Inter-cortical Modulation from Premotor to Motor Plasticity

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KEY POINTS SUMMARY

- Plasticity is involved in daily activities but abnormal plasticity may be deleterious. However, the way of manipulating plasticity has been obscure.
- In this study, we found that motor plasticity could be modulated by suppressing the premotor cortex with the theta burst form of repetitive transcranial magnetic stimulation.
- Such changes in motor plasticity was were associated with reduced learning of a simple motor task.
- We postulate that the premotor cortex adjusts the amount of motor plasticity to modulates motor learning through heterosynaptic metaplasticity.
- The present results provide an insight into how the brain physiologically coordinates two different areas to bring them into a functional network. This concept could be employed to intervene in diseases with abnormal plasticity.

ABSTRACT

Primary motor cortex (M1) plasticity is known to be influenced by the excitability and prior activation history of M1 itself. However, little is known about how its plasticity is influenced by other areas of the brain. In the present study on humans of either sex who were known to respond to theta burst stimulation from previous studies, we found plasticity of M1 could be modulated by suppressing the premotor cortex with the theta burst form of repetitive transcranial magnetic stimulation. Motor plasticity was distorted and disappeared 30 min and 120 min respectively after premotor ~~suppression~~excitability was suppressed. Further evaluation revealed that such changes in motor plasticity were associated with impaired learning of a simple motor task. We postulate that the premotor cortex modulates the amount of plasticity within M1 through heterosynaptic metaplasticity, and that this may impact on learning of a simple motor task previously shown to be directly affected by M1 plasticity. The present results provide an insight into how the brain physiologically coordinates two different areas to bring them into a functional network. Furthermore, such concepts could translated into therapeutic approaches for diseases with aberrant plasticity.

INTRODUCTION

The development of non-invasive technique of brain stimulation has made it possible to explore plasticity-like phenomena in human primary motor cortex (M1) (Ziemann, 2004; Nitsche *et al.*, 2005; Huang *et al.*, 2007; Hamada *et al.*, 2008). In the past few years, these have encompassed metaplasticity, describing how neuronal activities modulate subsequent synaptic plasticity (Siebner *et al.*, 2004; Muller *et al.*, 2007; Hamada *et al.*, 2008; Huang *et al.*, 2008; Hamada *et al.*, 2009; Murakami *et al.*, 2012; Ni *et al.*, 2014), and reversal of plasticity, i.e. depotentiation and de-depression, describing how recently induced plasticity can be abolished by a second period of stimulation (Huang *et al.*, 2010a; Huang *et al.*, 2011b). However, it is still unclear whether M1 plasticity can be modulated by other brain areas.

The brain is a complex network in which one brain area does not work alone. For instance, although M1 controls a large proportion of motor output, neuroimaging studies have revealed that a widespread network of cortical areas is involved in motor learning. Among these areas, the premotor cortex, particularly in the left hemisphere, is activated in many types of motor learning (Kantak *et al.*, 2012; Hardwick *et al.*, 2013).

A PET study showed that the rostral part of the premotor cortex participates in the early stage of visuomotor learning, and the caudal part of the left premotor cortex is activated during later stages (Inoue K et al. 2000). Changes in the premotor cortex were also found to be associated with motor learning in a resting-state study using functional MRI (Vahdat S et al. 2011). Meta-analyses have shown that the premotor area is consistently involved in motor sequence learning and may contribute to learning ~~at a level above~~by regulating motor performance that is mainly controlled by the primary motor cortex (Hardwick RM et al. 2013). The premotor area also contributes to on-line

error corrections (Lee JH and P van Donkelaar 2006) and smooth performance (Sosnik R et al. 2014) during learning.

Interactions between the premotor and primary motor cortices have been demonstrated using non-invasive brain stimulation techniques. A single pulse of transcranial magnetic stimulation (TMS) given to the premotor cortex inhibits motor-evoked potentials (MEPs) evoked by stimulation of contralateral M1 (Mochizuki *et al.*, 2004; Koch *et al.*, 2008). 1Hz and continuous theta burst stimulation (cTBS) forms of repetitive TMS (rTMS) over the premotor cortex reduce corticospinal excitability as well as the excitability of intracortical inhibitory and facilitatory circuits in ipsilateral M1 (Gerschlager *et al.*, 2001; Munchau *et al.*, 2002; Huang *et al.*, 2009a). Stimulation of the premotor cortex with 5Hz rTMS leads to opposite effects (Rizzo et al., 2004). Anodal, but not cathodal, transcranial direct current stimulation over the premotor cortex has been also shown to modify intracortical inhibition and facilitation, but not the MEP size or motor thresholds (Boros *et al.*, 2008).

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Furthermore, previous studies have found that the symptoms of dystonia, which may be due to excessive practice of a skilled movement pattern (Frucht, 2004; Byl, 2007) combined with excessive motor plasticity (Quartarone *et al.*, 2003; Edwards *et al.*, 2006), can be improved by reducing excitability/activity in ~~suppression of~~premotor cortex with ~~repetitive transcranial magnetic stimulation (~~rTMS~~)~~ (Murase *et al.*, 2005; Huang *et al.*, 2010b). Our group demonstrated that multi-session cTBS over premotor cortex ~~suppression~~ reduced excessive plasticity in the motor cortex of patients with genetic or focal hand dystonia (Huang *et al.*, 2012). We suggested that the premotor area may control motor learning by manipulating plasticity within the primary motor cortex. A disorder of this control could therefore underlie certain forms of dystonia.

Hence, the present study sought to test the physiological role of premotor cortex in motor plasticity and learning of healthy volunteers. In one experiment we suppressed its function temporarily with theta burst transcranial magnetic stimulation (TBS) (Huang *et al.*, 2009a) and evaluated the effect on the sensitivity of long-term potentiation (LTP) and long-term depression (LTD)–like phenomena within M1 using standard transcranial stimulation protocols. In a separate session, we measured whether this was accompanied by any change in learning of a simple motor task. The intention was to clarify the underlying mechanism of the effect of premotor suppression on motor plasticity.

MATERIAL AND METHODS

Subjects

Sixteen healthy right-handed volunteers, who were known to respond to TBS from previous studies, were recruited. Twelve (4 men and 8 women; mean age, 33.67±4.2 years) completed the two physiological experiments to assess motor plasticity; eleven (4 men and 7 women; mean age, 31.8±3.1 years) completed the behavioural experiment (Fig 1). Not all the participants participated in all the experiments because of the lengthy experimental protocol, which took approximately 4 months to complete all data collection. All volunteers gave their informed consent. The project protocol was approved by the Institutional Review Board of the Chang Gung Memorial Hospital in Taiwan and was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Stimulation and recordings

Subjects were seated in a comfortable chair. In experiment 1 and 2, EMGs were recorded from the right flexor pollicis brevis muscle (FPB). Signals were sampled at 5kHz (Power 1401; Cambridge Electronic Design, Cambridge, UK), amplified with a gain of 1000 and 5000 and filtered with a band-pass filter (3 Hz to 2k Hz) (Digitimer D360; Digitimer Ltd., Welwyn Garden City, Herts, UK). Single

pulse TMS was given using a 70mm figure-of-eight coil connected to a Magstim 200^2 (Magstim Co., UK), whereas TBS (Huang *et al.*, 2005; Huang *et al.*, 2008; Huang *et al.*, 2009b) was produced by a Magstim Rapid^2 Package through another 70mm figure-of-eight coil. The coil was placed over the left hemisphere tangentially to the scalp with the handle pointing backwards. The "motor hot-spot" was defined as the location where TMS produced the largest MEP from FPB. The active motor threshold (AMT) was defined as the minimum stimulation intensity over the "motor hot-spot" that could elicit an MEP of greater than 200μV in five out of ten trials during voluntary contraction of FPB at about 10% of maximum contraction.

In experiment 3, the right forearm, wrist and hand rested on a plate where they were tightly fixed in a cast with the shoulder adducted and the elbow in approximate 90 degrees flexion. Only the thumb was left free to move in all directions. A four-gram piezo-resistive accelerometer (Kistler Instrument Corp., Amherst, NY; sensitivity 20mV/g) was mounted on the dorsal aspect of the proximal phalanx of the right thumb close to the interphalangeal joint to detect flexion-extension of the metacarpophalangeal joint of the thumb. The signal was amplified with a gain of 100, filtered with 100 Hz cutoff (Kistler Instrument Corp.), sampled at 5kHz (Power 1401; Cambridge Electronic Design, UK) and fed into the computer for online visual display and off-line analysis.

Theta burst stimulation (TBS)

The protocols used for TBS are based on those that we have previously reported (Huang *et al.*, 2005; Fang *et al.*, 2010; Huang *et al.*, 2011a). The patterns of TBS all consist of bursts containing 3 pulses at 50Hz at 80% AMT repeated every 200ms. Three types of TBS were used in this study: 1) iTBS: a 2s train of TBS repeated every 10s for 20 repetitions to M1; 2) cTBS300: a 20s train of uninterrupted TBS to M1 and 3) cTBS600pm: a 40s train of uninterrupted TBS to the dorsal lateral premotor cortex (PMd) (Huang, 2010; Huang *et al.*, 2010b). cTBS600 was selected for ~~a stable~~reducing premotor ~~suppression~~excitability, while cTBS300 and iTBS were used for motor

plasticity assessment because they produce a plasticity effect that has a similar duration to motor practice performed in the experiment 3. PMd was located as being 2.5 cm anterior to the "motor hot-spot" (Samuel *et al.*, 1997; Gerschlager *et al.*, 2001; Cincotta *et al.*, 2004; Huang *et al.*, 2009a). For sham stimulation, the coil was flipped over and the stimulus intensity was reduced to 60% of AMT. This gave subjects an almost identical feeling to real stimulation, even though the stimulus in the brain is greatly reduced (to approximately 46.8% AMT) because the output of the flip side is about 78%of the normal side. (Huang *et al.*, 2012).

Assessment of plasticity in M1

LTP and LTD-like plasticity phenomena in M1 were assessed by quantifying the effect of iTBS and cTBS300 on the amplitude of MEPs evoked by single pulse TMS. Baseline MEPs were measured using 30 pulses delivered every 4.5-5.5 seconds. TBS (cTBS300 or iTBS) was then applied to M1. Following this, MEP size was assessed using single pulses of TMS delivered in trains of 12 pulses given every 4.5-5.5 seconds every 5 minutes until 20 minutes after the end of TBS. The intensity of stimulation for MEP assessment was set to that required to produce an MEP of approximately 1mV in the baseline condition before TBS over M1.

Motor practice (MP)

The learning task was adopted from previous studies (Ziemann *et al.*, 2004; Jung & Ziemann, 2009). Subjects were asked to perform flexion movements in the metacarpo–phalangeal joint of their right thumb as fast as possible when they heard an auditory cue given every 4 sec for 225 times in each block. After each movement, the thumb needed to return to the horizontal resting position marked by a pointer at the beginning of MP. As feedback, the actual acceleration curve (red) and all previous curves (grey) in the same block were displayed on a screen in front of the subject. Subjects were encouraged to perform fastest possible thumb flexion movements to exceed the first peak acceleration in previous trials.

Peak acceleration of thumb flexion movement

Peak acceleration of thumb flexion was used to quantify the motor learning (Muellbacher *et al.*, 2002; Ziemann *et al.*, 2004; Jung & Ziemann, 2009). Performance was assessed by recording 20 externally paced (1000 Hz tone at a rate 0.25 Hz) fastest possible thumb flexion movements without visual feedback (ACC) before cTBS600pm (time point B1 in Fig 1A), before MP (time point B2 in Fig 1A) and every ten min for the following 30 min starting 1 min after the 2 blocks of MP (time point P1-3 in Fig 1A). Peak accelerations during MP were averaged every 15 movements (1 min) for analysis.

Experimental design

This study included 3 experiments assessed in a random order: 1) the effect of cTBS600pm on LTP induced by iTBS, 2) the effect of cTBS600pm on LTD induced by cTBS300, 3) the effect of cTBS600pm on motor learning. Once an experiment was assigned, the experiment will be completed before moving to the next. Subjects and the data analyser (Miss Su-Chuan Lin) were blinded to the intervention.

Experiment 1: The effect of cTBS600pm on LTP induced by iTBS (Fig 1A)

In this experiment, we tested how the LTP-like effect induced by iTBS in M1 was affected by cTBS600 over PMd given 30 or 120 min beforehand. Subjects came for 3 sessions in a random order. In Session 1 & 2, 20 MEPs were recorded with the stimulation intensity adjusted to produce an MEP of approximately 1mV. Following this, either real (Session 1) or sham (Session 2) cTBS600pm was given. We then waited for 20 min for the effect to build up and then evoked 10 more MEPs in order to test whether the excitability of M1 had changed. We then adjusted the TMS intensity to produce an MEP of approximately 1mV in order to assess the LTP-like effect of motor cortex using iTBS as described above in the section of "*assessment of plasticity in M1*". We aimed to

start iTBS about 30 min after the end of cTBS600pm. In Session 3, we gave real cTBS600pm after 20 baseline MEPs were recorded. Subjects had 10 min rest then allowed to move before iTBS assessment. We aimed to start iTBS about 120 min after the end of cTBS600pm. We did not adjust the intensity of M1 TMS to assess M1 excitability in session 3, since previous work had shown there is no change in MEPs tested 2 hours after cTBS600pm (Huang *et al.*, 2009a; Huang *et al.*, 2012). Each session was performed at least 1 week apart.

Experiment 2: The effect of cTBS600pm on LTD induced by cTBS300 (Fig 1B)

In the experiment 2, we tested the effect of cTBS600 over PMd applied 30 or 120 min before cTBS300 to M1. Subjects came for 3 sessions in a random order. The 3 sessions were very similar to those in Experiment 1, except that iTBS was replaced by cTBS300 in each session that was performed at least 1 week apart.

Experiment 3: The effect of cTBS600pm on motor learning (Fig 1C)

In this experiment, 30 min and 120 min after delivery of cTBS600pm, we tested how cTBS600 to PMd influenced motor learning ~~30 min, when the effect of cTBS600 reached a stable state and is less vulnerable to physical activity (Huang *et al.*, 2008) and 120 min, when cTBS600pm was reported to reduce plasticity measured by the effect of M1 TBS (Huang *et al.*, 2012), after delivery of cTBS600pm~~. Subjects came for 3 sessions, which were tested at least 1 month apart to avoid carry-over effects, in a random order. In Session 1 & 2, one block of peak acceleration of thumb flexion movements (ACC B1) was assessed at the baseline condition. Then real (Session 1) or sham (Session 2) cTBS600pm was delivered. Subjects were asked to relax and rest for 10 min before measuring ACC B2. Two blocks of motor practice (MP) separated by 5 min rest began, aiming to have the first block of MP end at 30 min after cTBS600pm. Three blocks of ACC (P1, P2, P3) were assessed every 10 min beginning 1 min after the end of MP. In Session 3, baseline MEPs were assessed by single-pulse TMS delivered every 4.5-5.5 seconds for 20 pulses. Then real cTBS600pm

was given. After cTBS600pm, subjects had 10 min rest followed by recording 20 MEPs. They then allowed to move before ACC B2 that started 1.5 hr later followed by 2 blocks of MP and 3 blocks of ACC as in Session 1 & 2.

Data analysis

Data were analysed using SPSS. A two-way repeated measures ANOVA was used to compare the results of different conditions on the peak-to-peak amplitude of MEPs, ACC and the coefficient variability of ACC in MP. The coefficient of trial-by-trial variability of ACC peak acceleration was calculated to evaluate the possibility of different learning strategies adopted during motor practice (Jung & Ziemann, 2009). Trial-by-trial analysis was performed for all the trials of each of the two blocks of motor practice. The improvement of peak acceleration with practice was modelled by defining an implicit "target". The initial target was set as the peak acceleration of the first trial. Starting with the second trial, peak acceleration in each trial was tested in sequence. If it exceeded the previous target, the target was increased by 50% of the difference between the new maximum peak acceleration and the old target; if not, then the target remained unchanged. The difference between the actual peak acceleration and the current target was calculated for each trial, and the coefficients of variability were calculated as the standard deviation (SD) divided by the mean of these differences in bins of 1 min for each block of motor practice. ~~Following~~ Then, ~~one~~ one-way repeated measures ANOVA was used to examine the time course of changes in individual conditions. Paired t-tests were performed for the comparison between MEPs before and at 10 min, right before motor plasticity assessment at 30 min and at 120min after cTBS600pm. To evaluate the correlation between motor performance and physiological plasticity, we calculated the difference in the amplitude of MEPs between real and sham sessions at each time point after iTBS and averaged them. We then correlated this with the difference in the final peak acceleration in motor practice between real and sham sessions. Pearson's correlation was performed. A $P<0.05$ was

considered statistically significant. TwoStep cluster analysis was used to confirm the respond rate in iTBS and cTBS in sessions with sham premotor stimulation.

RESULTS

The effect of cTBS600pm on LTP measured in M1

M1 excitability, as measured by the MEP, was suppressed 30min after cTBS600pm (i.e. just before application of iTBS) ($t=2.970$, $p=0.013$), but had returned to baseline at 120 min ($t=0.696$, $p=0.501$).

A one-way ANOVA confirmed that the baseline MEPs did not differ between sessions ($F(2,22)=0.678$, $p=0.518$). A two-way ANOVA with the effects of CONDITION (sham, 30 min, 120 min after cTBS600pm) and TIME (before, 0, 5, 10, 15, 20 after) showed a significant CONDITION x TIME interaction ($F(10,110)=3.293$, $p=0.001$) and an effect of CONDITION ($F(2,22)=8.543$, $p=0.002$) (Fig 2). iTBS significantly enhanced the size of MEPs after sham ($F(5,55)= 3.745$, $p=0.005$) and at 30 min after real ($F(5,55)= 3.734$, $p=0.006$) premotor stimulation. In contrast, iTBS did not change the amplitude of MEPs 120 min after real cTBS600pm ($F(5,55)=1.496$, $p=0.206$). A further comparison of the time courses of the iTBS effect on MEPs between sham and real cTBS600pm given 30min beforehand revealed a significant CONDITION x TIME interaction ($F(5,55)=3.36$, $p=0.010$) and an effect of CONDITION ($F(5,40)=9.107$, $p=0.012$). The result suggests that cTBS600pm modified the effect of iTBS given 30 min later, although iTBS was still able to facilitate MEPs. TwoStep cluster analysis showed only one cluster in the results of iTBS in the sham cTBS600pm session, suggesting a 100% response rate in the participants.

The effect of cTBS600pm on LTD measured in M1

A one-way ANOVA confirmed that the baseline MEPs were not different between sessions ($F(2,22)=0.471$, $p=0.630$). A two-way ANOVA with the effects of CONDITION (sham, 30 min, 120 min

after cTBS600pm) and TIME (before, 0, 5, 10, 15, 20 after) showed a significant CONDITION x TIME interaction ($F(10,110)=2.049$, $p=0.035$) (Fig 3). This was because cTBS300 suppressed MEPs when given after sham premotor stimulation ($F(5,55)=6.955$, $p<0.001$), but had no effect if it was given 30 min ($F(5,55)=0.419$, $p=0.834$) or 2hr ($F(5,55)=0.851$, $p=0.376$) after real cTBS600pm. TwoStep cluster analysis showed only one cluster in the results of cTBS in the sham session, suggesting a 100% response rate in the participants.

The effect of cTBS600pm on peak acceleration during motor practice

Compared to baseline, MEPs were significantly suppressed 10 min after cTBS600pm ($t=4.098$, $p=0.003$), suggesting that cTBS600 had successfully suppressed the premotor cortex.

A two-way ANOVA with the effects of CONDITION (sham, 30 min, 120 min after cTBS600pm) and TIME (ACC B1 and B2) revealed no effect of CONDITION ($F(2, 20)=0.588$, $p=0.565$) or TIME ($F(1,10)=0.763$, $p=0.403$) and no interaction between CONDITION and TIME ($F(2,20)=2.176$, $p=0.106$), suggesting that cTBS600pm did not alter baseline peak acceleration prior to learning at 30 and 120 min after practice. There was also no difference in ACC B2 between the three conditions ($F(2, 20)=0.138$, $p=0.872$). A two-way ANOVA with the effects of CONDITION (sham, 30 min, 120 min after cTBS600pm) and TIME revealed a significant interaction of CONDITION and TIME ($F(58,580)=3.922$, $p<0.001$) on the peak acceleration (normalised to ACC B2) during the two 15-min motor practice sessions (Fig 4A). This was because real cTBS600pm given 120 min in advance of practice significantly reduced the increase in peak acceleration as compared with both sham (CONDITION effect: $F(1,10)=13.614$, $p=0.004$; interaction: $F(29, 290)=5.236$, $p<0.001$) and real cTBS600pm given 30 min in advance (CONDITION effect: $F(1,10)=9.839$, $p=0.011$; interaction: $F(29, 290)=6.505$, $p<0.001$). No significant difference was found between sham and real cTBS600pm at 30 min before practice. Further analysis revealed that peak acceleration increased with time in both blocks of motor practice ($F(14,140)=4.172$, $p<0.001$; $F(14,140)=7.193$, $p<0.001$) in the sham

condition and in the second block of MP at 30 min after cTBS600pm ($F(14,140)=7.986$, $p<0.001$), while no increase in peak acceleration was found in the first block at 30 min after cTBS600pm ($F(14,140)=1.471$, $p=0.129$) and in both blocks at 120 min after cTBS600pm ($F(14,140)=1.503$, $p=0.117$; $F(14,140)=1.082$, $p=0.379$). A significant correlation was found between the increase in final peak acceleration during MP and the difference in the amplitude of MEPs after iTBS given 30 min after real and sham cTBS600pm ($r=0.643$, $p=0.033$) and also between MEP amplitudes after sham cTBS600pm and at 120 min after real cTBS600pm ($r=0.739$, $p=0.009$).

We then compared the coefficient of variability of the peak acceleration, which reflects learning strategy, during motor practice between the three conditions using two-way ANOVA (Fig 4B). There was a significant effect of TIME ($F(29, 290)=21.765$, $P<0.001$), but no effect of CONDITION ($F(2,20)=2.474$, $p=0.110$) or CONDITION x TIME interaction ($F(58,580)=1.243$, $p=0.115$), suggesting no change in the learning strategy during motor practice in different sessions

The effect of cTBS600pm on peak acceleration after motor practice

The previous analysis concerned the improvement in task performance during practice; the next analysis asked whether performance has changed after the end of practice at 30 or 120min after premotor conditioning. We compared this "offline" effect of previous motor practice using a two-way ANOVA with main effects of CONDITION (sham, 30 min, 120 min after cTBS600pm) and TIME (ACC B2, P1, P2 and P3). This revealed a CONDITION x TIME interaction ($F(6,60)=4.258$, $p=0.001$) and an effect of TIME ($F(3,30)=11.340$, $p<0.001$) (Fig 5). This was because peak acceleration increased after motor practice and remained increased 30 and 120min later after sham ($F(3,30)=12.622$, $p<0.001$) or real ($F(3,30)=9.348$, $p<0.001$) cTBS600pm given 30min prior to practice. There was no improvement in performance at any time point in the session where practice had started 120 min after cTBS600pm ($F(3,30)=0.228$, $p=0.876$). A further comparison

revealed no difference in CONDITION (F(1,10)=0.010, p=0.823) nor CONDITION x TIMES (3,30)=0.300, p=0.825) interaction between the results at 30 min after real and sham cTBS600pm. We conclude that cTBS600pm given 120min prior to practice did not only prevent performance increments temporarily, for the duration of the motor practice, but also for the time period following the termination of motor practice.

DISCUSSION

TBS given to primary motor cortex can usually induce LTP- or LTD-like after-effects. The novel finding here was that it was no longer possible to recruit the LTP- or LTD-like after-effects of iTBS or cTBS (respectively) when tested 120 min after cTBS600 to the premotor area. A partial reduction of M1 plasticity could be observed 30 minutes after the delivery of cTBS600 to the premotor area. The LTD-like effects of cTBS were absent but the facilitatory effect of iTBS was distorted and the onset of LTP-like plasticity was delayed. It is possible that the effect could be due to spread of stimulus to the primary motor, since PMd is close to the hand area of motor cortex. However, in a previous study, we gave cTBS at an intensity adjusted to mimic the possible physical current spread to M1 and found no effect on MEPs, suggesting that the current spread is unlikely to be an important factor (Huang et al., 2009a).

The behavioural experiments revealed that this physiological effect was accompanied by a parallel effect of cTBS600pm on motor learning. Many previous reports as well as the present study show that people can usually increase the initial acceleration of thumb flexion after practice. This did not occur when participants performed the task 120min after having received cTBS600 to the premotor

cortex. Indeed, the timing was specific since acceleration increased as much as after sham if practice started earlier, 30min after cTBS600pm. However, it should be noted that even in the latter case, improvement took longer to emerge than after sham since the increase in thumb ACC was not significant until the second block of motor practice. The correlation between the changes in ACC and the size of MEPs further confirm the association between the physiological and behaviour results. Trial-by-trial variability of peak acceleration was not altered by premotor cTBS600, suggesting that premotor modulation of motor learning was not due to a change in learning strategy.

The present data show that the time course of the behavioural results is consistent with the physiological effects. Thus, practice no longer produced any increase in thumb acceleration 120 min after the cTBS600pm, at a time when the after-effects of motor cortex cTBS and iTBS were both abolished, while behavioural improvement was delayed but not absent at 30 min, when the plasticity effects were altered but not completely blocked. This suggests that the inability at this time to increase thumb acceleration is more likely to result from changes in plasticity caused by premotor suppression.

There is a good deal of evidence that the premotor cortex plays an important role in certain types of motor learning. It is involved in action selection when learning a cued sensorimotor task (O'Shea *et al.*, 2007; Picton *et al.*, 2007), and, in visuomotor adaptation it may contribute to on-line error correction, motion smoothness and retrieval process, but not trial-to-trial learning itself (Shadmehr & Holcomb, 1997; Lee & van Donkelaar, 2006; Sosnik *et al.*, 2014). Brain stimulation methods have shown that anodal transcranial direct current stimulation over the premotor cortex facilitates observational motor learning and improves sleep consolidation of motor sequence learning (Nitsche *et al.*, 2010; Wade & Hammond, 2015). In contrast, cTBS over PMd disrupts associative motor learning during object lifting (Nowak *et al.*, 2008).

However, there have been no previous studies of its role in the simpler task employed in the present experiments. This is often described as a “skill acquisition” task that involves “model-free” learning, in which there is no obvious rule that the motor system can use to improve task performance. Improvement can only come from randomly exploring task parameters in order to discover the optimal solution for producing the required movement.

Muellbacher et al found that M1 is involved in the protocol used here. They showed that increases in acceleration during practice were reduced or abolished after rTMS over M1, but not when rTMS was applied to occipital or dorsolateral prefrontal cortex (Muellbacher *et al.*, 2002). However, they did not probe premotor cortex. Although we cannot fully exclude the possibility that the effect of learning could be a direct effect of TBS on the premotor contribution to skill learning, the previous demonstration of the strong involvement of M1 of the simple task (Muellbacher *et al.*, 2002) and the identical trend in the changes in learning and motor plasticity suggest that in contrast to prefrontal cortex, premotor cortex can influence learning, perhaps via an effect on the plasticity of circuits in M1.

Premotor cTBS600 reduced both the LTP- and LTD-like effect of TBS on the primary motor cortex. This confirms our previous results showing that cTBS600 given to PMd abolished the after-effects of M1 cTBS in healthy subjects and that consecutive daily sessions for five days, but not a single session, of cTBS600 over PMd reduced excessive motor plasticity in patients with dystonia (Huang *et al.*, 2012). Similar modulation effects on plasticity have been repeatedly found by conditioning the same motor cortex with a preceding stimulation (Siebner *et al.*, 2004; Hamada *et al.*, 2008; Hamada *et al.*, 2009; Murakami *et al.*, 2012; Ni *et al.*, 2014). Such an effect on subsequent plasticity induction is considered to be due to metaplasticity mechanisms (Abraham, 2008). However, to our

knowledge, the inter-cortical modulation of plasticity as shown in the present study has seldom been investigated.

Metaplasticity may also explain the current findings. Metaplasticity is known to occur not only at the synapses activated by the priming stimulus (homosynaptic metaplasticity) but also at those which were not direct activated (heterosynaptic metaplasticity) (Holland & Wagner, 1998; Hulme *et al.*, 2014). Heterosynaptic metaplasticity may contribute to adjust the efficiency of not only adjacent synapses, but also synapses at remote areas throughout a network (Le Ray *et al.*, 2004). For example, priming activity at the basolateral amygdala can modulate subsequent plasticity in the dentate(Akirav & Richter-Levin, 2002). We therefore consider that cTBS to the premotor area may modulate or control the amount of motor learning through a form of heterosynaptic metaplasticity exerted on primary motor cortex.

Recent experiments have shown that one session of tDCS can affect plasticity produced by a second application of tDCS in different ways at different time intervals (Monte-Silva *et al.*, 2010; Monte-Silva *et al.*, 2013); a PAS study also found that metaplastic effects could be different at different time intervals between PAS and motor practice (Jung & Ziemann, 2009). We suggest that a similar phenomenon of different effects at different time intervals between conditioning and subsequent plasticity may explain the different results at 30 and 120 min after premotor suppression. Moreover, similar to the delayed effect at 120 min after premotor suppression, both studies found modulation occurs at a time when the effect of tDCS and PAS on M1 excitability had disappeared. However, the mechanisms of these delayed effects remain largely unknown. We can only speculate that after the priming stimulation there is a rapid modulation of channels or receptors and that this is then followed by slower process, e.g. changes in subunit composition of NMDA receptors as occurs in the rat visual cortex 2 hr after light exposure (Ireland et al., 2009), kicks in to generate the delayed metaplasticity.

Alternatively, modulation of motor plasticity could be due to a change in M1 excitability caused by premotor ~~suppression~~modulation. MEPs, which are a measure of ongoing cortical excitability rather than plasticity, were suppressed by cTBS over the premotor area as measured at 10 min and also immediately before the plasticity assessment at 30 min. We consider the change in MEPs after premotor conditioning to indicate successful suppression of the premotor area according to our previous studies (Huang *et al.*, 2009a; Huang *et al.*, 2012). It could therefore be that the effects on learning and plasticity were due to this rather than metaplasticity. However, as shown in the present and previous studies (Huang *et al.*, 2009a; Huang *et al.*, 2012), the effect of premotor cTBS on M1 excitability returns to baseline after 2 hours. In contrast, the effect of premotor cTBS on motor plasticity as well as on task performance is if anything more obvious at 120 min than at 30 min after the conditioning.

Note that the study was performed on individuals who are known to respond to TBS in previous experiments. It remains unclear if the same approach is reproducible in the generally population.

CONCLUSION

Premotor cortex may influence plasticity occurring in the primary motor cortex. In parallel, simple motor learning was modified and reduced by prior suppression of the premotor ~~area~~activity. One potential mechanism to account for the interaction might be heterosynaptic metaplasticity. The present results provide an insight into how the brain physiologically coordinates different areas and functions as a network from the view point of plasticity. Moreover, such concept could be applied therapeutically in diseases with aberrant plasticity.

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1. Conception and design of the experiments
2. Collection, analysis and interpretation of data
3. Drafting the article or revising it critically for important intellectual content

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FIGURE LEDENDS

Fig 1. Experimental design. In experiment 1, the effect of premotor suppression on long-term potentiation (LTP) in M1 at 30 and 120 min after premotor conditioning was assessed in 3 separate sessions, including one sham control session. In experiment 2, the effect of premotor suppression on long-term depression (LTD) in M1 at 30 and 120 min after premotor conditioning with cTBS600 (cTBS600pm) was assessed in 3 separated sessions, including one sham control session. In experiment 3, the effect of premotor suppression on motor learning measured by peak acceleration of thumb flexion (ACC) at 30 and 120 min after premotor conditioning was assessed in 3 separate sessions, including one sham control session. All experiments were tested in a pseudorandom order. Note that in experiment 3, behavioural learning occurred over two 15min practice periods, whereas measurement of physiological plasticity in experiments 1 and 2 took only 20-192 sec. We therefore tested the latter at a time after premotor cTBS that was equivalent to the end of the first period of motor practice.

Fig 2. The effect of cTBS600 over the premotor cortex (cTBS600pm) on long-term potentiation (LTP). cTBS600pm given 30 min ahead distorted LTP induced by iTBS in M1 to have a delayed onset of potentiation, while the effect of iTBS on M1 was reduced or disappeared at 120 min after cTBS600pm. The error bars represent the standard error. (∗ = significant difference)

Fig 3. The effect of cTBS600 over the premotor cortex (cTBS600pm) on long-term depression (LTD). The cTBS-induced LTD in M1 disappeared at either 30 or 120 min after cTBS600pm preconditioning. The error bars represent the standard error. (∗ = significant difference)

Fig 4. The effect of cTBS600 over the premotor cortex (cTBS600pm) on peak acceleration (ACC) during motor practice. At 30 min after premotor suppression, the increase in peak acceleration that

was seen in both motor practice blocks in the sham control session was only seen in the second block of motor practice. At 120 min after premotor suppression, no increase in peak acceleration was observed in both practice blocks (A). The coefficient of variability of the peak acceleration was similar in all three sessions, indicating the same learning strategy during motor practice in all sessions (B). The error bars represent the standard error. (∗ = significant difference)

Fig 5. The effect of cTBS600 over the premotor cortex (cTBS600pm) on the learning effect of motor practice. Peak acceleration (ACC) increased after motor practice in the sessions that real and sham cTBS600pm was given 30 min beforehand. In contrast, at 120 min after premotor conditioning by cTBS600pm, motor practice did not improve peak acceleration. The error bars represent the standard error. (∗ = significant difference)