of variance tests with the Shapiro-Wilk's test. All TEP and MEP data was non-significant at $\alpha = .05$, indicating normal distributions. Mean MEP amplitudes from the first two blocks were averaged together as a baseline of cortical excitability and differences from these values were analyzed with a 2 (condition: iTBS, sham) × 2 (hemisphere of stimulation: right, left) × 4 (time: Post-iTBS blocks) repeated measures ANOVA. Separate 2 × 2 × 4 repeated measures ANOVA were conducted in the same manner for each of the five TEP components. Electrodes for analysis were determined by inspecting scalp maps of grand averages and selecting the electrode where the signal was centered at the component's time window (Figures 3 and 4). In the rare occasions that a subject did not have at least 30 trials (50%) in a block, MEP and TEP values were imputed by adding the mean difference from baseline for that block to the subject's baseline value. Statistics were run both without the subject in the analysis and with the missing values imputed. There were no differences in the pattern of results between the two methods, so all results reported are with the imputed values included. Sphericity was assessed by means of the Mauchly's test and the Greenhouse-Geisser correction used where applicable. Follow-up pairwise comparisons were corrected with the Bonferroni method.

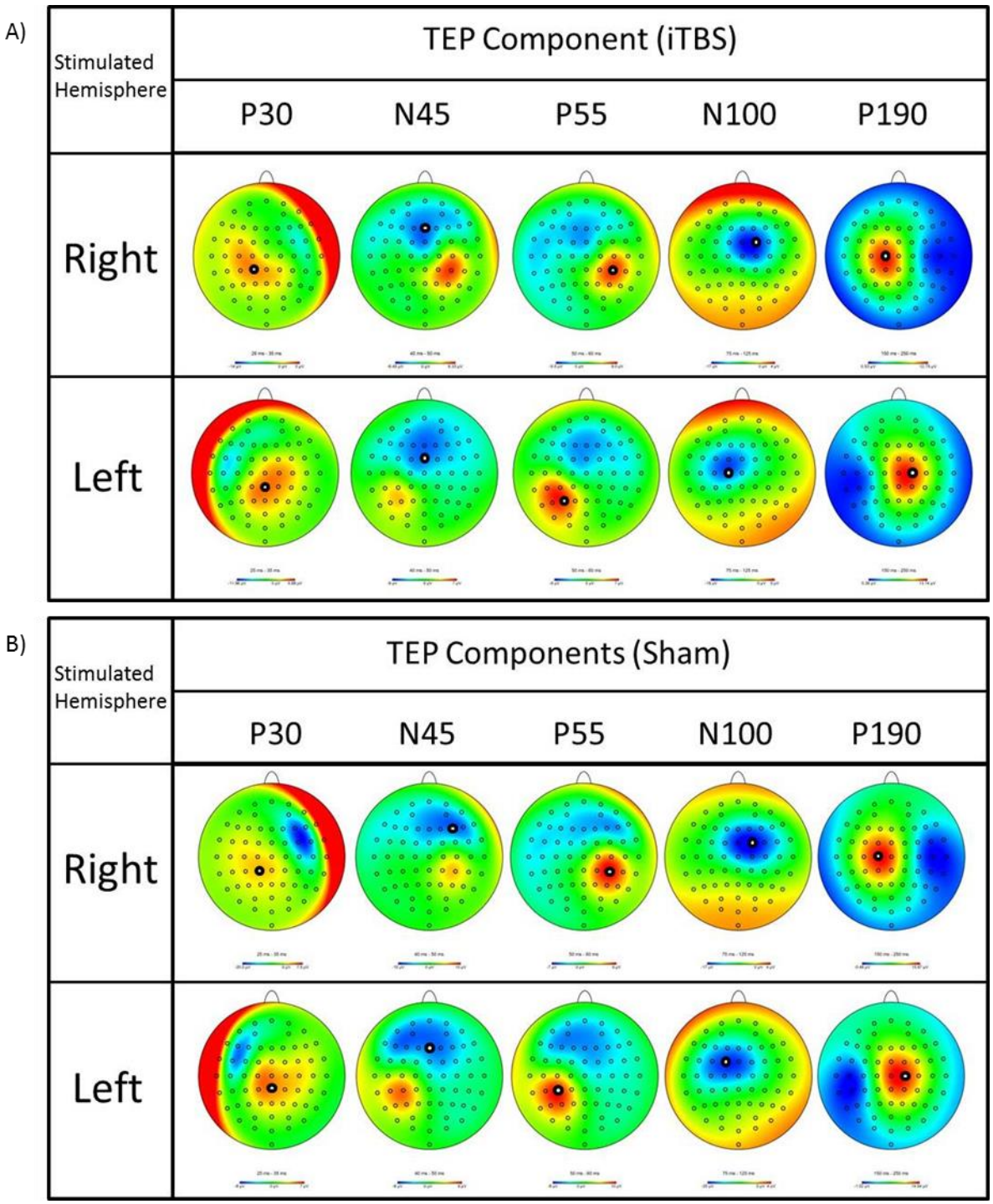

Figure 3: *Scalp maps of component time windows from grand averages and electrodes selected for analysis for (A) iTBS condition and (B) Sham condition.*

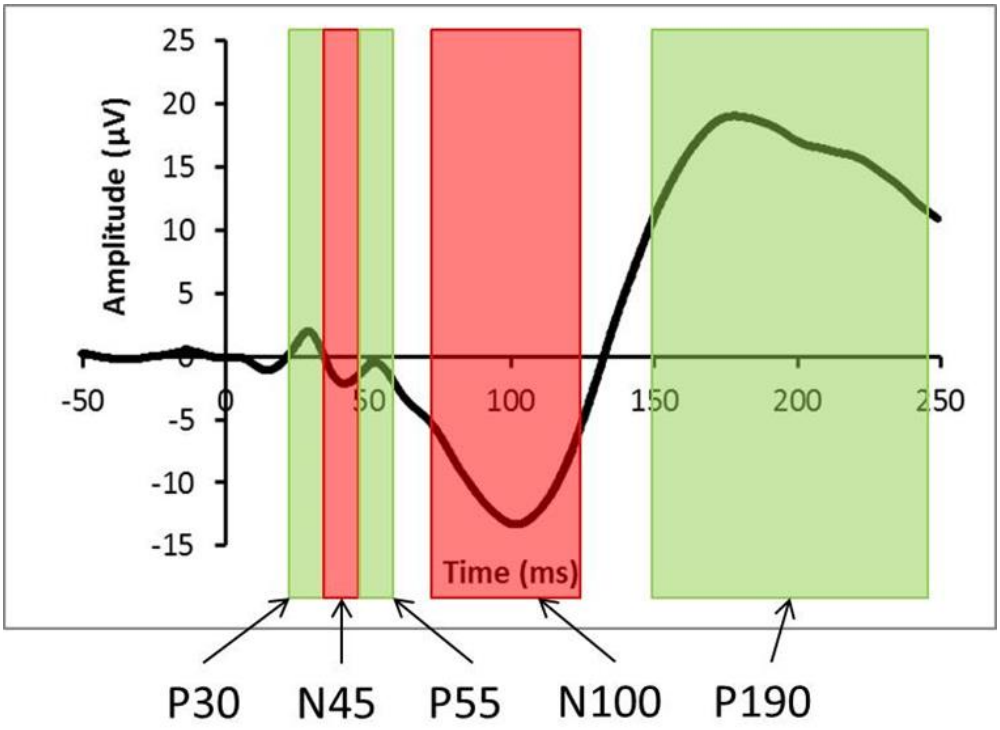

Figure 4: *Grand average waveform and component time windows*

Results

Motor-evoked potentials (MEPs)

Baseline MEP amplitudes did not differ between condition (p = .419) or hemisphere of stimulation (p = .519) and there were also no differences in the average number of outliers between the iTBS (12.58, 1.75%) and sham (10.17, 1.41%) conditions (p = .155). The omnibus ANOVA test revealed no significant differences between condition (p = .890) or hemisphere of stimulation (p = .619) due to iTBS stimulation. However, there was a significant effect of time, $F(3,33) = 2.959$, $p = .046$, $\eta^2 = .212$, that was qualified by a significant linear trend, $F(1,11) = 5.981$, $p = .032$, $\eta^2 = .352$. Mean amplitudes increased relative to baseline in both hands and in both conditions, with the largest increases in the final block. Pairwise comparisons between the final block and baseline revealed that only the right hemisphere stimulation in the iTBS

condition had a significant increase, t(11) = 2.95, p = .013. However, there were no differences in amplitude between the iTBS and sham condition (Figure 5).

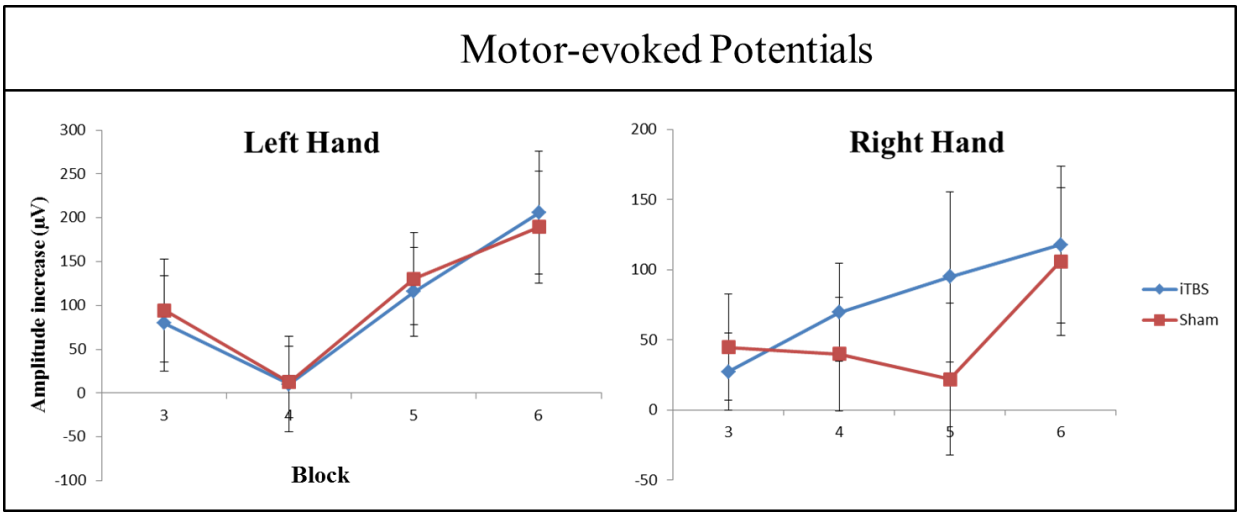

Figure 5*. Amplitude difference from baseline in motor-evoked potentials (MEPs). Condition × Hemisphere × Block ANOVA revealed significant effect of time, F(3,33) = 2.959, p = .046, η^2 = .212*

*TMS-evoked potentials (TEPs***)**

Analysis of baseline TEP amplitudes revealed no initial differences between condition (p = .149) or hemisphere of stimulation (p = .670). The repeated measures ANOVA for the P30 component revealed a marginally significant condition × hemisphere interaction, F(1,11) = 4.021, p = .070, η^2 = .268 (Figure 6.). This interaction appeared to be driven by an immediate potentiation of the component after iTBS stimulation in right hemisphere, while there was a sustained suppression in left hemisphere post-iTBS stimulation. This interaction suggests that the effect of condition appears to have differing effects in each hemisphere, therefore separate, post-hoc tests were run to evaluate the effect of condition in each hemisphere individually. Post-iTBS, the P30 component was significantly smaller in the left hemisphere relative to the sham condition, t(11) = 2.743, p = .019 (Figure 7.). There was no significant effect of condition in

right hemisphere. Analysis of the P190 component revealed a marginally significant condition × hand interaction, $F(1,11) = 3.297$, $p = .097$, $\eta^2 = .231$ (Figure 6.). No differences were observed during right hemisphere stimulation, but there was a marginal effect of condition during left hemisphere stimulation, $F(1,11) = 3.706$, $p = .080$, $\eta^2 = .252$, with enhancement in the iTBS condition relative to sham. There was also a strong main effect of block for the P190, $F(3,33) = 6.072$, $p = .002$, $\eta^2 = .356$, which was qualified by a significant cubic trend, $F(1,11) = 9.023$, $p = .012$, $\eta^2 = .451$. No effects of condition were observed for the N45, P55, or N100 components.

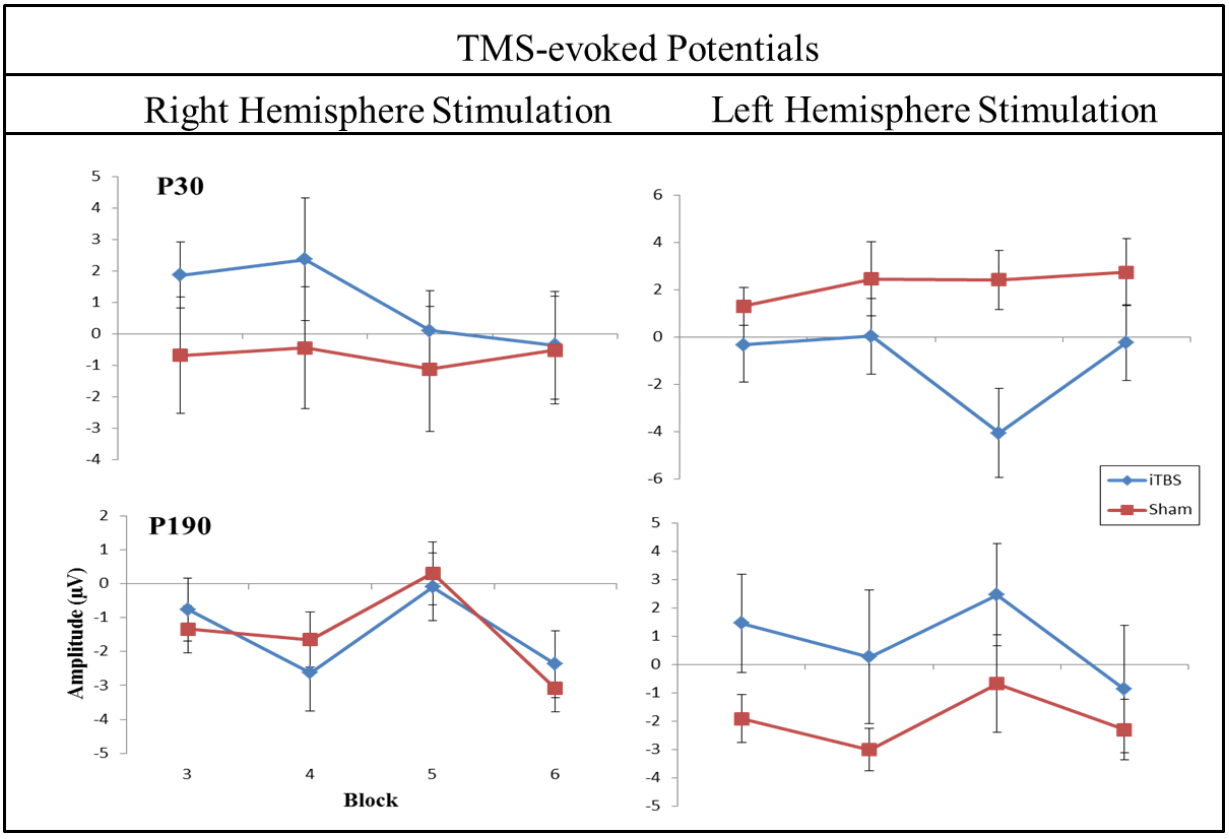

Figure 6*. Amplitude difference from baseline for P30 and P190 components. Marginally significant condition × hemisphere interaction, F(1,11) = 4.021, p = .070 in P30 component; condition × hand interaction, F(1,11) = 3.297, p = .097 in P190 component.*

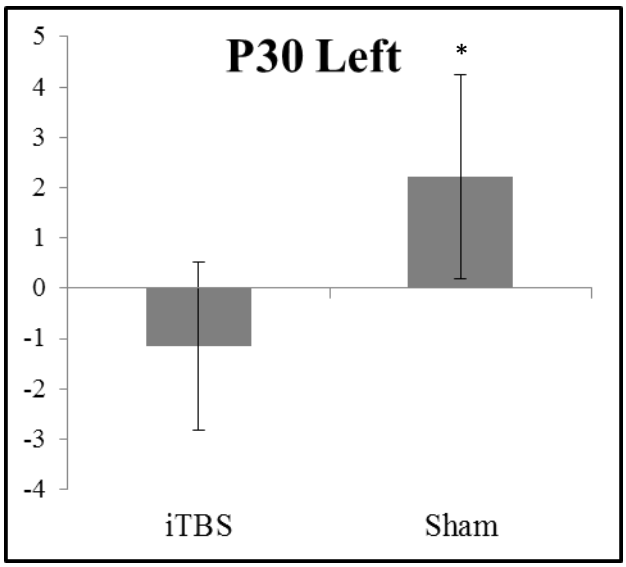

Figure 7. *Effect of condition on P30 component in right hemisphere, t(11) = 2.743, p = .019*

Discussion

The present study examined changes in the cortical excitability of left and right motor cortices after conducting an iTBS protocol over right motor cortex. The iTBS procedure uses trains of high-frequency TMS pulses and has been to shown increase the excitability of the underlying cortical tissue (Huang et al., 2005). I indexed changes in cortical excitability by applying single-pulse TMS to right and left motor cortices to elicit motor-evoked potentials, measured from the hands, and TMS-evoked potentials, measured from the scalp.

Our results indicated that MEP amplitudes were larger post-iTBS intervention, but this occurred in the sham condition as well as the experimental condition and there were no differences between the conditions at any time points. Results from scalp measures of cortical excitability were equally murky. Significant differences from sham were apparent for the P30 component in each hemisphere, suggesting that the iTBS had some measure of effect in the contralateral hemisphere. I observed differing effects of iTBS stimulation on the P30 between the

two hemispheres; an initial enhancement of the component in right hemisphere and a sustained suppression in left hemisphere. Although interpretation of the generation of this component is not well known, evidence suggests that this component reflects a combination of both EPSPs (Maki & Ilmoniemi, 2010) and IPSPs (Ferrarelli et al., 2010). Consistent with previous research (Paus et al., 2001), I found this component to be distributed centrally over the scalp and activations contralateral to the stimulation site. For this reason, this has been proposed to represent spreading of activation via transcallosal and subcortical pathways (Bonato et al., 2006). A few studies have found a positive trial-by-trial relationship between the P30 and MEP amplitudes (Maki & Ilmoniemi, 2010; Ferreri et al., 2011; Vernet et al., 2013). Our finding that the P30 was suppressed compared to sham when stimulating contralateral to the iTBS stimulation site would therefore expect to be accompanied by suppression of MEPs. On the other hand, these studies did not examine this relationship in regard to iTBS stimulation, so it could be interpreted that iTBS has a differential effect on this association. Di Lazarro et al., (2010) demonstrated that cTBS reduced the first wave of corticospinal I-waves, while iTBS enhanced later I-waves, further suggesting different mechanisms of action and not opposite effects on the same mechanism. A recent animal study also showed iTBS, cTBS, and 1Hz-rTMS all had differential effects on markers of GABAergic neurotransmission (Trippe, Mix, Aydin-Abidin, Funke, & Benali, 2009). If iTBS is suspected to work through weakened inhibition, specifically at fast-acting $GABA_A$ sites, release of sub-cortical inhibition to the contralateral motor cortex could be accompanied by an opposing increase in inhibition in this pathway when probing the contralateral motor cortex. In this way there could be a suppression of the P30 component in left motor cortex, while leaving the MEPs relatively unaffected.

This data also indicated a significant enhancement of the P190 component due to iTBS during single-pulse probes of the left, but not right, hemispheres. Very little is known about this component and it was initially believed to be a response to the click sound that the coil makes when discharging (Nikouline, Ruohonen, & Ilmoniemi, 1999; Tiitinen et al., 1999). However, later studies have sufficiently masked the coil sound without fully eliminating the P190 peak, concluding that this does indeed have some cortical contributions (Komssi, Kähkönen, & Ilmoniemi, 2004; ter Braack, de Jonge, & van Putten, 2013). The scalp distribution for this component was centrally located, entirely contralateral to the stimulation site. The timing and scalp distributions of our results align closely with the P2 component of the auditory-evoked potential (AEP). However, the P2 is centrally located at the midline and it would be unlikely that an AEP from the coil click would produce such a lateralized distribution as our results. It would also be unlikely that there would be differences between our experimental and sham conditions if it were entirely the result of an AEP. I cannot however tease apart how much of this signal may be auditory processing, but it seems clear that some part of the signal is being driven by our cortical stimulation. Prior studies of iTBS modulated plasticity may not have seen this effect because few have examined effects in the hemisphere contralateral to stimulation site, nevertheless more research on this component is needed to determine its generation.

The data failed to show any enhancement of MEPs after the iTBS protocol above what was seen in the sham condition. There was however an overall effect of time on MEP amplitudes, such that MEPs were larger at the end of the experiment than at baseline across both groups. These results seem to indicate that the iTBS procedure had no effect on cortical excitability; however, there may be a number of explanations for this. The effects of rTMS protocols have recently been found to have high individual differences (Ridding & Ziemann,

2010). Consistent with our findings, a number of recent studies have found no group-level differences in the after effects of iTBS on measures of RMT or MEP amplitudes (Hamada et al., 2012; Lopez-Alonso, Cheeran, Río-Rodríguez, & Fernández-del-Olmo, 2014). Indeed, even if a study by Nettekoven and colleagues (2014) group-level differences in MEP amplitudes were seen despite more than 50% of subjects not responding in the "canonical" fashion. Due to the high variability in responsiveness to TBS protocols, research has begun examining what factors may predict responsiveness in the individual. Hamada et al. (2012) completed a large (n = 52) study in which subjects completed both iTBS and cTBS protocols in separate sessions. Approximately a quarter of these subjects responded to both of these procedures in the expected manner, while only half responded to one and not the other. Their experiment analyzed the role that recruitment of early and late I-waves had on the efficacy of TBS protocols and concluded that individuals that exhibited easier recruitment of late I-waves responded best to each TBS procedure.

Other studies have observed that functional connectivity within the motor network is correlated with rTMS after effects. "Non-responders" to iTBS stimulation had significantly higher resting-state functional connectivity (rsFC) between pre-motor and primary motor cortex at baseline than "responders" (Nettekoven et al., 2014). Salomons et al (2014) also found that higher rsFCs in those with major depressive disorder that did not respond to rTMS treatment. These findings could suggest that there is a ceiling effect, in that high functional connectivity's between regions prohibits excitability modulations. Preferential recruitment of late I-waves has also been linked to individuals with weaker rsFC of the motor network indicating responsiveness to TBS, recruitment of late I-waves, and rsFC may be linked through the same circuitry (Volz et al., 2014). Here, I do not have measures of rsFC nor recruitment of late I-waves, however

exploratory analyses between responders and non-responders could reveal other potential individual difference markers.

Other potential confounds that can produce differing after effects of rTMS are time of day, attention, genetics, and prior motor activity (Ridding & Ziemann, 2010). In our experiment, I did not control for time of day when our participants came in, so I am unable to determine if this could have caused potential inter-individual variability or differences in cortical reactivity between sessions within individuals. Our participants also engaged in a set of motor tasks before iTBS, but after their initial MEP and TEP baseline measurements. Short periods of active motor training have been shown to increase excitability in cortex (Lotze, Braun, Birbaumer, Anders, & Cohen, 2003) and some studies have shown reversals of the expected TBS effects when the stimulation is applied following motor training (Iezzi et al., 2008; Gentner, Wankerl, Reinsberger, Zeller, & Classen, 2008). These results are akin to several priming studies that have shown that short primes of facilitatory or inhibitory brain stimulation lead to reversal or negation of effects when followed by a protocol of the same nature (facilitatory or inhibitory) and enhanced effects of protocols of the opposite kind (Müller, Orekhov, Liu, & Ziemann, 2007; Hamada et al., 2009; Todd, Kimber, Ridding, & Semmler 2009). It could be interpreted that I did not see enhancement effects in our MEP data because the iTBS effects were negated by an increased excitability in cortex due to motor training. It is also important to keep in mind that MEPs are a composite measure of the state of the entire cortico-spinal tract and behavioral motor tasks may cause modulations in the excitability of this network beyond just cortical changes. Examination of EEG data during these behavioral tasks will help elucidate this problem and give a fuller picture of potential iTBS-induced plasticity.

Conclusion

In this study I demonstrated differential patterns of cortical modulation in left and right hemispheres, evidenced by the P30 and P190 components, after an iTBS protocol was administered over right motor cortex. Although MEP amplitudes were not altered, I believe that this could be accounted for by a combination of prior motor training and an expectation that 25%-50% of subjects will not be "responders". Further, TEP measures should indicate more subtle changes in cortex as it is a direct measure from the scalp, whereas MEP measures are a composite measure of the state of the cortico-spinal tract to the peripheral muscle. Further analyses are required of the brain states during motor tasks to determine differences in behavioral measures due to iTBS stimulation.

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Appendix

UNIVERSITY OF ARKANSAS

Office of Research Compliance
Institutional Review Board

March 17, 2014

MEMORANDUM

TO:	Nathan Parks Matthew Gannon Ashley Knapp
FROM:	Ro Windwalker IRB Coordinator
RE:	PROJECT CONTINUATION
IRB Protocol #:	13-03-556
Protocol Title:	*Neurophysiological Investigations of Human Perceptual and Motor Systems*
Review Type:	☐ EXEMPT ☐ EXPEDITED ☒ FULL IRB
Previous Approval Period:	Start Date: 05/20/2013 Expiration Date: 04/11/2014
New Expiration Date:	04/11/2015

Your request to extend the referenced protocol has been approved by the IRB. If at the end of this period you wish to continue the project, you must submit a request using the form *Continuing Review for IRB Approved Projects*, prior to the expiration date. Failure to obtain approval for a continuation on or prior to this new expiration date will result in termination of the protocol and you will be required to submit a new protocol to the IRB before continuing the project. Data collected past the protocol expiration date may need to be eliminated from the dataset should you wish to publish. Only data collected under a currently approved protocol can be certified by the IRB for any purpose.

This protocol has been approved for 130 total participants. If you wish to make *any* modifications in the approved protocol, including enrolling more than this number, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 210 Administration Building, 5-2208, or irb@uark.edu.

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