The Contribution of Primary Motor Cortex is Essential for Probabilistic Implicit Sequence Learning: Evidence from Theta Burst Magnetic Stimulation

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Abstract

■ Theta burst transcranial magnetic stimulation (TBS) is considered to produce plastic changes in human motor cortex. Here, we examined the inhibitory and excitatory effects of TBS on implicit sequence learning using a probabilistic serial reaction time paradigm. We investigated the involvement of several cortical regions associated with implicit sequence learning by examining probabilistic sequence learning in five age- and IQ-matched groups of healthy participants following continuous inhibitory TBS over primary motor cortex (M1); or the supplementary motor area (SMA) or dorsolateral prefrontal cortex (DLPFC) or following intermittent excitatory TBS of M1; or after sham TBS. Relative to sham TBS, probabilistic sequence learning was abolished by inhibitory TBS over M1, demonstrating that this region is critical for implicit motor sequence learning. Sequence learning was not significantly affected by inhibitory TBS over the SMA, DLPFC or excitatory TBS over M1. These results demonstrate that the M1 mediates implicit sequence learning. ■

INTRODUCTION

It has been suggested that implicit (unconscious) and explicit (conscious) memory are separable learning systems (Squire & Zola, 1996). The implicit system is believed to be involved in motor skill learning acquired incidentally with practice (e.g., riding a bicycle, playing golf), whereas the explicit system is considered to play a role in the acquisition of knowledge in a more intentional way (e.g., remembering lists of words). Furthermore, it has been proposed that the striatal structures with their cortical projections support implicit learning, whereas the cortico-limbic-diencephalic structures are the substrate for explicit (conscious) learning (Cohen & Squire, 1980).

One paradigm that has been developed to study implicit learning in the laboratory is the serial reaction time (SRT) task (Nissen & Bullemer, 1987). Typically, on each trial of the SRT task, a target appears in one of four 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., 10 blocks of 100 trials) and reaction times (RTs) are measured. Unknown to participants, the majority of targets actually appear in a predetermined repeating sequence of box locations (e.g., 3-4-2-3-1-2-1-4-3-2-4-1). The sequence can be presented in either a deterministic or a probabilistic way. If mean RTs across blocks become faster for the sequence relative to the random or pseudorandom trials, then it can be inferred that participants learned the trained sequence.

Imaging studies have revealed the functional anatomy of implicit motor sequence learning and have shown that such learning is associated with activation of primary motor cortex, supplementary motor area (SMA), premotor cortex, dorsolateral prefrontal cortex (DLPFC), as well as the putamen and caudate (Poldrack et al., 2005; Seidler et al., 2005; Schendan, Searl, Melrose, & Stern, 2003; Hazeltine, Grafton, & Ivry, 1997; Grafton, Hazeltine, & Ivry, 1995). However, functional imaging does not reveal whether the contribution of these various brain regions to implicit motor sequence learning is essential or not. To address this question, the technique of repetitive transcranial magnetic stimulation (rTMS) to induce "virtual lesions" has been used in several studies. Early studies have suggested that deterministic SRT learning was impaired by rTMS over DLPFC but not by rTMS over the SMA (Pascual-Leone, Wassermann, Grafman, & Hallett, 1996) or the primary motor cortex (M1) (Pascual-Leone et al., 1999). However, more recent studies on the effect of stimulation of primary motor cortex contradict this result. Motor sequence learning was enhanced by 5 Hz rTMS over primary motor cortex (Kim, Park, Ko, Jang, & Lee, 2004), whereas anodal transcranial direct current stimulation (atDCS) over this area enhanced learning if it was delivered during the task (Nitsche et al., 2003) but had no effect if it was delivered prior to learning (Kuo et al., 2008).

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Similarly, although rTMS over the SMA was previously shown not to affect SRT learning (Pascual-Leone et al., 1996), it affected transfer of knowledge to the nonperforming hand (Perez et al., 2007). The role of DLPFC in sequence learning also appears to be more complex. Although rTMS over DLPFC delivered during a deterministic SRT task (Pascual-Leone et al., 1996, 1999) impaired normal learning, this impairment disappeared if there was no spatial component to the visual cues (Robertson, Tormos, Maeda, & Pascual-Leone, 2001). Furthermore, an impairment of SRT learning in the left hand of a patient with a focal lesion of the left cerebellum was shown to be restored by rTMS over both the cerebellum and DLPFC delivered prior to SRT learning (Torriero et al., 2007).

This inconsistent pattern of results partly relates to the different methodologies of delivering brain stimulation. As mentioned previously, some experimenters delivered stimulation during the SRT task (Nitsche et al., 2003; Pascual-Leone et al., 1996, 1999) or interspersed with the SRT task (Kim et al., 2004). The delivery of stimulation during performance of the SRT task is compounded by problems of distraction as rTMS produces a palpable scalp sensation and a loud "click," which may interfere with task performance and learning. This problem does not apply to tDCS. Finally, all of the above studies used a deterministic SRT task, which is a less sensitive index of learning and less likely to foster learning that is truly explicit compared to the probabilistic SRT task, which provides an "on-line" index of learning on every block and the element of noise in the probabilistic sequence blocks explicit knowledge and promotes implicit learning of the sequence.

Theta burst rTMS (TBS) is a more recent rTMS technique (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005) which can be delivered relatively rapidly (<3 minutes) and has been shown to produce excitatory (intermittent TBS) or inhibitory (continuous TBS) effects on cortical excitability lasting for 40 min after the stimulation has ended. This longer-lasting stimulation effect allows an "off-line" approach with the participant being able to perform the SRT task undistracted by concurrent stimulation and unconstrained by the stimulating coil. TBS has been shown to produce plastic changes in human motor cortex (Huang et al., 2005), but to date, the effects of TBS on learning have not been investigated. In this study, we had two objectives. Our first aim was to assess whether the contribution of the M1, SMA, and DLPFC to implicit sequence learning are essential by applying continuous inhibitory TBS over these areas immediately before performance of a probabilistic SRT task. We predicted that if the contribution of these areas to learning is essential, then inhibitory TBS over the area would impair subsequent implicit sequence learning. Our second aim was to assess whether implicit sequence learning could be improved by intermittent excitatory TBS over M1.

METHODS

Participants

Forty right-handed healthy volunteers were recruited, all of whom met the safety criteria for TMS (Keel, Smith, & Wassermann, 2001). None of the participants had any neurological disorder or history of psychiatric illness, drug or alcohol abuse, or were on any drug treatments that might influence performance. The study was approved by the Joint Ethics Committee of the Institute of Neurology and The National Hospital for Neurology and Neurosurgery. Informed consent was obtained from all participants. Participants were randomly assigned either to:

(a) Sham stimulation group (n = 8, 4 women) aged 22–36 years (M = 27.63, SD = 4.44).
(b) Continuous (inhibitory) TBS over the M1 group (n = 8, 5 women) aged 24–37 years (M = 30.63, SD = 4.57).
(c) Continuous (inhibitory) TBS over the SMA group (n = 8, 4 women) aged 20–33 years (M = 25.38, SD = 4.78).
(d) Continuous (inhibitory) TBS over the DLPFC group (n = 8, 5 women) aged 24–38 years (M = 30.38, SD = 5.48).
(e) Intermittent (excitatory) TBS over the M1 group (n = 8, 6 women) aged 22–36 years (M = 27.63, SD = 4.44).

SRT Task

In the deterministic SRT task (employed in the majority of SRT studies), this sequence is repeatedly presented during all blocks with the exception of a single "transfer" block (e.g., Block 9) in which different random or pseudorandom trials are introduced. It is often concluded that knowledge acquired during the deterministic SRT task is implicit or unconscious; however, in some studies, participants have been shown to develop conscious sequence knowledge during supposedly implicit SRT learning (Wilkinson & Shanks, 2004). To address this concern, experimenters have attempted to minimize the chance that SRT learning will be explicit by adopting probabilistic, rather than deterministic, sequence presentation. In the probabilistic SRT task (employed here), the sequence is presented such that on any single trial there is an 85% chance that the target will appear according to the sequence and a 15% chance that it will appear in a pseudorandomly determined location. Hence, the element of noise during the probabilistic sequence presentation reduces the chance of participants developing explicit knowledge of the sequence and allows for a more sensitive on-line measure of learning across all blocks (rather than just one) by comparing RTs on probable versus improbable trials.

The probabilistic SRT task was performed immediately after the TBS procedure was completed. Stimulus presentation, response recording, and RT measurement were all

implemented on a PC with a 33-cm color monitor connected to a four-button box. The four buttons were arranged in a row and will be referred to as 1–4 from left to right. Stimulus presentation involved four boxes arranged horizontally along the middle of the computer screen in white against a gray background. The boxes were 26 mm wide and 26 mm high. On each trial of the SRT task, a black "X" appeared in the center of one of the boxes, to which participants had to respond.

Two second-order conditional sequences, SOC1 = 3–1–4–3–2–4–2–1–3–4–1–2 and SOC2 = 4–3–1–2–4–1–3–2–1–4–2–3, were used in the probabilistic SRT task. These sequences are equated with respect to location 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 differed in their second- and higher-order conditional structure. For approximately half the participants in each condition, SOC1 was the training sequence and for the remainder it was SOC2.

In the course of the probabilistic SRT task, the location of the target was specified by the assigned training sequence with a probability of .85 and by the alternate sequence with a probability of .15. The probabilistic sequences were implemented by using the two most recent events to select the next event. There was a probability of .85 that the next target would be the event in the training sequence specified by the last two locations and a probability of .15 that it would be the event in the alternate sequence specified by the last two locations. For example, for a given participant trained on SOC1, the transition 4–1 was followed by a target at Location 2 (following the specified sequence of SOC1) with a probability of .85, and it was followed by a target at Location 3 (following the specified sequence of SOC2) with a probability of .15. This algorithm was applied on each trial and determined the location of the current target simply based on the two preceding targets.

The probabilistic SRT task comprised 10 blocks, each block with 100 trials during which participants were exposed to a four-choice SRT task. On each trial, participants reacted to the location of the target as quickly as possible by pressing the corresponding button on the four-button response box. Buttons A, B, C, and D corresponded to Locations 1 to 4, in that order. Participants were required to respond to Locations 1 to 4 with the first four fingers, respectively, of their right hand. Participants were instructed to respond to the target as fast and as accurately as possible.

Each block began at a random point in the sequence. A trial ended when a participant pressed the correct key, at which time the target disappeared from the screen. The next target appeared after a 250-msec interval. Response latencies were measured in milliseconds from the onset of the target to the completion of a response.[1] In total, participants took 16–24 min to complete 10 blocks.

Theta Burst Stimulation

Stimulation was delivered using a Magstim Rapid stimulator (Magstim Co., Dyfed, UK) connected to a figure-eight cased coil with an internal wing diameter of 70 mm, held with the handle pointing posterolaterally. Electromyographic (EMG) recordings were made using a belly-to-tendon montage from the right first dorsal interosseous (FDI) muscle. The location of the hand representation in the left hemisphere was determined, defined as the position at which stimulation produced optimal muscle-evoked potentials (MEPs) in the right FDI. The active motor threshold was assessed during voluntary contraction of the target FDI at approximately 10% of maximum force, and was defined as the lowest stimulus intensity required to evoke an MEP of >200 μV in 5 out of 10 trials.

Theta burst stimulation was given according to the continuous (cTBS) or intermittent (iTBS) protocol described by Huang et al. (2005). A theta burst consists of three pulses at 50 Hz, at an intensity of 80% active motor threshold. For the cTBS protocol, theta bursts were given every 200 msec (i.e., 5 Hz) for a total of 600 pulses (200 theta bursts or 600 pulses) in the cTBS protocol. The stimulation lasted for 40 sec and has been shown to produce a decrease in corticospinal excitability lasting up to 40 min (Huang et al., 2005). For the iTBS protocol, theta bursts were given every 200 msec for 2 sec (i.e., 10 theta bursts or 30 pulses), followed by a pause of 8 sec before another 2 sec of theta bursts. This was repeated 20 times, thereby producing a total of 200 theta bursts or 600 pulses. The stimulation lasted in total of 200 sec and has been shown to produce an increase in corticospinal excitability lasting up to 20 min (Huang et al., 2005).

For sham stimulation, the coil was held rotated 90° over the hand representation of motor cortex so that the point of contact with the scalp was unchanged but the handle pointed vertically upward.

For cTBS (inhibitory) over M1, cTBS was delivered as described above to the hand representation of motor cortex as identified above with the coil handle in the posterolateral position.

For cTBS (inhibitory) over the SMA, the coil center was placed over a point 3 cm anterior and 0.5 cm to the left of the standard 10–20 electrode position, Cz, with the coil handle pointed laterally to the left (Matsunaga et al., 2005). At this point, there was no discernable twitch in the muscles of the leg of the participant.

For cTBS (inhibitory) over DLPFC, the coil center was placed over a point 5 cm anterior to the hand representation of motor cortex as identified above with the coil handle in the posterolateral position (Pascual-Leone et al., 1996).

For iTBS (excitatory) over M1, iTBS was delivered as described above to the hand representation of motor cortex as identified above with the coil handle in the posterolateral position.

SRT Task Data Analysis

For each participant, mean overall RT, mean overall errors, and mean RTs and errors for both probable and improbable trials at each block were calculated. Any RTs shorter than 200 msec or longer than three standard deviations above an individual's overall mean RT were excluded from the analysis. The analysis of RT data included trials on which errors were made because the presence of significantly more error trials in the improbable data is caused by anticipation (see analysis of error data), therefore, it is informative and contributes to the developing difference between probable and improbable RTs across blocks. The standard deviations of RTs for probable and improbable trials at each block were calculated as a measure of variability of RTs.

In all subsequent analyses: (i) RTs or errors for participants trained on one of the two possible sequences were combined; (ii) RTs or errors to the first two targets of each block were excluded because their locations cannot be predicted; (iii) 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; and (iv) a significance criterion of $\alpha = .05$ was used.

RESULTS

Participants randomly assigned to the sham, inhibitory M1, inhibitory SMA, inhibitory DLPFC, or excitatory M1 groups did not differ in terms of either age [$F(4, 39) = 1.69, p > .05$], IQ [$F(4, 39) = 1.13, p > .05$], or sex distribution [$\chi(4) = 2.13, p < .05$]. Prior to the analysis of learning effects, one-way ANOVAs established that overall mean RTs [$F(4, 39) = 1.17, p > .05$] and overall mean errors ($F < 1, p > .05$) were not significantly affected by group. Therefore, nonspecific effects of TBS on overall RTs or accuracy did not confound the following analysis of learning.

Reaction Times

Figure 1A–E depicts mean RTs obtained over the training phase, plotted separately for the five groups and for each type of target location, probable or improbable. First, to establish whether RTs for probable trials changed significantly across blocks in the five groups, an ANOVA was performed on mean RT for probable trials, with block (1–10) as a within-subject variable and group (sham vs. inhibitory M1 vs. inhibitory SMA vs. inhibitory DLPFC vs. excitatory M1) as a between-groups variable. This analysis revealed a significant main effect of block [$V = 0.50, F(9, 27) = 2.95, p = .01$] because RTs for probable trials significantly changed across blocks. The main effect of group [$F(4, 35) = 1.01$] and interaction between Group × Block ($F < 1$) were not significant. For the main effect of block, there was a significant quadratic trend [$F(1, 35) = 8.72, p = .01$] reflecting the fact that across all groups RTs for probable trials increased across the first couple of blocks, followed by a period of leveling off, after which they showed a decrease. The eventual speed-up in RTs for probable trials was seen in all groups and could either be the result of learning the probable sequence or be due to a nonspecific effect of task practice.

Second, to examine whether learning was present in the five groups and to compare patterns of learning across blocks and in the five groups, an ANOVA was performed on mean RT with probability (probable vs. improbable) and block as within-subject variables and group as a between-groups variable. This analysis revealed a significant main effect of probability [$F(1, 35) = 52.25, p < .001$] because, overall, probable targets performed faster than improbable targets, which is indicative of sequence learning. There was also a significant main effect of block [$F(5.4, 188.3) = 3.97, p = .001$] and significant interactions between Probability × Block [$F(9, 315) = 3.96, p < .001$] and Group × Probability × Block [$F(9, 315) = 1.53, p = .03$], showing that the magnitude of differentiation between RTs for probable and improbable targets (i.e., extent of learning) changed across blocks—and this pattern differed significantly between the groups. The main effect of group [$F(4, 35) = 1.17\, p > .05$] and interactions between Group × Probability and Group × Block were not significant (Fs $< 1, p > .05$).

In view of the different patterns of learning demonstrated by the five groups across blocks, composite measures of learning for epochs at the beginning, middle, and end of the training phase were obtained by calculating a difference score (improbable − probable trials) and comparing the mean difference score across Blocks 1–4, 5–7 and 8–10. If learning has occurred, probable trials should be performed faster than improbable trials, therefore, a positive difference score, which is also significantly different from zero, is evidence of learning.

Evidence of Learning and the Progression of Learning across Blocks in Each Group

Figure 2A–C depicts the mean of the difference scores for the three training epochs, plotted separately—relative to the sham groups' performance—for each of the TBS groups. An ANOVA was performed on difference scores with epoch (1–3) as a within-participant variable and group as a between-groups variable. This analysis revealed a significant interaction between Group × Epoch [$F(8, 70) = 2.61, p = .02$], again indicating that the magnitude of RT differences between probable and improbable targets changed across blocks and between groups. The main effect of epoch [$F(2, 70) = 7.68, p = .01$] was also significant, whereas the main effect of group failed to reach significance [$F(4, 35) = 1.00$].

In light of the significant Group × Epoch interaction and to establish whether learning occurred at each epoch, in each group, we compared mean difference scores to

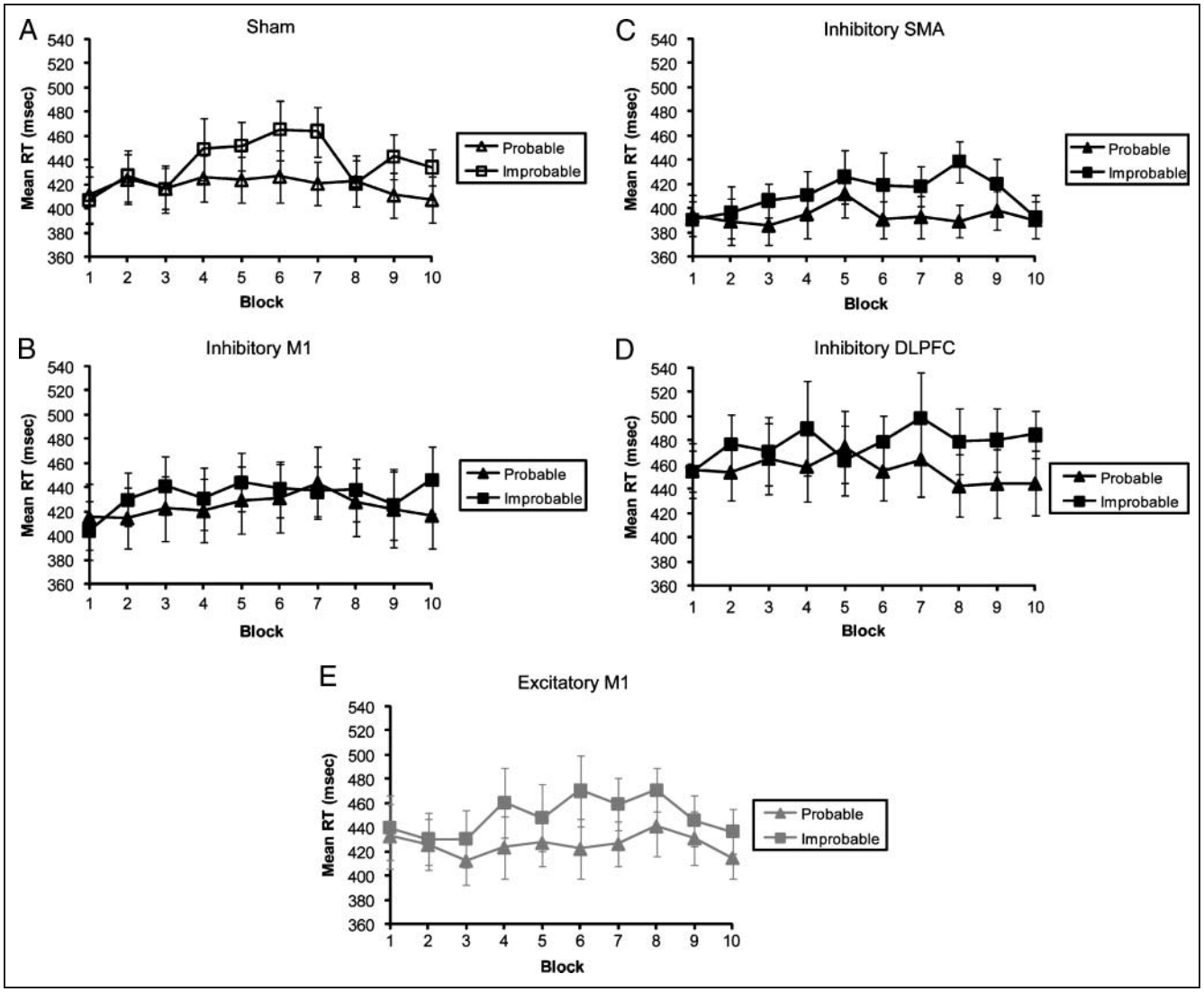

Figure 1. (A–E) Mean RTs across training blocks for the implicit sequence learning task plotted on separate figures for the (A) sham, (B) inhibitory M1, (C) inhibitory SMA, (D) inhibitory DLPFC, and (E) excitatory M1 groups. Probable targets were consistent with the generating sequence, whereas improbable targets were not. Error bars represent standard errors.

zero. Then, to examine the progression of learning across blocks, for each group we compared mean difference scores across each epoch. For the sham group, mean difference scores were all positive and for the second [$t(7) = 5.34$, $p = .001$] and third [$t(7) = 3.05$, $p = .02$] epochs, the mean difference scores were significantly different from zero, indicating that the sham group showed significant evidence of learning in the middle and the end of the training phase. For the first epoch, t was <1 for this comparison. The second epoch score was significantly greater than the first [$t(7) = -4.00$, $p = .01$], but the first versus third [$t(7) = -1.43$] and second versus third [$t(7) = 2.09$] scores did not differ significantly.

In contrast, for the inhibitory M1 group, mean difference scores were all positive, but none of the difference scores for the three epochs were significantly different from zero [first: $t(7) = 1.47$; third: $t(7) = 1.80$; second: $t < 1$], demonstrating that inhibitory TBS of the M1 abolished SRT learning across all phases of training. The difference scores for the first versus second, first versus third, and second versus third epoch scores did not differ significantly from each other (all ts < 1).

For the inhibitory SMA group, the mean difference scores were all positive, and for all epochs, these scores were significantly different from zero [first: $t(7) = 3.08$, $p = .02$; second: $t(7) = 8.13$, $p < .001$; third: $t(7) = 3.20$, $p = .02$], indicating that this group showed significant learning of the sequence across all epochs. Similar to the sham group, the difference score for the second epoch was significantly greater than the first [$t(7) = -4.62$, $p = .002$], but the difference scores for the first versus third [$t(7) = -2.11$] and second versus third ($t < 1$) scores did not differ significantly.

For the inhibitory DLPFC group, mean difference scores were all positive, and the scores for the first [$t(7) = 3.20$, $p = .02$] and third [$t(7) = 5.04$, $p = .001$] epochs were

significantly different from zero [second: $t(7) = 1.66$], indicating that this group showed significant evidence of learning at the beginning and the end of learning. Perhaps our failure to observe significant evidence of learning in the middle blocks in this group can be explained by the low number of participants and the resulting large standard errors. In contrast to the sham group, there was no significant difference between the scores for the first and second epochs ($t < 1$). The score for the third epoch was significantly greater than both the first [$t(7) = -4.22$, $p = .004$] and second [$t(7) = -3.34$, $p = .01$].

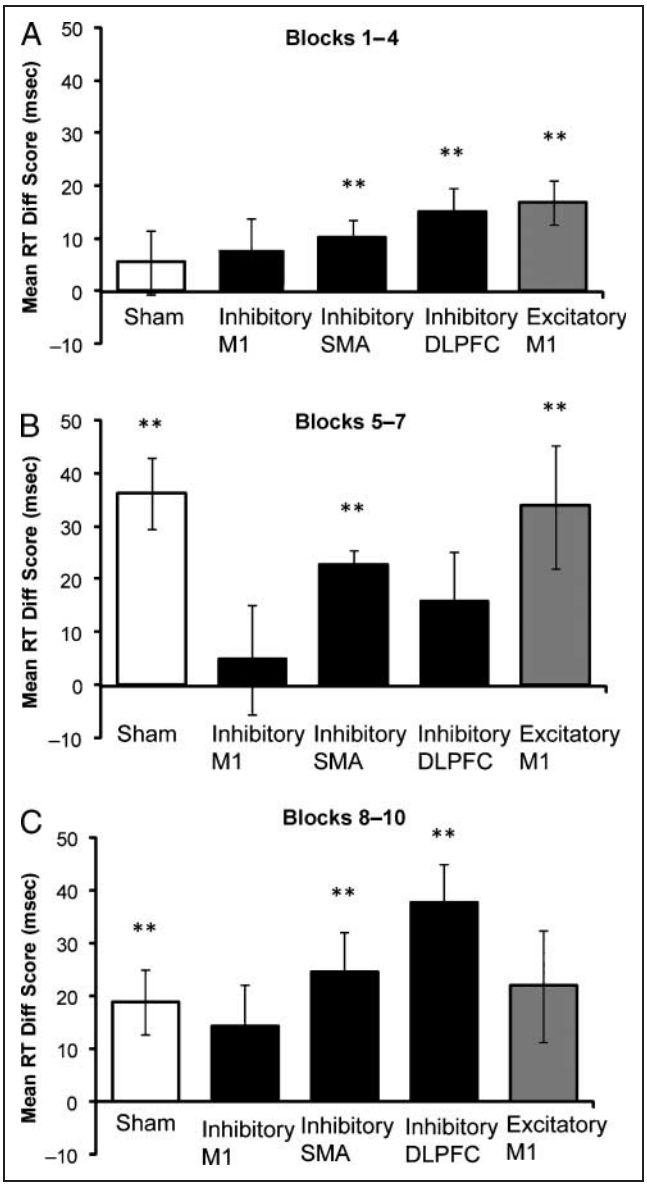

Figure 2. (A–C) Mean RT difference scores plotted separately for the sham, inhibitory M1, inhibitory SMA, inhibitory DLPFC, and excitatory M1 groups and plotted on separate figures for (A) the first epoch (Blocks 1–4), (B) the second epoch (Blocks 5–7), and (C) the third epoch. A positive RT difference score indicates better learning and a double asterisk indicates scores that were significantly different from zero (two-tailed). Error bars depict standard errors.

For the excitatory M1 group, mean difference scores were all positive, and scores the first [$t(7) = 4.00$, $p = .01$] and second [$t(7) = 2.91$, $p = .02$] epochs were significantly different from zero. The score for the third epoch was marginally significantly different from zero [$t(7) = 2.08$, $p = .08$], indicating that this group showed significant learning of the sequence across all epochs. Scores for the first versus second [$t(7) = -1.71$], first versus third ($t < 1$), and second vs. third [$t(7) = 1.23$] epochs did not differ significantly.

The TBS Groups' Performance Relative to the Sham Group

Next, for each TBS group, we compared mean difference scores at each epoch with the sham groups' performance. For the inhibitory M1 group, mean difference scores were significantly different during the second epoch because the sham group learned significantly more than the inhibitory M1 group during the middle stage of the training phase [$t(14) = -2.54$, $p = .03$]. During the first and third epochs, the differences between the groups were not significant (ts < 1).

For all other groups, mean difference scores were not significantly different from the sham group across all epochs [inhibitory SMA: second, $t(14) = -1.83$, first and third, ts < 1; inhibitory DLPFC: first, $t(14) = 1.23$, second, $t(14) = -1.74$, third, $t(14) = 1.93$; excitatory M1: first $t(14) = 1.53$, second and third, ts < 1].

Comparison of the TBS Groups Who Showed Learning to the Inhibitory M1 Group

We compared mean difference scores at each epoch for each TBS group that showed learning (inhibitory SMA, inhibitory DLPFC, and excitatory M1) with the learning in the inhibitory M1 group. For the inhibitory DLPFC group, mean difference scores were significantly different during the third epoch because the inhibitory DLPFC group learned significantly more than the inhibitory M1 group during the later stage of the training phase [$t(14) = 2.14$, $p = .05$]. During the first and second epochs, the differences between these two groups were not significant (ts < 1). The excitatory M1 group showed greater learning than the inhibitory M1 group during the middle stage of the training phase, a difference which approached significance [$t(14) = 1.86$, $p = .08$]. During the first [$t(14) = 1.22$] and second ($t < 1$) epochs, the differences between these two groups were not significant.

For the inhibitory SMA group, mean difference scores were not significantly different from the inhibitory M1 group across any of the three epochs [second: $t(8.0) = 1.66$; first and third: ts < 1].

Variability of RTs

To establish whether the TBS manipulation changed variability of RTs across blocks, an ANOVA was performed

on mean standard deviation of RTs with probability and block as within-subject variables and group as a between-groups variable. This analysis revealed a significant main effect of block [$V = 0.54$, $F(9, 27) = 3.56$, $p = .01$]. The main effect of group [$F(4, 35) = 1.12$] and all other main effects and interaction were not significant (all Fs < 1, ps $> .05$).

Errors

Overall mean error rates were as follows: sham = 0.04, $SD = 0.04$; inhibitory M1 = 0.04, $SD = 0.03$; inhibitory SMA = 0.07, $SD = 0.12$; inhibitory DLPFC = 0.05, $SD = 0.03$; and excitatory M1 = 0.04, $SD = 0.03$. Recall, the TBS manipulation made no difference to overall accuracy. To compare the rate of errors across blocks and in the five groups, an ANOVA was performed on mean error rate with probability and block as within-subject variables and group as a between-groups variable. This analysis revealed a significant main effect of probability [$F(1, 35) = 24.20$, $p < .001$] because, overall, more errors were made for improbable relative to probable targets; this reflects the fact that participants were able to develop expectations about the location of the probable target which caused anticipations, and thus, errors when the target appeared in the unanticipated location. The main effect of block [$V = 0.40$, $F(9, 27) = 1.97$], interaction between Probability × Block [$V = 0.30$, $F(9, 27) = 1.26$], and all other main effects and interactions were not significant (all Fs < 1, ps $> .05$).

To compare the rate of errors across blocks in the inhibitory M1 group alone, an ANOVA was performed on mean error rate with probability and block as within-subject variables. In contrast to the above, this analysis did not produce a significant "expectancy effect" because the main effect of probability [$F(1, 9) = 1.36$, $p > .05$] was not significant. This finding is consistent with our failure to find evidence of sequence learning based on the RT data in this group. The main effect of block and the Probability × Block interaction were not significant either (Fs < 1, ps $> .05$).

DISCUSSION

In the present study, we demonstrate, for the first time, that inhibitory TBS delivered over the M1 impaired subsequent probabilistic implicit sequence learning in healthy participants. In the inhibitory M1 group, sequence learning as indexed by the RT and error data was abolished. In contrast, inhibitory TBS to the DLPFC and SMA, and excitatory TBS to M1 did not affect the overall extent of probabilistic implicit sequence learning. The difference between both the sham and the excitatory M1 groups from the inhibitory M1 group was most apparent during the middle stage of training, whereas the difference between the inhibitory DLPFC group and the inhibitory M1 group was most evident during later blocks. Despite the fact that the inhibitory SMA group showed evidence of learning relative to chance, there was no significant difference between the extent of learning achieved by this group and the inhibitory M1 group.

Methodological Differences across Studies of the Effects of rTMS and tDCS on SRT Learning

Our findings with TBS rTMS have similarities but also important differences from those of Nitsche et al. (2003) with atDCS. The consistent conclusions across studies are that although M1 is implicated in implicit sequence learning, DLPFC is not. Furthermore, if TBS is delivered to M1 prior to learning, as was the case here, subsequent learning is impaired, whereas if atDCS is delivered during the task, it enhances concurrent learning (Nitsche et al., 2003).

The common results from this study and the study of Nitsche et al. (2003) also stand in contrast to some of the findings of other studies that did not show an effect of rTMS over M1, delivered concurrently during SRT learning (Pascual-Leone et al., 1999) or of atDCS over M1 delivered prior to SRT learning (Kuo et al., 2008), but, did show an effect of rTMS over DLPFC (Pascual-Leone et al., 1996). Nevertheless, our finding that inhibitory TBS over the SMA did not affect SRT learning is consistent with one of these studies (Pascual-Leone et al., 1996).

There are several possible explanations for the differences in the current results and those seen previously by Robertson et al. (2001) and Pascual-Leone et al. (1996, 1999). First, it is possible that the inhibitory rTMS procedures used in previous studies were not of sufficient intensity/frequency to induce changes in plasticity in M1. Second, the possibility remains that in previous studies (Pascual-Leone et al., 1996, 1999), concurrently delivered rTMS modified learning because of other reasons such as interference with attentional focusing.

Furthermore, one limitation of this study is the lack of stereotaxic coregistration of the site of the TMS. Although M1 localization is quite reliable due to presence of MEPs in the hand muscles, localization of the SMA and DLPFC is less reliable. For the SMA, this study used landmarks from previous rTMS studies which produced an effect (Matsunaga et al., 2005) and here we established that participants did not make any leg movements during stimulation over the SMA to make sure that stimulation did not affect the leg motor area (just posterior to the SMA). For DLPFC, 5 cm from the motor hotspot was used. The lack of stereotaxic coregistration may have meant that the stimulation was insufficiently specific to DLPFC and may have affected dorsal premotor cortex. Another possibility is that the lower intensity of stimulation used in TBS may have reduced the potency of the effect. Thus, our failure to find an effect of inhibitory TBS over DLPFC on SRT learning is less conclusive than the presence of an effect in M1.

Progression of Learning across Blocks

For the sham group, learning gradually developed in the course of the training, and significant evidence of learning emerged in the middle stage of the task, which was maintained in later blocks. It could be argued that if sequence learning was the only factor involved, then one would have expected that the dissociation between probable and improbable trials should gradually get larger across training blocks. However, one possibility is that, in the sham group, learning developed gradually across training blocks and then plateaued. Another possible explanation might be that participants learn not only the probable sequence but also the reoccurrence of improbable stimuli, which may reduce their distractive effect across blocks, and thus, reduce RTs. In this case, however, the RT for the improbable sequence and the RT differences between probable and improbable sequences would not be a pure measure of sequence learning. It is important to note that, for the sham group, the difference between learning scores for the middle and late epochs was not actually statistically significant. Instead, it appears that, for this group, learning emerged during the middle stage and then stabilized across the middle and later blocks. We observed a similar pattern of the development of learning in healthy elderly controls during the probabilistic SRT task (Wilkinson & Jahanshahi, 2007).

Out of the three TBS groups that showed significant evidence of learning, one (excitatory M1) showed a similar pattern—with respect to the middle and later stages of learning—to the sham group. Whereas the other two groups (SMA and DLPFC) showed an improvement of learning scores across the middle and later blocks, for the inhibitory DLPFC group, this improvement was significant.

With regard to the development of learning across all blocks, the inhibitory SMA group showed a similar pattern to the sham group—a significant improvement of learning across the early and middle blocks, followed by a stabilization of learning across the middle and later blocks. In contrast, for the excitatory M1 group, learning stabilized early on and remained constant throughout the task, whereas, for the inhibitory DLPFC group, a significant improvement of learning relative to the beginning and middle of the task was not seen until the later blocks. Thus, although excitatory TBS over M1 and inhibitory TBS over DLPFC may not have an influence on the extent of learning achieved overall, it appears that these manipulations may have affected the progression of learning across blocks.

Neural Basis of Motor Sequence Learning

Imaging evidence for the contribution of brain regions to implicit sequence learning during the SRT task is inconsistent. Some studies have demonstrated activation of the M1, SMA, and putamen during the SRT task (Seidler et al., 2005; Hazeltine et al., 1997; Grafton et al., 1995), whereas other studies have revealed activation in different areas including the caudate and PFC (Poldrack et al., 2005; Schendan et al., 2003). Similar to the rTMS and tDCS studies of SRT learning, differences in patterns of brain activation associated with the SRT task across studies relate to several important methodological variations. First, most imaging studies have used deterministic SRT tasks and some have employed a dual-task approach (e.g., tone counting concurrently with the SRT) to block awareness of the repeating sequence. It is likely that studies differ in the extent to which learning on the SRT task was truly implicit, with activation of PFC likely to reflect awareness and explicit learning of the sequence (Seidler et al., 2005). Second, studies differ in the extent to which their designs allow successful isolation of brain activity specifically associated with learning per se rather than performance of sequential movements.

From the results of imaging studies of implicit and explicit sequence learning, it is possible to suggest that two distinct fronto-striatal circuits are involved. It is plausible that intentional learning of motor sequences with explicit knowledge activates both the associative circuit between the dorsal caudate and the DLPFC, and the motor circuit between the putamen and M1, SMA and lateral premotor cortex. Once performance of such intentionally and explicitly learned sequences becomes skilled and automatic, then control is passed on to the motor circuit alone. Incidental sequence learning without explicit knowledge that there is a repeating sequence also appears to be mediated by the motor circuit which subserves skilled performance of motor sequences (Brown, 1999). These proposed substrates of implicit and explicit sequence learning, combined with the important methodological differences between TMS studies of sequence learning noted above (degree of explicit knowledge, extent of training and skilled performance, intensity/frequency of stimulation), shed some light on the discrepant pattern of findings across studies.

Patients with Parkinson's disease (PD) have impaired sequence learning on the SRT task (Wilkinson & Jahanshahi, 2007; Jackson, Jackson, Harrison, Henderson, & Kennard, 1995; Ferraro, Balota, & Connor, 1993; Pascual-Leone et al., 1993). Furthermore, posteroventral pallidotomy, which alters basal ganglia output to M1 and SMA, completely abolishes SRT sequence learning in PD patients, which was present, albeit at an attenuated level preoperatively (Brown et al., 2003). These findings on the effects of PD and the further negative impact of posteroventral pallidotomy on the SRT, similar to our results from TBS rTMS, further support the role of M1 in implicit sequence learning.

The Role of M1 in Sequential Learning

It has been suggested that M1 is specifically involved in long-term consolidation and storage of sequential knowledge (Matsuzaka, Picard, & Strick, 2007; Robertson, Press, & Pascual-Leone, 2005; Karni et al., 1995). For instance, in a study of primates who had already completed 2 years

of SRT task learning, Matsuzaka et al. (2007) identified differential patterns of neuronal firing in M1 during performance of sequential relative to random trials. Furthermore, Robertson et al. (2005) showed that rTMS to M1, delivered after SRT learning, disrupted subsequent consolidation of sequential knowledge. However, in contrast to the view that the role of M1 is restricted to long-term consolidation of sequence learning, Seidler et al. (2005) demonstrated learning-related activation in M1 during the early encoding phase of the SRT task, and Nitsche et al. (2003) modified early SRT learning using anodal DCS over M1. Our findings also demonstrate that M1 is directly involved in the initial encoding and acquisition stage of sequence learning.

The precise nature of M1 involvement in the SRT is unclear. Overall RTs were the same in all five groups of participants, implying that finger movements themselves were unaffected by the preceding cTBS. One possibility is that cTBS over M1 interferes with the short-term memory trace of preceding movements that becomes linked during learning to the most probable subsequent movement. This might be analogous to the memory trace that could contribute to the "repetition effect" (Pashler & Baylis, 1991; Bertelson, 1965), where there is a speed advantage when the same stimulus and response are repeated on two consecutive trials. However, further experiments would be required to test this fully.

Why Didn't Excitatory TBS Produce Enhanced Sequence Learning?

Despite our finding that inhibitory TBS over the M1 impaired subsequent SRT learning, we failed to observe a significant improvement of implicit sequence learning following excitatory TBS over M1. It is possible that learning during the SRT task is dependent on a more complex process than simply changes in motor cortical excitability. Interestingly, Nitsche et al. (2003) reported significant improvement of SRT learning with concurrent excitatory atDCS to M1, whereas excitatory atDCS over M1 impaired subsequent learning when delivered prior to the SRT task (Kuo et al., 2008) and there may be similar temporal effects of excitatory TBS on learning. Furthermore, it is also possible that the between-subjects design used in the present study to minimize potential transfer effects that can occur with a within-subjects design reduced the power of detecting such an enhanced learning effect for the excitatory M1 group.

Conclusion

We have presented evidence that continuous inhibitory TBS over M1 impairs implicit sequence learning in a probabilistic SRT task. It remains a task for future studies to examine the time course of these inhibitory TBS effects on the development and progression of learning examined and using a within-subject design to further investigate the potential of intermittent excitatory TBS over M1 to enhance sequence learning in the SRT task.

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Note

1. SOC1 and SOC2, and SOC3 and SOC4 are different but parallel pairs of SOC sequences. For counterbalancing purposes, for half of the participants in the implicit sequence learning task, SOC1 and SOC2 were substituted by SOC3 and SOC4.

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