Published in final edited form as:

Cortex. 2016 January ; 74: 134–148. doi:10.1016/j.cortex.2015.10.004.

Shifts in connectivity during procedural learning after motor cortex stimulation; a combined transcranial magnetic stimulation /functional magnetic resonance imaging study

Adam Steel^a, Sunbin Song^b, Devin Bageac^a, Kristine M. Knutson^a, Ziad S. Saad^c, Stephen J. Gotts^d, Eric M. Wassermann^{a,*}, and Leonora Wilkinson^a

Adam Steel: adam.steel@nih.gov; Sunbin Song: songss@mail.nih.gov; Devin Bageac: devin.bageac@nih.gov; Kristine M. Knutson: knutsonk@ninds.nih.gov; Ziad S. Saad: saadz@mail.nih.gov; Stephen J. Gotts: gottss@mail.nih.gov; Leonora Wilkinson: Leonora.Wilkinson@nih.gov

^aBehavioral Neurology Unit, National Institute of Neurological Disorders and Stroke, National Institutes of Health, 10 Center Dr., MSC 1440, Bethesda, Maryland, 20892-1440, USA

^bHuman Cortical Physiology Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, 10 Center Dr., MSC 1440, Bethesda, Maryland, 20892-1440, USA

^cScientific and Statistical Computing Core, National Institute of Mental Health, National Institutes of Health, 10 Center Dr., MSC 1440, Bethesda, Maryland, 20892-1440, USA

^dLaboratory of Brain and Cognition, National Institute of Mental Health, National Institutes of Health, 10 Center Dr., MSC 1440, Bethesda, Maryland, 20892-1440, USA

Abstract

Inhibitory transcranial magnetic stimulation, of which continuous theta burst stimulation (cTBS) is a common form, has been used to inhibit cortical areas during investigations of their function. cTBS applied to the primary motor area (M1) depresses motor output excitability via a local effect and impairs procedural motor learning. This could be due to an effect on M1 itself and/or to changes in its connectivity with other nodes in the learning network. To investigate this issue, we used functional magnetic resonance imaging to measure changes in brain activation and connectivity during implicit procedural learning after real and sham cTBS of M1. Compared to sham, real cTBS impaired motor sequence learning, but caused no local or distant changes in brain activation. Rather, it reduced functional connectivity between motor (M1, dorsal premotor & supplementary motor areas) and visual (superior & inferior occipital gyri) areas. It also increased connectivity between frontal associative (superior & inferior frontal gyri), cingulate (dorsal & middle cingulate), and temporal areas. This potentially compensatory shift in coupling, from a motor-based learning network to an associative learning network accounts for the behavioral effects of cTBS of M1. The findings suggest that the inhibitory transcranial magnetic stimulation

*Correspondence to: Eric M. Wassermann, MD, 10 Center Dr., MSC 1440, Bethesda, Maryland, 20892-1440, USA; wassermann@ninds.nih.gov, telephone: + (1) 301-496-0151, + (1) fax: 301-480-2909.

The authors declare no conflict of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

affects behavior via relatively subtle and distributed effects on connectivity within networks, rather than by taking the stimulated area “offline.”

Keywords

continuous theta burst stimulation; functional connectivity; primary motor cortex; probabilistic sequence learning; procedural learning

1.1 Introduction

Analysis of patients with focal lesions from brain injury or disease is a classical means of assigning function to structure. The introduction of non-invasive brain stimulation techniques, notably inhibitory transcranial magnetic stimulation (TMS), has made it possible to alter processing in regions of the healthy human cortex temporarily, creating what have been called “virtual lesions” and establishing the involvement of brain regions in laboratory tasks (Breton & Robertson, 2014; Grafman & Wassermann, 1999; Jahanshahi & Rothwell, 2000; Pascual-Leone, Walsh, & Rothwell, 2000; Song, Sandrini, & Cohen, 2011).

Inhibitory TMS of the primary motor area reduces corticospinal excitability, measured as a decrease in the amplitude of the motor evoked potential (MEP). This effect resembles long-term synaptic depression (R. Chen et al., 1997; Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005; Iyer, Schleper, & Wassermann, 2003; Wassermann, Wedegaertner, Ziemann, George, & Chen, 1998; Wilkinson et al., 2015) and likely reflects a change in the efficacy of synapses downstream from the stimulated neurons. In addition, inhibitory TMS over M1 during various phases of procedural memory formation reduces learning, confirming the involvement of M1 in acquisition, consolidation, and retention of visuo-motor skill knowledge (Hadipour-Niktarash, Lee, Desmond, & Shadmehr, 2007; Robertson, Press, & Pascual-Leone, 2005; Rosenthal, Roche-Kelly, Husain, & Kennard, 2009; Wilkinson et al., 2015; Wilkinson, Teo, Obeso, Rothwell, & Jahanshahi, 2010). These findings are consistent with those of animal studies showing that motor skill learning is associated with synaptic potentiation in M1 (Rioult-Pedotti, Friedman, Hess, & Donoghue, 1998) and changes in the organization of local circuits involved in trained movements (Nudo, Milliken, Jenkins, & Merzenich, 1996). In humans as well, M1 changes after motor training suggest that remodeling of M1 circuits is a component of motor learning (Karni et al., 1995; Muellbacher et al., 2002).

Although M1 inhibition could disrupt learning via its effect on local synapses, perhaps by blocking the reassignment and strengthening of local connections, TMS can also affect activity in distant brain regions via connections from the stimulated site (Andoh & Zatorre, 2013; Baumgartner, Knoch, Hotz, Eisenegger, & Fehr, 2011; Bestmann, Baudewig, Siebner, Rothwell, & Frahm, 2004; Bestmann et al., 2008; Cardenas-Morales, Groen, & Kammer, 2011; Lee & D'Esposito, 2012; Ruff et al., 2008; Ward et al., 2010; Watanabe et al., 2014). Changes to network connectivity are also a plausible mechanism for the effects on learning. Connectivity between motor areas increases with skill learning (Debas et al., 2014; Sun, Miller, Rao, & D'Esposito, 2007), and the connectivity of this network can be changed by TMS (Bestmann et al., 2008). In another study (Wilkinson et al., 2015), we found no

correlation between the degree of MEP depression and the effect on learning within individuals, suggesting that the two effects have different origins. If inhibitory TMS of M1 produces synaptic changes in a network responsible for procedural motor learning, tracing those changes might provide new information on its function.

One paradigm that has been developed to study procedural learning is the serial reaction time task (SRTT) (Nissen & Bullemer, 1987). Typically, on each trial of the SRTT, a target appears in one of four box locations, and participants must respond as quickly as possible by pressing a corresponding key on a keypad. Participants perform several blocks of trials (e.g., 20 blocks of 100 trials). Reaction times (RTs) and errors are measured. Unknown to participants, the majority of targets appear in a predetermined repeating sequence of box locations. A sequence can be presented deterministically or probabilistically. Probabilistic presentation on the probabilistic SRTT (pSRTT) involves two sequences shown concurrently across blocks with one of two sequences occurring more frequently. When RT and error rates decrease differentially between the sequences it can be inferred that the more frequent, “probable,” sequence has been learned. The advantage of pSRTT relative to the classical, deterministic, version is that it provides a continuous index of learning across blocks. The element of noise in the pSRTT also makes the development of explicit sequence knowledge less likely.

Continuous theta burst stimulation (cTBS) appears to be a particularly efficient way of reducing M1 excitability and motor sequence learning. This reduction has consistently been seen for durations up to 50 - 60 min when cTBS is delivered in a 40-second train of 600 pulses (Gamboa et al., 2011; Gentner, Wankerl, Reinsberger, Zeller, & Classen, 2008; Huang et al., 2005; Wilkinson et al., 2015). A 20-second train of 300 pulses delivered to M1 reduces the MEP for up to 20-30 minutes (Di Lazzaro et al., 2005; Gentner et al., 2008; Huang et al., 2005). Both cTBS durations temporarily impair motor sequence learning on the pSRTT when delivered to M1 (Rosenthal et al., 2009; Wilkinson et al., 2015; Wilkinson et al., 2010).

Here, we used functional magnetic resonance imaging (fMRI) to examine the effects of real and sham cTBS to M1 (using a 40-second train of 600 pulses) on whole-brain activation and functional connectivity during procedural motor sequence learning on the pSRTT. We expected that real cTBS would reduce connectivity among motor regions during learning. As we anticipated that cTBS would have an effect on global connectivity, we used an unbiased, data-driven method (Gotts et al., 2012) to evaluate changes in functional connectivity during the learning process.

1.2 Materials and Methods

1.2.1 Participants

We recruited 38 right-handed, healthy volunteers, all of whom met safety criteria for TMS (Keel, Smith, & Wassermann, 2001). All gave written informed consent and were free of neurological and psychiatric illness. None were on continuous medication other than oral contraceptives. We estimated IQ with the National Adult Reading Test. The study was approved by the NIH Combined Neuroscience Institutional Review Board.

Sixteen participants were excluded for the following reasons: (a) failure to return for the second fMRI session ($n = 7$); (b) self-reported inattention to the task during fMRI ($n = 3$); (c) excessive motion during fMRI sessions (i.e., greater than 3 mm displacement between successive volumes; $n = 2$); (d) delay in scanning after TMS (outliers relative to average across both sessions of 390 sec; $n = 2$); (e) inability to see the fMRI stimuli ($n = 1$); and (f) technical malfunction during fMRI data collection ($n = 1$). This left 22 participants who completed both of the TMS/fMRI sessions. The fact that we chose a longitudinal design for the effect of stimulation contributed substantially to the high dropout rate; however, as mentioned in the discussion, there are significant differences in the effects of 40 seconds of 600 cTBS pulses of M1 on MEP between individuals (Hamada, Murase, Hasan, Balaratnam, & Rothwell, 2013). Consequently, we considered the benefits of increased sensitivity for the effects of stimulation to be of greatest importance here.

1.2.2 Procedure

The experimental timeline is shown in Figure 1. During an initial visit, we located M1 and made TMS measurements required for stimulation dosing. All participants were tested with real and sham inhibitory cTBS in separate sessions at least one week apart in a counterbalanced fashion. At the beginning of each session, either real or sham cTBS was delivered to M1 in a room adjacent to the scanner using MRI-guided neuronavigation. The mean time between the end of the TMS treatment and beginning of the first fMRI run was 330 ± 80 sec for the real and 450 ± 28 sec for the sham conditions ($t_{(21)} = 1.7$, n.s.). As mentioned before, the inhibitory effect of 40 seconds of 600 cTBS pulses of M1 on the MEP lasts for up to 50-60 min (Gamboa et al., 2011; Huang et al., 2005). For all participants, the real cTBS/fMRI session lasted less than one hour.

In each of the two TMS/fMRI sessions, participants completed four fMRI runs during which they performed the pSRTT. Each run consisted of seven task-blocks of 100 trials: five blocks in which stimuli appeared according to a pair of probabilistic sequences (S blocks) and two in which the stimuli appeared randomly so that sequence learning could not occur (R blocks). In each run, blocks were ordered R-S-S-S-R-S, there was a 20 sec break period between each block to allow the hemodynamic response to return to baseline and each run was completed in 704 sec. Therefore, 20 S and 8 R blocks were completed in each of the two sessions. After the second session, participants were told that there was a sequence in the S blocks and then performed the process dissociation procedure (PDP) to assess their conscious sequence awareness.

1.2.3 pSRTT

In the scanner, stimulus presentation was rear-projected to the participant who responded using a four-button response box. The screen displayed four boxes arranged horizontally, corresponding to the response keys. On each trial, an X appeared in the center of one of the boxes, indicating the finger for the response. Participants were instructed to respond to the X as fast and accurately as possible. After 600 msec, the X disappeared from the screen. The next X appeared after a 200-msec interval. RTs were measured from onset of the X to button press.

During S blocks, cues appeared according to two different sequences that are closely related and intermixed. The ordering rule for the “probable” sequence was followed more frequently (85%) than the rule for the other “improbable” one (15%), causing participants to learn to anticipate targets from the probable sequence. In the pSRTT, intercalation of improbable sequence trials creates noise and inhibits the development of explicit knowledge of the probable sequence. As sequence learning occurs, RT and error rate also increase for the improbable sequence relative to the probable one.

As mentioned previously, a critical advantage of the pSRTT over the standard, deterministic version is that it provides a continuous measure of sequence learning, rather than a single measure at the end (Wilkinson & Shanks, 2004). In the deterministic version, RT and error rates are compared across separate random and sequence cue presentation blocks at the end of the task. We used the pSRTT in to provide information on learning on a block-by-block basis. Nevertheless, as this was a blocked fMRI design, we also implemented additional random blocks during training so we could use them for the purpose of the fMRI analyses. While the behavioral data could be explored at the level of each block, the imaging analysis was based on the comparison of S and R blocks across runs.

For the S blocks, we used two pairs of different but parallel, second-order conditional (SOC) sequences:

- a. Pair 1 (SOC1 = 2-4-2-1-3-4-1-2-3-1-4-3 & SOC2 = 2-3-4-3-1-2-4-1-3-2-1-4)
- b. Pair 2 (SOC3 = 3-4-2-3-1-2-1-4-3-2-4-1 & SOC4 = 3-4-1-2-4-3-1-4-2-1-3-2).

These sequence pairs are equivalent with respect to cue frequency (each location occurs three times), first-order transition frequency (each location is preceded once by each of the other three locations), and repetitions (no repetitions in either sequence) (Reed & Johnson, 1994). The sequences in each pair differ only in their second- and higher-order conditional structure. That is, the SOC in each pair contain 12 identical pairs of cues or first-order transitions. (For SOC1 and 2, the pairs are 2-4, 4-2, 2-1, 1-3, 3-4, 4-1, 1-2, 2-3, 3-1, 1-4, 4-3, 3-2). However, the sequences *do* differ in their possible triplets. In SOC1, 2-4 is always followed by 2; whereas, in SOC2, it is followed by 1 and so on. This second-order rule distinguished the probable and improbable sequences in each pairing.

Sequences and sequence pairs were counterbalanced across real and sham cTBS sessions and participants. Each S block began at a random point in the sequence. During R blocks, participants performed 100 trials of the task except that the order of the cues was random.

To promote the development of implicit knowledge, we employed a dual-task learning environment (e.g. A. Cohen, Ivry, & Keele, 1990). For the secondary task, participants were told to pay attention to a running tally of pseudo-feedback that appeared on the screen after each trial. On each probable trial, there was a 50% chance of seeing a smiley face and 50% chance of seeing a sad face. Running counts of sad and smiley faces were displayed on the screen between blocks. On improbable sequence trials, the faces were always neutral. This pseudo-feedback task was meant to match the stimulus conditions from our previous study on the effect of cTBS and reward on the pSRTT (Wilkinson et al., 2015).

1.2.3. 1. Awareness testing—It was not possible to do awareness testing after the first session, as it would have required informing participants of the presence of a sequence before the second session. Therefore, the PDP was completed after the second session and only in a subset of participants ($n = 18$), as four participants did not complete awareness testing. This estimate of awareness of sequence knowledge is based on the assumption that explicit knowledge is both reportable on request *and* under intentional control. Implicit knowledge may or may not be reportable, but is *not* under intentional control (Destrebecqz et al., 2005; Karabanov et al., 2010; Wilkinson & Jahanshahi, 2007; Wilkinson, Khan, & Jahanshahi, 2009; Wilkinson & Shanks, 2004). The PDP applied to sequence learning is specifically designed to test the above assumptions to test whether sequence knowledge meets the above criteria. The PDP comprises inclusion and exclusion test phases, which take place immediately after learning. Participants are informed of the presence of a sequence during training and told that their sequence knowledge will be tested. In the inclusion test, they are required to make 100 key presses, including as much as possible of the sequence they learned during training. In the exclusion test, they are requested to generate 100 key presses while avoiding generating the sequence. Performance on both tests is usually assessed by counting the number of triplets generated from the 12-item SOC sequence on which they predominately trained (with a maximum score of 98/100). The reasoning of this approach is that if participants are able to score above chance on the inclusion test (i.e., knowledge is reportable), while at the same time, withholding sequence knowledge on the exclusion test (i.e. knowledge is under intentional control), then this is evidence of explicit learning. In contrast, if they have neither reportable knowledge nor intentional knowledge control, this is evidence of implicit sequence knowledge. Finally, if inclusion and exclusion performance are *both* above chance (subject can generate, but not withhold the sequence), this indicates implicit knowledge under the assumptions of this paradigm.

However, as previously reported (Wilkinson & Shanks, 2004), the free generation nature of the standard PDP allows participants to adopt various perseverative or “lazy” response strategies. For example, a participant could generate the sequence 1–2–3–4 repeatedly throughout the test phase. Because this run contains the triplet 2–3–4 (which appears in SOC2), this would achieve a test score of 98 if trained on SOC2. It may be tempting for participants to persevere in the exclusion test if they assume that the SOC sequences do not contain runs. The temptation to persevere is presumably not as strong in the inclusion test. Another possibility is that the production of simple runs is a convenient way for an unmotivated participant to finish the experiment as quickly as possible. To avoid these potential problems with the standard version of the PDP, we developed a trial-by-trial testing method (Wilkinson & Shanks, 2004) in which participants observe a short sequence of targets from the training sequence and then produce a single generation response. This version of the PDP allows us to look more specifically at participants' sequence knowledge and their capacity for intentional control and avoid the potential contaminating effect of perseverative response strategies.

1.2.3. 1.1. Trial-by-trial PDP—This included two sequence generation tests, each based on 12 6-item fragments of previously learned probable sequences. In the inclusion test, participants responded to five targets and then were required to produce the sixth target in

the sequence (third target in the second triplet). In the exclusion test, participants were told to make a response different from the next item of the sequence. There was a 1/3 probability per trial (or 4/12 test items) of making the correct answer by chance. In the exclusion test, there was a 2/3 probability per trial (or 8/12 test items) of making the correct answer by chance. The order of tests was counterbalanced across participants.

1.2.4 Behavioral data analysis

For each participant, mean RTs as well as commission (within the target presentation period) and omission (outside the target presentation period) error rates for probable and improbable trials were calculated for each S block. Incorrect responses and RTs < 200 or > 600 msec (outside target presentation period) were counted as errors and excluded from the RT analysis. Data from the first two trials of each S block were excluded from all analyses because their target locations could not be predicted from sequence knowledge. If there was a violation of the sphericity assumption, Pillai's multivariate test of significance was employed. Thus, if the Greenhouse–Geisser was less than 1.0, Pillai's exact F is reported. We used a significance criterion of $\alpha = .05$, unless otherwise specified. R block behavioral data were not included in the behavioral data analysis.

1.2.5 TMS

Stimulation was delivered to the left M1 with a Magstim Rapid stimulator (Magstim Co., Dyfed, UK) connected to a figure-of-eight coil with an internal wing diameter of 70 mm and held with the handle pointing posterolaterally. The electromyogram was recorded with a belly-tendon montage from the right first dorsal interosseous (FDI) muscle. The left M1 was located by finding the best scalp site for producing MEPs in the right FDI. We usedBrainsight, MRI-guided neuronavigation software (Rogue Research Inc., Dyfed, UK), to record the location of M1 in each individual and ensure the same stimulation site would be used for real and sham cTBS across sessions.

Resting motor threshold was defined as the minimum stimulus intensity that produced a MEP (50 μ V or greater in five of 10 trials) at rest. Active motor threshold (AMT) was defined as the minimum stimulus intensity that produced an MEP (about 200 μ V) in 5 of 10 trials and was assessed during voluntary contraction of the right FDI at approximately 10% of maximum voluntary contraction.

We administered cTBS as described by Huang et al. (2005). Each burst consisted of 3 pulses at 50 Hz at an intensity of 80% of AMT. The mean intensity used was $31.68 \pm 7.47\%$ (range 19–50%) for the real condition. The same intensity was employed in the sham condition. Bursts of three were given every 200 msec (50 Hz) for a total of 600 pulses. The stimulation lasted 40 sec. For sham stimulation, we used a coil that was identical to the real coil in appearance and acoustic properties but did not produce the physiological effect of TMS. Participants but not experimenters were blind to the stimulation condition.

1.2.6 fMRI acquisition and preprocessing

The experiment was performed in a 3.0T GE HDx scanner using an 8-channel coil (GE Medical Systems, Milwaukee, WI). Blood oxygen level-dependent (BOLD) data were

obtained using a gradient-echo echoplanar imaging sequence (EPI: repetition time (TR) = 2000 msec, echo time (TE) = 27 msec, flip angle = 60°, 38 axial contiguous interleaved slices per volume, 3.0 mm slice thickness, 2.67 mm in-plane resolution, field of view (FOV) = 24 × 24 cm, matrix size 80 × 80). In addition, a high-resolution T1-weighted anatomical image was acquired (magnetization-prepared rapid gradient echo (MPRAGE), TR = 6.504 msec, TE = 2.796 msec, 198 slices per volume, 1 mm thickness, FOV = 24 × 24 cm², 256 × 256 acquisition matrix).

Image pre-processing was done with AFNI software (Cox, 1996). We removed the first six EPI volumes from each run to ensure that all remaining volumes were at magnetization steady state. Signal outliers were attenuated on a voxel-wise basis using AFNI's 3dDespike as described (Jo et al., 2013). Volumes were slice-time corrected and motion parameters were estimated using rigid body transformations. We then co-registered the volumes to the anatomical scan and resampled to 3mm isotropic voxels. We applied the basic ANATICOR procedure (Jo, Saad, Simmons, Milbury, & Cox, 2010) for removing nuisance physiological and non-physiological artifacts from the data as follows: the anatomical scan was segmented into tissue compartments using Freesurfer (Fischl et al., 2002). Ventricle and white-matter masks were created, and the white matter masks were eroded by one voxel to prevent partial volume effects with grey matter. Masks were then applied to the volume-registered EPI data to yield pure nuisance time series for the ventricles and local estimates of the BOLD signal in white matter, which were averaged within a 20-mm radius sphere. All nuisance time-series in each run were detrended with fourth-order polynomials before least-squares model fitting to each voxel's time series. Additional filtering, as is often done in resting-state fMRI preprocessing, was not applied due to a lack of knowledge of the appropriate spectrum of the task-based signals and to corresponding concerns that the BOLD effects of interest might be removed. Nuisance variables for each voxel included an average ventricle time series, a local average white-matter time series, and six parameter estimates for head motion. The time-course of these variables was removed from the full voxel time series to yield a time series for statistical analyses. Brain masks excluding white matter, the ventricles, and other non-brain areas were created for each participant. This clean time-series was then smoothed with an isometric 6-mm full-width half-maximum Gaussian kernel and normalized to the mean signal intensity in each voxel to reflect signal percentage. For group analysis, participant data were aligned by affine registration to AFNI's TT_N27 template which is in the standardized Talairach and Tournoux (1988) space.

1.2.7 Imaging analysis

For analysis of task-based increases in BOLD activation, task regressors were modeled as boxcar functions convolved with the hemodynamic response. Multi-subject analysis was based on the general linear model treating subjects as random-effects. Global brain signal was not included among the nuisance variables (Saad et al., 2012). To control for family-wise type I error, we used a cluster threshold adjustment method based on Monte Carlo simulations (3dClustSim) using total data smoothness of the time series residuals (3dFWHMx). We determined that a minimum cluster size of 44 voxels at $p < .005$ would be used to correct for multiple comparisons (at $p < .05$). Only voxels determined to be grey matter in 80% of subjects were considered for analysis.

1.2.8 Whole-brain correlation analysis

To obtain an unbiased measure of whole brain connectivity changes due to real cTBS, we split the residual time series of each voxel into its task condition (Sequence: S vs. Random 1 [1st random block per run]: R1 vs. Random 2 [2nd random block per run]: R2). Task conditions were shifted by two TRs to allow for hemodynamic lag and concatenated. We computed the Pearson's r correlation of the time series within each condition for each voxel to all other voxels and stored the mean r -value for each voxel (3dTcorrMap). We calculated whole-brain mean connectedness in each condition for each participant (Gotts et al., 2012) and then transformed all connectedness values using Fisher's Z to yield normally distributed values.

We then compared whole-brain mean connectedness values with a 2×3 ANOVA (Stimulation condition: real vs. sham cTBS $\times$ pSRTT Block type: S vs. R1 vs. R20 (G. Chen, Saad, Britton, Pine, & Cox, 2013). Because we lacked measures of cardiac and respiratory cycles for these scans, we added the grand average correlation among all voxel time series for each participant (referred to as “GCOR” for “global correlation”) as a nuisance covariate to these analyses to rule out the contribution of un-modeled global artifacts to the condition differences (Gotts et al., 2013; Saad et al., 2013).

The time courses for sequence blocks were taken from each of the regions of interest (ROIs) and used separately in seed-based correlation measures (3dTcorr1D). As a post-hoc follow-up, we used the peak value of the ROIs resulting from seed-based correlations to determine regions contributing to the difference in overall connectedness between TMS conditions (subsequently referred to as “derived ROIs”). The resulting maps were z -transformed and then subjected to pair wise t -tests to compare each TMS condition (3dTtest++). Because each seed map was considered separately, results were Bonferroni corrected by adjusting for the number of seeds tested using a cluster threshold adjustment method (3dClustSim). This method for multiple corrections is identical to those performed previously (Gotts et al., 2012; Song, Gotts, Dayan, & Cohen, 2015).

To visualize the differences in region-by-region correlation, we determined the seed voxel and peak voxels from both seed and derived ROI. A 5-mm radius sphere was drawn at the peak voxel of each ROI. The correlation of time course for sequence blocks for each ROI was computed, and the conditions were compared using paired t -tests. For both real TMS over M1 and sham, we extracted the voxel-averaged BOLD time-series during S blocks for each of these regions and computed the region-by-region correlation matrix. We transformed the resulting correlation matrices to normal distributions and compared between stimulation conditions using paired t -tests, which were then corrected for multiple comparisons using Holm-Bonferroni correction (Fogel et al., 2014; Hamilton et al., 2011; Holm, 1979).

1.3 Results

Twenty-two participants (12 female) aged 22–36 years ($M = 26$, $SD = 4.2$) with an average of 17 years of education ($SD = 1.4$) and mean IQ of 109 ± 4.7 completed two combined TMS/fMRI sessions. Twelve participants underwent the real stimulation condition first.

1.3.1 Behavioral results: effects of TMS on motor sequence learning

1.3.1. 1. RTs across learning blocks—Figure 2A shows mean RT during the scanning phase as a function of trial-type and S-blocks plotted separately for each stimulation condition. The main behavioral index of sequence learning in this task for S-blocks is the difference in RT between probable and improbable trials. We expected that RT would decrease across blocks for probable relative to improbable trials as participants came to anticipate the probable sequence. To examine the effects of feedback and cTBS on sequence learning across S-blocks, we performed a repeated measures ANOVA on mean RTs with Trial-type (probable vs. improbable), S-block (1-20), and Stimulation condition (real vs. sham) as within-subject variables. Across all S-blocks, mean RT to probable sequence stimuli ($M = 388.92$, $\pm SE = 8.4$) was faster than to improbable sequence stimuli ($M = 400.65$, $\pm SE = 7.8$) (main effect of Trial-type; ($F_{(1,21)} = 43.49$, $p < .0005$), indicating specific learning of the probable sequence. Importantly, real cTBS also caused a significant change in RT-based evidence of sequence learning across S-blocks (Stimulation condition $\times$ Trial-type $\times$ S-block; $F_{(19,3)} = 8.39$, $p = .05$). Finally, there was no nonspecific influence of real cTBS on overall RTs across S-blocks, which could have confounded the analysis of sequence learning (main effect of Stimulation condition, n. s., $F < 1$). The main effect of S-block ($F_{(19,3)} = 1.84$, $p = .34$), interactions between Trial-type $\times$ S-block ($F_{(19,3)} = 2.92$, $p = .21$), Stimulation condition $\times$ S-block ($F_{(19,3)} = 1.16$, $p = .52$), and all other interactions were not significant (all F s < 1).

In light of the significant interaction between Trial-type, S-block and Stimulation condition, we calculated measures of sequence learning in the RT data (RT difference scores; see Figure 2B) for each S-block by subtracting the mean RT for the probable trials from the mean RT for improbable trials. This procedure was conducted for each stimulation condition separately. A positive RT difference score that significantly differed from zero was considered evidence of sequence learning. Twenty comparisons to zero were made per stimulation condition, and we used an α of .0025 (.05/20) to determine significance. In the sham condition, RT difference scores were significantly greater than zero at S-block 13 ($t_{(21)} = 4.43$, $p < .0005$), reflecting the emergence of RT based sequence learning, and for 3 subsequent S-blocks (15,16,17, all p s $< .0025$). In the real condition, the RT difference score was greater than zero for the first time at S-block 14 ($t_{(21)} = 5.19$, $p < .0005$) and for just one subsequent S-block (20, $p < .0005$).

As it has already been demonstrated that RT based sequence learning is impaired by cTBS to M1 before pSRTT learning (Rosenthal et al., 2009; Wilkinson et al., 2015; Wilkinson et al., 2010), we used a one-tailed approach for the next analysis. RT-based evidence of sequence learning was significantly lower in the real condition relative to sham in S-blocks 1 ($t_{(21)} = 2.33$, $p = .04$, 2-tailed) and 15 ($t_{(21)} = 2.33$, $p = .04$, 1-tailed).

Finally, analysis using Tukey's HSD test on performance in the sham condition indicated significantly better performance in the 13th ($p = .02$), 15th ($p = .003$), 16th ($p = .001$), 17th ($p = .003$), and 19th ($p = .03$) S-blocks of trials relative to S-block 1. The same analysis of the real condition showed a markedly different trend as all S-blocks apart from S-block 7 were significantly better than S-block 1 (all p s $< .05$). This difference in trends was significant (5

vs. 18 S-blocks; $\chi^2_{(1, N=40)} = 17.29, p < .0005$). In the real condition, participants achieved significantly less learning in S-block 1, leading to better relative performance in later blocks. So, it was the initial impairment of learning in the real cTBS condition across the first 100 trials of the task that caused a significant change in the learning trend relative to the sham condition.

1.3.1. 2. Errors across learning blocks—We predicted that both omission and commission error rates would decrease across S-blocks for probable relative to improbable trials as participants came to anticipate the probable sequence, providing a second assay of sequence learning. Figure 2C shows mean omission error rates during the scanning phase as a function of trial-type and S-block, plotted separately for each stimulation condition. An identical analysis on omission error rates across S-blocks revealed a main effect of Trial-type over S-blocks 1-20 ($F_{(1,21)} = 363.05, p < .0005$) as more omission errors were produced on improbable ($M = .16, \pm SE = .01$) relative to probable ($M = .09, \pm SE = .01$) sequence trials, again indicating sequence-specific learning in the omission error data as we predicted. Real cTBS led to significantly altered error-based evidence of sequence learning across blocks (Stimulation condition $\times$ Trial-type $\times$ S-block; $F_{(19,3)} = 8.58, p = .051$). Main effects of S-block ($F_{(19,3)} = 4.94, p = .11$), Stimulation condition and all other the other interactions were not significant (all F s < 1).

In light of the significant interaction between Trial-type, S-block and Stimulation condition, we calculated measures of sequence learning in the omission error data (error difference scores; Figure 2D) for each S-block by subtracting the omission error rate for the probable trials from that for improbable trials. This procedure was performed for each stimulation condition separately. A positive error difference score that was also significantly different from zero was evidence of sequence learning. In both stimulation conditions, error difference scores were significantly greater than zero for the first time at S-block 1 (sham, $t_{(21)} = 4.60, p < .0005$; real, $t_{(21)} = 6.05, p < .0005$), reflecting the emergence of error-based sequence learning at the same time according to this measure of learning and early on in training. However, participants in the sham condition achieved more subsequent learning blocks in which the error difference was significantly greater than zero (18: all except S-block 19, p s $< .0025$; $\alpha = .0025$) relative to the real condition (16: all except S-blocks 6, 10 & 14, p s $< .0025$; $\alpha = .0025$).

As it has already been demonstrated that error-based sequence learning is impaired by cTBS to M1 before pSRTT learning (Wilkinson et al., 2015; Wilkinson et al., 2010), we used a one-tailed approach in the next analysis. Error-based evidence of sequence learning was significantly decreased in the real condition, relative to the sham, in the 6th S-block ($t_{(21)} = 1.74, p = .049$, 1-tailed).

Finally, according to Tukey's HSD test, performance in the sham condition was significantly better relative to the first S-block in the sixth S-block ($p = .02$). The same analysis of the real condition showed S-block 11 was significantly better than S-block 1 ($p = .02$). It took significantly longer in the real condition than the sham condition for the first evidence of an improvement in error-based sequence learning relative to S-Block 1 to emerge across S-blocks (11 vs. 6 S-blocks; $\chi^2_{(1, N=20)} = 7.01, p = .01$).

An equivalent analysis of commission errors across S-blocks revealed a main effect of Trial-type over S-blocks ($F_{(1,21)} = 21.96, p < .0005$) with more commission errors made on improbable ($M = .09, +/- SE = .01$) relative to probable ($M = .07, +/- SE = .01$), showing commission error-based sequence learning. However, the effect of Stimulation condition on sequence learning did not reach significance (Stimulation condition $\times$ Trial-type $\times$ S-block, $F_{(19,3)} = 3.23, p = .18$; Stimulation condition $\times$ Trial-type, $F < 1$). Main effects of S-block ($F_{(19,3)} = 2.01, p = .31$), Simulation condition ($F_{(21,1)} = 1.57, p = .23$) and the interactions between Simulation condition $\times$ S-block ($F_{(19,3)} = 2.12, p = .29$), Trial-type $\times$ S-block ($F_{(19,3)} = 4.01, p = .14$) were not significant.

1.3.1. 3. Sequence awareness test—We calculated the number of correct probable sequence triplet completions generated on the inclusion and exclusion tests for each stimulation condition for a subset of 18 participants who completed awareness testing. To test for explicit sequence knowledge, we compared PDP performance to chance. For inclusion, performance was at chance level in both the real ($M = 4.6 +/- SE = .4; (t_8) = 1.25, p = .25$) and sham ($M = 4.6 +/- SE = .5; (t_9) = 1.15, p = .28$) conditions. Furthermore, for exclusion, performance was below chance level in both real ($M = 4.6 +/- SE = .5$) and sham ($M = 4.3 +/- SE = .4$) conditions ($ps < .0005$). These results indicate that the probable sequence was not acquired explicitly. Compared to a previous study using the same trial-by-trial PDP paradigm to test awareness after pSRTT learning in a group of healthy controls (see Wilkinson & Shanks, 2004, Experiment 3 for details), the current study has statistical power of 90.7% in the sham and 87.9% in the real group to detect evidence of conscious sequence knowledge in the inclusion condition. These values are above the criterion of 80% recommended by Cohen (1988) as an acceptable level of power. As such, we can be confident in our conclusion that sequence knowledge is implicit in both cTBS groups is not simply a failure to detect an effect that exists in the population.

1.3.2. Imaging results

1.3.2. 1. Activation analysis—Beta values from each participant were submitted to a 2×3 ANOVA (Stimulation condition (real vs. sham cTBS) $\times$ Block-type (S, R1, R2)). We found a main effect of Block-type in cortical areas including M1, premotor, supplementary motor (SMA), cerebellar motor, putamen, and ventral tegmental areas as well as the occipital and parietal regions (Table 1). Post-hoc analyses revealed $S > R1$ and $S > R2$ in all of these regions and were consistent with previous findings of fMRI studies of sequence learning (e.g., Doyon, Penhune, & Ungerleider, 2003). There was no main effect or interaction with Stimulation condition in any brain regions.

1.3.2. 2. Global connectedness—A whole brain, data-driven search for functional connectivity differences was conducted using global connectivity (Gotts et al., 2012). When global connectivity results were submitted to a 2×3 ANOVA (Stimulation condition (real vs. sham cTBS) $\times$ Block-type (S, R1, R2), there was a main effect of Stimulation condition in several brain regions (Figure 3A and 3B; shown in bold in Table 2). Post-hoc analyses revealed decreased global connectivity in real relative to sham stimulation in left primary visual cortex and in the left dorsal premotor area (PMd). Global connectivity increased in

the middle cingulate gyrus (mCC), dorsal anterior cingulate (dACC), and superior frontal gyrus (SFG). There was no main effect or interaction with Block-type in any brain region.

To determine which brain regions drove the differences in global connectivity, we employed seed-based correlations using the five regions determined in the global connectivity analysis above as seeds (in bold in Table 2; also see Gotts et al., 2012 for the same approach). Four new regions showed a main effect of stimulation condition at a p -level corrected for multiple comparisons ($p < .05/5$) (Table 2 plain type, in green in Figure 4), including the left middle temporal area (MT), left inferior occipital gyrus, right inferior frontal gyrus (IFG), and a region encompassing the left M1 and SMA. There was no main effect or interaction with Block-type in any brain region.

To visualize and quantify the relationships among these ROIs, we calculated the region $\times$ region correlation matrices for the time series during the S blocks for each participant in both stimulation conditions. Time-series from the two distinct local maxima within the M1/SMA cluster were considered as separate ROIs. The t -values from the resulting real vs. sham correlation matrix comparisons are shown in the correlation contrast matrix in Figure 5. After real relative to sham cTBS stimulation, coupling decreased between the left inferior occipital gyrus and PMd, SMA, and M1. Coupling also decreased between the superior occipital gyrus and M1 as well as the SMA ($p < .05$, Holm-Bonferroni corrected). Moreover, after real cTBS, coupling increased relative to sham between the MT and dACC and between the SFG and inferior frontal gyrus.

1.4 Discussion

In this study, we showed that TMS applied to M1 in a way that depresses corticospinal excitability and motor learning produced no change in learning-related activation locally or distantly. However, it induced a shift in functional connectivity away from a network containing M1 toward another, non-motor network. The shift in global connectedness caused by real cTBS was seen in both the random and sequential phases of the task. However, the TMS-related behavioral effect on the RT and error rates was specific to sequence learning.

We and others found disruptive effects of M1 cTBS on sequence acquisition (Rosenthal et al., 2009; Wilkinson et al., 2015; Wilkinson et al., 2010) and early consolidation (Wilkinson et al., 2015), using a similar task and identical or similar brain stimulation. Here, we also observed a negative effect of cTBS on learning, but it was less robust than in previous studies, possibly due to changes in the task required to adapt it to the scanning environment. In particular, imaging required a set inter trial interval, longer than that occurring in the usual, self-paced version of the task. Furthermore, while the MEP suppression from 40 sec of cTBS M1 lasts approximately one hour, the duration of the learning effect has not been measured and may be different. Nevertheless, we feel confident in interpreting the shift in connectivity as a response to cTBS of M1.

Real cTBS reduced connectivity among motor (M1, SMA and PMd) and visual (superior and inferior occipital gyri) areas, while increasing it among frontal, cingulate (dorsal and middle cingulate cortex), and temporal areas.

This is consistent with theories positing parallel acquisition of information by multiple networks during procedural learning (Nakahara, Doya, & Hikosaka, 2001). Parallel processing may preserve function following perturbations (Bullmore & Sporns, 2012), such as the one we produced here. There are several theories positing parallel systems for visuomotor sequence learning. In the model proposed by Keele, Ivry, Mayr, Hazeltine, and Heuer (2003), two networks are recruited during learning: a dorsal network including the superior parietal lobule and cortical motor regions, M1 and premotor cortex, and a ventral network comprising the temporal and lateral prefrontal areas. The dorsal network acquires implicit information in an attention-independent manner, while the ventral network requires attention to the task but can operate either implicitly (as here) or explicitly. A similar model, based on primate studies (Hikosaka, Nakamura, Sakai, & Nakahara, 2002), suggests that parallel, associative, and motor cortico-striatal loops are involved in sequence learning. The associative loop integrates sensory information and acquires sequential knowledge early in learning, but the sequence information decays rapidly after the learning period when restricted to this network. The motor network develops long-term motor plans and acquires sequence information more slowly. In our sham condition, we found greater connectivity between motor areas and other regions of the brain, suggesting that real cTBS decreased these structures' connectivity with the rest of the cortex. The increase in coupling between the middle temporal area and frontal regions, including SFG, dACC, and IFG, might reflect higher-level processing of visuo-spatial information required to maintain learning after cTBS. Interestingly, we found that the SMA decreased its connectivity with occipital areas after cTBS but increased its connectivity with mCC. This switch may indicate that the SMA plays a role in translating information between these two learning networks, a role hypothesized by Hikosaka et al. (2002).

Human study data also suggest that multiple networks support sequence learning. For instance, humans can acquire complex sequence information by observation as efficiently as by motor practice (Song, Howard, & Howard, 2008). To evaluate the contribution of non-motor regions to sequence learning, Rosenthal and colleagues (2009) compared the effects of cTBS over the angular gyrus and M1 and found that cTBS to the angular gyrus disrupted visuo-spatial learning early in sequence acquisition, while M1 stimulation impaired acquisition of motoric information, sparing visuo-spatial knowledge. This suggests that visuo-spatial areas can acquire sequence knowledge and partially compensate for deactivation of the motor network during learning, and is consistent with shifting functional connectivity toward a perceptual learning network after deactivation of M1.

This shift in connectivity is consistent with our earlier finding (Wilkinson et al., 2015) that M1 cTBS impairs retention of sequence knowledge, since the associative, ventral network is retains information less efficiently than the dorsal network (Hikosaka et al., 2002). Visuo-spatial sequence learning requires attention; whereas, motoric learning is more robust and attention-independent (Song et al., 2008). In this model, inhibitory stimulation applied during acquisition would have a negative effect on sequence learning, which becomes greater on delayed tests because of the decay of visuo-spatial information. Stimulation applied during the consolidation phase or after training would also prevent consolidation of the motoric aspects of the memory, producing a similar effect on retention (Hadipour-Niktarash et al., 2007).

It is noteworthy that here we found a significant reduction of connectivity between motor and between visual networks after simulation of a motor region, suggesting strong connections between the M1 and the visual network. Parietal visual and frontal motor areas are strongly interconnected and visual and motor areas are also linked by a pathway from pontine cells receiving visual input from the cortex and superior colliculus, to the cerebellum (Glickstein, 2000). Either or both of these pathways could allow communication between the motor and visual networks.

Although there is evidence that this shift in connectivity is related to the task, it is possible that the change in connectedness after cTBS could be due to simple competition between mutually inhibitory networks. That is, reducing transmission efficacy in the motor network effectively increases it in the associative network irrespective of task. While the tendency of SMA to increase coupling with mCC and the associative network after real cTBS suggests that the shift in connectivity has functional relevance, we cannot rule out the more general possibility.

While cTBS caused changes in functional brain connectivity, it produced no detectable changes in local or distant brain activation. M1 is involved in both the generation and learning of movement and blood flow during hand movement scales with basic task demands, e.g., tapping frequency (Sadato et al., 1996) and grip force (Sehm, Perez, Xu, Hidler, & Cohen, 2010). One study, (Conchou et al., 2009) found changes in movement-related blood flow in contralateral M1 and connected areas, after inhibitory (1 Hz) rTMS in a comparison to rest. Our paradigm did not contain a resting condition and the motoric demands of the task were constant across conditions. Moreover, there was no main effect of learning condition (Block Type) on activation in M1 or elsewhere, showing that there was no major immediate effect of exposure to sequence information on the BOLD signal, either. Inclusion of a resting condition might have made detection of subtle activation effects possible. However, we believe a focal or distributed “virtual lesion” would still have been apparent with the existing design.

In this experiment, we showed that the motor sequence learning we observed was implicit in both real and sham learning conditions. Previous behavioral studies have tested awareness after pSRTT learning using similar methods and have found awareness to be intact in healthy subjects (Wilkinson & Jahanshahi, 2007). Others have found awareness to be intact following sham cTBS but not after real cTBS of M1 (Rosenthal et al., 2009; Wilkinson et al., 2015). Our findings differ with these results, and, as for the unusually small implicit learning effects, we attribute this to the adaptation of the task to the imaging environment and the disruptive nature of that environment itself.

It is beyond the scope of the present study to determine whether the connectivity changes are specific to task performance or also present at rest. However, a recent study by Cocchi et al. (2015) showed that M1's functional connectivity to regions outside its network, decreased after cTBS; whereas, the same measure for insular cortex, temporal cortex, and subcortical regions increased. These areas also showed an increase in within-network connectivity. In contrast, the changes in functional connectivity observed here included regions involved in movement production and a visual network. This suggests that the changes in functional

connectivity that we observed were specific to motor and visual integration required by the SRTT. Further work should directly compare the functional connectivity changes induced by cTBS prior to task and during rest.

This work adds to the evidence that TMS inhibitory effects on behavior are mediated by relatively subtle and distributed changes in network connectivity rather than taking local or distant areas “offline” as previously proposed (Pascual-Leone et al., 2000). Moreover, the behavioral data imply that parallel networks for acquisition can preserve task performance, at least partially, after disruptive interventions. This principle has already been applied by using TMS to suppress competition from the intact hemisphere to foster recovery of function in the damaged hemispheres of patients (Khedr, Abdel-Fadeil, Farghali, & Qaid, 2009; Kirton et al., 2008; Naeser et al., 2010). Our data suggest that it might also be used to reroute learning among networks to enhance recovery after brain damage. Inhibitory TMS has been used to implicate cortical areas in cerebral functions other than learning, notably primary visual cortex during tactile sensation in individuals with early-onset blindness (L. G. Cohen et al., 1997). While the technique may still be used to identify cortical areas as contributors to a particular function, our findings indicate that it may be simplistic to impute a central role to a single target area based on such paradigms. Behavioral changes from focal inhibitory stimulation may be due to effects on the targeted area itself, a set of downstream regions or connections, or an influence on the interaction of networks.

Forty sec of cTBS has been shown in some studies to reduce M1 excitability for up to 60 min (Gamboa et al., 2011; Gentner et al., 2008; Huang et al., 2005; Wilkinson et al., 2015) and for 20-30 minutes after a 20 sec train (Di Lazzaro et al., 2005; Gentner et al., 2008; Huang et al., 2005). However, there is contradictory evidence concerning the neurophysiological effects of cTBS on M1. In the study by Gentner et al. (2008), 20 sec of cTBS had no inhibitory effect on the MEP unless it was preceded by 5 min of isometric muscle contraction. In another experiment, (Cocchi et al., 2015) a trend for a reduction of the MEP after 40 sec of cTBS but did not significance. A third study (Hamada et al., 2013) found that a large proportion of individuals showed no lasting reduction in MEP amplitude after 40 sec. Instead, the cTBS effect was correlated with the latency of MEPs produced by an anterior-posterior current across the central sulcus, suggesting differential effects of cTBS depending on individual anatomical differences

In the current study, the requirement to place participants in the scanner as soon as possible after cTBS prevented us from measuring the effect of cTBS on the MEP. To overcome this limitation, we used a within subjects design, but it is likely that individual differences in the response to cTBS influenced the results. We also note that in our behavioral study (Wilkinson et al., 2015), we found significant group effects on both learning and the MEP. Interestingly, however, these did not correlate within individuals. Therefore, we do not believe the effects of cTBS on the corticospinal tract and effects on learning are the same or even closely related. In our opinion, it is more likely that the learning effect is related to altered activity in a network of which M1 is a node and the current study constitutes functional imaging evidence of this.

1.5 Conclusion

Implicit motor sequence learning, which recruits brain areas commonly identified with motor function, can be partially disrupted by inhibition of M1. Underlying this disruption is a shift in connectivity from a motor to a visuospatial learning network, but not a detectable change in local activity, even in the stimulated area. These findings show that TMS may exert its effects on cognitive performance via subtle synaptic changes at a distance from its target and need not make local functional ablations to be effective.

Acknowledgments

We thank Angad Uppal for helping with data collection and entry. We thank Drs. Aysha Keisler, David Pitcher, and Sule Tinaz for their insightful comments on the manuscript. We are extremely grateful to Mr. Phil Koshy for proof-reading and editing this manuscript. This study used the high-performance computational capabilities of the Biowulf Linux cluster at the National Institutes of Health, Bethesda, Md. (<http://biowulf.nih.gov>).

Funding came from the Clinical Neuroscience Program of the National Institute of Neurological Disorders and Stroke, National Institutes of Health and the National Institute of Mental Health Intramural Research Program, National Institutes of Health (Dr. S.J. Gotts and Dr. Z.S. Saad).

The sponsors played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

References

- Andoh J, Zatorre RJ. Mapping interhemispheric connectivity using functional MRI after transcranial magnetic stimulation on the human auditory cortex. *Neuroimage*. 2013; 79:162–171.10.1016/j.neuroimage.2013.04.078 [PubMed: 23631993]
- Baumgartner T, Knoch D, Hotz P, Eisenegger C, Fehr E. Dorsolateral and ventromedial prefrontal cortex orchestrate normative choice. *Nat Neurosci*. 2011; 14(11):1468–1474.10.1038/nn.2933 [PubMed: 21964488]
- Bestmann S, Baudewig J, Siebner HR, Rothwell JC, Frahm J. Functional MRI of the immediate impact of transcranial magnetic stimulation on cortical and subcortical motor circuits. *European Journal of Neuroscience*. 2004; 19(7):1950–1962.10.1111/j.1460.9568.2004.03277.x [PubMed: 15078569]
- Bestmann S, Swayne O, Blankenburg F, Ruff CC, Haggard P, Weiskopf N, et al. Ward NS. Dorsal premotor cortex exerts state-dependent causal influences on activity in contralateral primary motor and dorsal premotor cortex. *Cerebral Cortex*. 2008; 18(6):1281–1291.10.1093/cercor/bhm159 [PubMed: 17965128]
- Breton J, Robertson EM. Flipping the switch: mechanisms that regulate memory consolidation. *Trends Cogn Sci*. 2014; 18(12):629–634.10.1016/j.tics.2014.08.005 [PubMed: 25240672]
- Bullmore E, Sporns O. The economy of brain network organization. *Nat Rev Neurosci*. 2012; 13(5): 336–349.10.1038/nrn3214 [PubMed: 22498897]
- Cardenas-Morales L, Groen G, Kammer T. Exploring the After-Effects of Theta Burst Magnetic Stimulation on the Human Motor Cortex: A Functional Imaging Study. *Human Brain Mapping*. 2011; 32(11):1948–1960.10.1002/hbm.21160 [PubMed: 21181786]
- Chen G, Saad ZS, Britton JC, Pine DS, Cox RW. Linear mixed-effects modeling approach to FMRI group analysis. *Neuroimage*. 2013; 73:176–190.10.1016/j.neuroimage.2013.01.047 [PubMed: 23376789]
- Chen R, Classen J, Gerloff C, Celnik P, Wassermann EM, Hallett M, Cohen LG. Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. *Neurology*. 1997; 48(5): 1398–1403.10.1212/WNL.48.5.1398 [PubMed: 9153480]
- Cocchi L, Sale MV, Lord A, Zalesky A, Breakspear M, Mattingley JB. Dissociable effects of local inhibitory and excitatory theta-burst stimulation on large-scale brain dynamics. *J Neurophysiol*. 2015; 113(9):3375–3385.10.1152/jn.00850.2014 [PubMed: 25717162]

- Cohen A, Ivry RI, Keele SW. Attention and structure in sequence learning. *Journal of Experimental Psychology-Learning Memory and Cognition*. 1990; 16(1):17–30.10.1037//0278-7393.16.1.17
- Cohen, J. Statistical power analysis for the behavioral sciences. 2nd. New York: Academic Press; 1988.
- Cohen LG, Celnik P, PascualLeone A, Corwell B, Faiz L, Dambrosia J, et al. Hallett M. Functional relevance of cross-modal plasticity in blind humans. *Nature*. 1997; 389(6647):180–183.10.1038/38278 [PubMed: 9296495]
- Conchou F, Loubinoux I, Castel-Lacanal E, Le Tinnier A, Gerdelat-Mas A, Faure-Marie N, et al. Simonetta Moreau M. Neural substrates of low-frequency repetitive transcranial magnetic stimulation during movement in healthy subjects and acute stroke patients. A PET study. *Hum Brain Mapp*. 2009; 30(8):2542–2557.10.1002/hbm.20690 [PubMed: 19072894]
- Cox RW. AFNI: Software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research*. 1996; 29(3):162–173.10.1006/cbmr.1996.0014 [PubMed: 8812068]
- Debas K, Carrier J, Barakat M, Marrelec G, Bellec P, Tahar AH, et al. Doyon J. Off-line consolidation of motor sequence learning results in greater integration within a cortico-striatal functional network. *Neuroimage*. 2014; 99:50–58.10.1016/j.neuroimage.2014.05.022 [PubMed: 24844748]
- Destrebecqz A, Peigneux P, Laureys S, Degueldre C, Del Fiore G, Aerts J, et al. Maquet P. The neural correlates of implicit and explicit sequence learning: Interacting networks revealed by the process dissociation procedure. *Learning & Memory*. 2005; 12(5):480–490.10.1101/lm.95605 [PubMed: 16166397]
- Di Lazzaro V, Pilato F, Saturno E, Oliviero A, Dileone M, Mazzone P, et al. Rothwell JC. Theta-burst repetitive transcranial magnetic stimulation suppresses specific excitatory circuits in the human motor cortex. *J Physiol*. 2005; 565(Pt 3):945–950.10.1113/jphysiol.2005.087288 [PubMed: 15845575]
- Doyon J, Penhune V, Ungerleider LG. Distinct contribution of the cortico-striatal and cortico-cerebellar systems to motor skill learning. *Neuropsychologia*. 2003; 41(3):252–262.10.1016/S0028-3932(02)00158-6 [PubMed: 12457751]
- Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, et al. Dale AM. Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. *Neuron*. 2002; 33(3):341–355.10.1016/s0896-6273(02)00569-x [PubMed: 11832223]
- Fogel SM, Albouy G, Vien C, Popovicci R, King BR, Hoge R, et al. Doyon J. fMRI and Sleep Correlates of the Age-Related Impairment in Motor Memory Consolidation. *Human Brain Mapping*. 2014; 35(8):3625–3645.10.1002/hbm.22426 [PubMed: 24302373]
- Gamboa OL, Antal A, Laczó B, Moliadze V, Nitsche MA, Paulus W. Impact of repetitive theta burst stimulation on motor cortex excitability. *Brain Stimul*. 2011; 4(3):145–151.10.1016/j.brs.2010.09.008 [PubMed: 21777874]
- Gentner R, Wankerl K, Reinsberger C, Zeller D, Classen J. Depression of human corticospinal excitability induced by magnetic theta-burst stimulation: Evidence of rapid polarity-reversing metaplasticity. *Cerebral Cortex*. 2008; 18(9):2046–2053.10.1093/cercor/bhm239 [PubMed: 18165282]
- Glickstein M. How are visual areas of the brain connected to motor areas for the sensory guidance of movement? *Trends Neurosci*. 2000; 23(12):613–617. [PubMed: 11137151]
- Gotts SJ, Saad ZS, Jo HJ, Wallace GL, Cox RW, Martin A. The perils of global signal regression for group comparisons: a case study of Autism Spectrum Disorders. *Front Hum Neurosci*. 2013; 7(356)10.3389/fnhum.2013.00356
- Gotts SJ, Simmons WK, Milbury LA, Wallace GL, Cox RW, Martin A. Fractionation of social brain circuits in autism spectrum disorders. *Brain*. 2012; 135:2711–2725.10.1093/brain/awb160 [PubMed: 22791801]
- Grafman J, Wassermann E. Transcranial magnetic stimulation can measure and modulate learning and memory. *Neuropsychologia*. 1999; 37(2):159–167.10.1016/S0028-3932(98)00090 [PubMed: 10080373]
- Hadipour-Niktarash A, Lee CK, Desmond JE, Shadmehr R. Impairment of retention but not acquisition of a visuomotor skill through time-dependent disruption of primary motor cortex.

- Journal of Neuroscience. 2007; 27(49):13413–13419.10.1523/jneurosci.2570-07.2007 [PubMed: 18057199]
- Hamada M, Murase N, Hasan A, Balaratnam M, Rothwell JC. The role of interneuron networks in driving human motor cortical plasticity. *Cereb Cortex*. 2013; 23(7):1593–1605.10.1093/cercor/bhs147 [PubMed: 22661405]
- Hamilton JP, Furman DJ, Chang C, Thomason ME, Dennis E, Gotlib IH. Default-Mode and Task-Positive Network Activity in Major Depressive Disorder: Implications for Adaptive and Maladaptive Rumination. *Biological Psychiatry*. 2011; 70(4):327–333.10.1016/j.biopsych.2011.02.003 [PubMed: 21459364]
- Hikosaka O, Nakamura K, Sakai K, Nakahara H. Central mechanisms of motor skill learning. *Current Opinion in Neurobiology*. 2002; 12(2):217–222.10.1016/s0959-4388(02)00307-0 [PubMed: 12015240]
- Holm S. A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*. 1979; 6(2):65–70.
- Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. *Neuron*. 2005; 45(2):201–206.10.1016/j.clinph.2007.01.021 [PubMed: 15664172]
- Iyer MB, Schleper N, Wassermann EM. Priming stimulation enhances the depressant effect of low-frequency repetitive transcranial magnetic stimulation. *Journal of Neuroscience*. 2003; 23(34):10867–10872. [PubMed: 14645480]
- Jahanshahi M, Rothwell J. Transcranial magnetic stimulation studies of cognition: an emerging field. *Experimental Brain Research*. 2000; 131(1):1–9.10.1007/s002219900224 [PubMed: 10759166]
- Jo HJ, Gotts SJ, Reynolds RC, Bandettini PA, Martin A, Cox RW, Saad ZS. Effective Preprocessing Procedures Virtually Eliminate Distance-Dependent Motion Artifacts in Resting State fMRI. *Journal of Applied Mathematics*. 2013; 10.1155/2013/935154
- Jo HJ, Saad ZS, Simmons WK, Milbury LA, Cox RW. Mapping sources of correlation in resting state fMRI, with artifact detection and removal. *Neuroimage*. 2010; 52(2):571–582.10.1016/j.neuroimage.2010.04.246 [PubMed: 20420926]
- Karabanov A, Cervenka S, de Manzano O, Forssberg H, Farde L, Ullen F. Dopamine D2 receptor density in the limbic striatum is related to implicit but not explicit movement sequence learning. *Proc Natl Acad Sci U S A*. 2010; 107(16):7574–7579. doi: <http://dx.doi.org/10.1073/pnas.0911805107>. [PubMed: 20368439]
- Karni A, Meyer G, Jezzard P, Adams MM, Turner R, Ungerleider LG. Functional Mri Evidence for Adult Motor Cortex Plasticity During Motor Skill Learning. *Nature*. 1995; 377(6545):155–158. doi: <http://dx.doi.org/10.1038/377155a0>. [PubMed: 7675082]
- Keel JC, Smith MJ, Wassermann EM. A safety screening questionnaire for transcranial magnetic stimulation. *Clinical Neurophysiology*. 2001; 112(4):720–720. doi: [http://dx.doi.org/10.1016/S1388-2457\(00\)00518-6](http://dx.doi.org/10.1016/S1388-2457(00)00518-6). [PubMed: 11332408]
- Keele SW, Ivry R, Mayr U, Hazeltine E, Heuer H. The cognitive and neural architecture of sequence representation. *Psychological Review*. 2003; 110(2):316–339.10.1037/0033-295x.110.2.316 [PubMed: 12747526]
- Khedr EM, Abdel-Fadeil MR, Farghali A, Qaid M. Role of 1 and 3 Hz repetitive transcranial magnetic stimulation on motor function recovery after acute ischaemic stroke. *European Journal of Neurology*. 2009; 16(12):1323–1330.10.1111/j.1468-1331.2009.02746.x [PubMed: 19780802]
- Kirton A, Chen R, Friefeld S, Gunraj C, Pontigon AM, deVeber G. Contralesional repetitive transcranial magnetic stimulation for chronic hemiparesis in subcortical paediatric stroke: a randomised trial. *Lancet Neurology*. 2008; 7(6):507–513.10.1016/s1474-4422(08)70096-6 [PubMed: 18455961]
- Lee TG, D'Esposito M. The dynamic nature of top-down signals originating from prefrontal cortex: a combined fMRI-TMS study. *J Neurosci*. 2012; 32(44):15458–15466. doi: <http://dx.doi.org/10.1523/JNEUROSCI.0627-12.2012>. [PubMed: 23115183]
- Muellbacher W, Ziemann U, Wissel J, Dang N, Kofler M, Facchini S, et al. Hallett M. Early consolidation in human primary motor cortex. *Nature*. 2002; 415(6872):640–644.10.1038/nature712 [PubMed: 11807497]

- Naeser MA, Martin PI, Treglia E, Ho M, Kaplan E, Bashir S, et al. Pascual-Leone A. Research with rTMS in the treatment of aphasia. *Restorative Neurology and Neuroscience*. 2010; 28(4):511–529.10.3233/rnn-2010-0559 [PubMed: 20714075]
- Nakahara H, Doya K, Hikosaka O. Parallel cortico-basal ganglia mechanisms for acquisition and execution of visuomotor sequences - a computational approach. *J Cogn Neurosci*. 2001; 13(5): 626–647. doi: <http://dx.doi.org/10.1162/089892901750363208>. [PubMed: 11506661]
- Nudo RJ, Milliken GW, Jenkins WM, Merzenich MM. Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. *Journal of Neuroscience*. 1996; 16(2):785–807. [PubMed: 8551360]
- Pascual-Leone A, Walsh V, Rothwell J. Transcranial magnetic stimulation in cognitive neuroscience - virtual lesion, chronometry, and functional connectivity. *Current Opinion in Neurobiology*. 2000; 10(2):232–237.10.1016/s0959-4388(00)00081-7 [PubMed: 10753803]
- Reed J, Johnson P. Assessing Implicit Learning with Indirect Tests -Determining What Is Learned About Sequence Structure. *Journal of Experimental Psychology-Learning Memory and Cognition*. 1994; 20(3):585–594. doi: <http://dx.doi.org/10.1037/0278-7393.20.3.585>.
- Rioutl-Pedotti MS, Friedman D, Hess G, Donoghue JP. Strengthening of horizontal cortical connections following skill learning. *Nature Neuroscience*. 1998; 1(3):230–234.10.1038/678 [PubMed: 10195148]
- Robertson EM, Press DZ, Pascual-Leone A. Off-line learning and the primary motor cortex. *Journal of Neuroscience*. 2005; 25(27):6372–6378. doi:10.1523/jneurosci.1851-05.2005[issn 0270-6474. [PubMed: 16000627]
- Rosenthal CR, Roche-Kelly EE, Husain M, Kennard C. Response-Dependent Contributions of Human Primary Motor Cortex and Angular Gyrus to Manual and Perceptual Sequence Learning. *Journal of Neuroscience*. 2009; 29(48):15115–15125.10.1523/jneurosci.2603-09.2009 [PubMed: 19955363]
- Ruff CC, Bestmann S, Blankenburg F, Bjoertomt O, Josephs O, Weiskopf N, et al. Driver J. Distinct causal influences of parietal versus frontal areas on human visual cortex: Evidence from concurrent TMS-fMRI. *Cerebral Cortex*. 2008; 18(4):817–827.10.1093/cercor/bhm128 [PubMed: 17652468]
- Saad ZS, Gotts SJ, Murphy K, Chen G, Jo HJ, Martin A, Cox RW. Trouble at rest: how correlation patterns and group differences become distorted after global signal regression. *Brain Connect*. 2012; 2(1):25–32. doi: <http://dx.doi.org/10.1089/brain.2012.0080>. [PubMed: 22432927]
- Saad ZS, Reynolds RC, Jo HJ, Gotts SJ, Chen G, Martin A, Cox RW. Correcting brain-wide correlation differences in resting-state FMRI. *Brain Connect*. 2013; 3(4):339–352. doi: <http://dx.doi.org/10.1089/brain.2013.0156>. [PubMed: 23705677]
- Sadato N, Ibanez V, Deiber MP, Campbell G, Leonardo M, Hallett M. Frequency-dependent changes of regional cerebral blood flow during finger movements. *J Cereb Blood Flow Metab*. 1996; 16(1): 23–33.10.1097/00004647-199601000-00003 [PubMed: 8530552]
- Sehm B, Perez MA, Xu B, Hidler J, Cohen LG. Functional neuroanatomy of mirroring during a unimanual force generation task. *Cereb Cortex*. 2010; 20(1):34–45.10.1093/cercor/bhp075 [PubMed: 19435709]
- Song S, Gotts SJ, Dayan E, Cohen LG. Practice Structure Improves Unconscious Transitional Memories by Increasing Synchrony in a Premotor Network. *J Cogn Neurosci*. 2015; 27(8):1503–1512.10.1162/jocn_a_00796 [PubMed: 25761004]
- Song S, Howard JH Jr, Howard DV. Perceptual sequence learning in a serial reaction time task. *Exp Brain Res*. 2008; 189(2):145–158. doi: <http://dx.doi.org/10.1007/s00221-008-1411-z>. [PubMed: 18478209]
- Song S, Sandrini M, Cohen LG. Modifying somatosensory processing with non-invasive brain stimulation. *Restorative Neurology and Neuroscience*. 2011; 29(6):427–437.10.3233/rnn-2011-0614 [PubMed: 22124034]
- Sun FT, Miller LM, Rao AA, D'Esposito M. Functional connectivity of cortical networks involved in bimanual motor sequence learning. *Cerebral Cortex*. 2007; 17(5):1227–1234.10.1093/cercor/bhl033 [PubMed: 16855008]

- Talairach, J.; Tournoux, P., editors. Coplanar stereotaxic atlas of the human brain. New York: Thieme, Verlag; 1988.
- Ward NS, Bestmann S, Hartwigsen G, Weiss MM, Christensen LOD, Frackowiak RSJ, et al. Siebner HR. Low-Frequency Transcranial Magnetic Stimulation over Left Dorsal Premotor Cortex Improves the Dynamic Control of Visuospatially Cued Actions. *Journal of Neuroscience*. 2010; 30(27):9216–9223.10.1523/jneurosci.4499-09.2010 [PubMed: 20610756]
- Wassermann EM, Wedegaertner FR, Ziemann U, George MS, Chen R. Crossed reduction of human motor cortex excitability by 1-Hz transcranial magnetic stimulation. *Neuroscience Letters*. 1998; 250(3):141–144.10.1016/s0304-3940(98)00437-6 [PubMed: 9708852]
- Watanabe T, Hanajima R, Shirota Y, Ohminami S, Tsutsumi R, Terao Y, et al. Ohtomo K. Bidirectional effects on interhemispheric resting-state functional connectivity induced by excitatory and inhibitory repetitive transcranial magnetic stimulation. *Human Brain Mapping*. 2014; 35(5):1896–1905.10.1002/hbm.22300 [PubMed: 23897535]
- Wilkinson L, Jahanshahi M. The striatum and probabilistic implicit sequence learning. *Brain Research*. 2007; 1137(1):117–130. doi: <http://dx.doi.org/10.1016/j.brainres.2006.12.051>. [PubMed: 17229409]
- Wilkinson L, Khan Z, Jahanshahi M. The role of the basal ganglia and its cortical connections in sequence learning: Evidence from implicit and explicit sequence learning in Parkinson's disease. *Neuropsychologia*. 2009; 47(12):2564–2573.10.1016/j.neuropsychologia.2009.05.003 [PubMed: 19447121]
- Wilkinson L, Shanks DR. Intentional control and implicit sequence learning. *Journal of Experimental Psychology-Learning Memory and Cognition*. 2004; 30(2):354–369. doi: <http://dx.doi.org/10.1037/0278-7393.30.2.354>.
- Wilkinson L, Steel A, Mooshagian E, Zimmermann T, Keisler A, Lewis JD, Wassermann EM. Online feedback enhances early consolidation of motor sequence learning and reverses recall deficit from transcranial stimulation of motor cortex. *Cortex*. 2015; 71:134–147.10.1016/j.cortex.2015.06.012 [PubMed: 26204232]
- Wilkinson L, Teo JT, Obeso I, Rothwell JC, Jahanshahi M. The Contribution of Primary Motor Cortex is Essential for Probabilistic Implicit Sequence Learning: Evidence from Theta Burst Magnetic Stimulation. *Journal of Cognitive Neuroscience*. 2010; 22(3):427–436. doi: <http://dx.doi.org/10.1162/jocn.2009.21208>. [PubMed: 19301999]

Abbreviations

AMT	active motor threshold
BOLD	Blood oxygen level-dependent
cTBS	continuous theta burst stimulation
cTBS 300	a 20 sec uninterrupted train of theta burst of 300 pulses
cTBS 600	a 40 sec uninterrupted train of theta burst of 600 pulses
dACC	dorsal anterior cingulate
EPI	echo planar imaging sequence
FDI	first dorsal interosseous muscle
fMRI	functional magnetic resonance imaging
FOV	field of view
GCOR	global correlation
imp	improbable

IFG	inferior frontal gyrus
M1	primary motor area
mCC	middle cingulate gyrus
MEP	motor evoked potential
MPRAGE	magnetization-prepared rapid gradient echo
MRI	magnetic resonance imaging
MT	middle temporal area
PDP	process dissociation procedure
PMd	dorsal premotor cortex
prob	probable
pSRTT	probabilistic serial reaction time task
R	Random block
ROIs	regions of interest
RT	reaction time
S	Sequence block
SFG	superior frontal gyrus
SOC	second order conditional
SMA	supplementary motor area
SRTT	serial reaction time task
TE	echo time
TMS	transcranial magnetic stimulation
TR	repetition time

Figure 1.

Experimental design. Each participant underwent two TBS-fMRI sessions, during which they received either real or sham cTBS over M1. The order of stimulation conditions was counterbalanced. Experimental sessions occurred at least one week apart.

Figure 2.

A. Mean RT for probable (prob) and improbable (imp) trials across learning (S1-20) blocks and mean RT for random (rand; R1-8) blocks, plotted by stimulation condition. B. Mean RT difference scores (improbable - probable trials) for the real and sham conditions over S blocks 1-20. Positive RT difference score = better learning of probable trials. **: difference between stimulation conditions, $p < .05$, 2-tailed, uncorrected. *: difference between stimulation conditions, $p < .05$, 1-tailed, uncorrected. #: Tukey's HSD, significant difference from Block 1, $p < .05$, 2-tailed. ♦: All blocks with difference between prob and imp trials, $p < .003$, 2-tailed. C. Mean omission error rates across S blocks and mean RT for random (rand; R1-8) blocks, plotted by stimulation condition. D. Mean error difference scores (improbable - probable trials) for the real and sham conditions. Positive RT difference score = better learning of probable trials. *: blocks showing significant differences between stimulation conditions, $p < .05$, 1-tailed, uncorrected. #: Tukey's HSD, significant difference

from Block 1, $p < .05$, 2- tailed. ♦: All blocks with difference between prob and imp trials, $p < .003$, 2-tailed. Bars show standard error.

Figure 3.

A. Regions differing in overall functional connectedness between real and sham cTBS. Decreases in global connectedness with real relative to sham are shown in blue; increases are shown in red. B. Graphs show the connectedness measure for the peak of each significant cluster for each stimulation condition and do not reflect additional statistical testing.

Figure 4.

Map of seed and derived ROIs showing differences in connectivity between the real and sham cTBS conditions. Decreases in global connectivity with real cTBS are in blue; increases are in red. Clusters of voxels in green showed differences in connectivity with these seed regions and were included as additional regions of interest.

Figure 5.

Correlation contrast matrix from a 5-mm sphere around the peak voxel in each significant cluster showing a decrease in connectivity between visual and motor regions after real cTBS. *: $p < .05$, corrected. **: $p < .05$, uncorrected.

Table 1

Results from the sequence vs. random contrast (all $ps < .05$ corrected). Coordinates are in Talairach-Tournoux space.

Volume (mm ³)	Peak x	Peak y	Peak z	Region
Seq > Rand				
40716	37.5	25.5	50.5	Left primary motor area, premotor area, sensorimotor area
33804	-16.5	49.5	-21.5	Left cerebellum
28188	10.5	-37.5	47.5	Left superior frontal gyrus
23436	7.5	16.5	-6.5	Left ventral tegmental area
6669	-31.5	7.5	44.5	Right premotor area
6156	-49.5	61.5	26.5	Right middle temporal gyrus
5670	55.5	34.5	-3.5	Left middle temporal gyrus
4914	-1.5	58.5	29.5	Right posterior cingulate
3294	-25.5	55.5	47.5	Right superior parietal lobule
2781	7.5	4.5	53.5	Supplementary motor area
2079	22.5	4.5	11.5	Left putamen
1593	-61.5	10.5	-15.5	Right inferior temporal gyrus
1539	-58.5	37.5	-0.5	Right middle temporal gyrus
1458	-37.5	-13.5	44.5	Right middle frontal gyrus
Rand > Seq				
59184	-13.5	34.5	38.5	Middle cingulate
42768	10.5	76.5	26.5	Left cuneus, superior parietal lobule
7695	34.5	-1.5	14.5	Left insula
2592	-28.5	-34.5	35.5	Right middle frontal gyrus
2430	28.5	-31.5	38.5	Left middle frontal gyrus
2133	58.5	34.5	29.5	Left inferior parietal lobule

Regions of interest (ROIs) showing differences in functional connectivity. Seed ROIs producing significant results for the whole brain connectedness comparison are in bold. Peak = peak *t*-value voxel in the ROI for group comparisons of functional connectedness. Coordinates are in Talairach-Tournoux space. ROIs are listed in order of their row/column presentation in Figure 5.

Table 2

	Volume (mm ³)	Peak x	Peak y	Peak z	Region
1	2943	10.5	91.5	8.5	Left cuneus/Superior occipital gyrus
2	2052	22.5	91.5	-9.5	Left inferior occipital gyrus
3	1998	43.5	-1.5	50.5	Left dorsal premotor area
4		16.5	28.5	65.5	Left precentral gyrus, dorsal M1
5	7965	7.5	22.5	50.5	SMA
6	1458	28.5	73.5	20.5	Left middle temporal area
7	2430	-4.5	28.5	35.5	Middle cingulate cortex
8	1890	1.5	-37.5	5.5	Dorsal anterior cingulate cortex
9	1647	10.5	-58.5	26.5	Left superior frontal gyrus
10	2376	-52.5	-25.5	14.5	Right inferior frontal gyrus