Phasic Voluntary Movements Reverse the Aftereffects of Subsequent Theta-Burst Stimulation in Humans

Ennio Iezzi,[1,2] Antonella Conte,[1,2] Antonio Suppa,[1,2] Rocco Agostino,[2] Loredana Dinapoli,[2] Alessandra Scontrini,[1,2] and Alfredo Berardelli[1,2]

[1]*Department of Neurological Sciences and* [2]*Neuromed Institute, "Sapienza" University of Rome, Italy*

Iezzi E, Conte A, Suppa A, Agostino R, Dinapoli L, Scontrini A, Berardelli A. Phasic voluntary movements reverse the aftereffects of subsequent theta-burst stimulation in humans. *J Neurophysiol* 100: 2070–2076, 2008. First published August 27, 2008; doi:10.1152/jn.90521.2008. Theta-burst stimulation (TBS) is a technique that elicits long-lasting changes in the excitability of human primary motor cortex (M1). Tonic contraction of the target muscle modifies the aftereffects of TBS, whereas interactions between phasic muscle contraction and the aftereffects of TBS are unknown. In this paper, we investigated whether phasic voluntary movements influence TBS-induced changes in M1 excitability. We examined whether a brief sequence of phasic finger movements performed by healthy humans before both intermittent TBS (iTBS) and continuous TBS (cTBS) influences TBS-induced aftereffects. Ten healthy subjects underwent iTBS and cTBS. To evaluate the TBS-induced aftereffects on M1 excitability, single TMS pulses were given over the FDI motor area before (T0) and 5 (T1), 15 (T2), and 30 min (T3) after TBS. To find out whether finger movements influenced the TBS-induced aftereffects, we tested motor-evoked potentials (MEPs) size by single TMS pulses at T0, immediately after movements, and at T1–T3. We also measured the kinematic variables mean amplitude and mean peak velocity of the movements. When no phasic voluntary movements preceded TBS, iTBS elicited facilitatory and cTBS elicited inhibitory aftereffects on MEP size. Conversely, movements performed before TBS elicited significant changes in the direction of the TBS-induced aftereffects. iTBS produced inhibitory instead of facilitatory aftereffects and cTBS produced facilitatory instead of inhibitory aftereffects. Finger movements alone had no effects on MEPs size tested with single-pulse TMS. Peripheral electrical stimulation had no effect on iTBS-induced aftereffects. Repeated phasic finger movements interfere with TBS-induced aftereffects probably by modulating mechanisms of brain metaplasticity.

INTRODUCTION

Theta-burst magnetic stimulation (TBS) is a repetitive transcranial magnetic stimulation (rTMS) technique that elicits long-lasting changes in the excitability of human primary motor cortex (M1) (Huang et al. 2005). The aftereffects of TBS depend on how TBS is delivered: continuous (cTBS) produces inhibitory, whereas intermittent TBS (iTBS) leads to excitatory aftereffects. Convincing evidence shows that the TBS-induced aftereffects originate from the cortex. For example, TBS requires low, subthreshold intensities [80% of the active motor threshold (AMT)], elicits no descending activity in the corticospinal tract and leaves H-reflexes unaffected (Huang et al. 2005). Experiments recording the cortico-spinal volleys

evoked by single-pulse TMS in conscious humans after conditioning cTBS suggested that cTBS acts on neural circuits at the level of M1 (Di Lazzaro et al. 2005). The mechanisms underlying TBS-induced aftereffects on cortical excitability resemble the synaptic plasticity phenomena described after electrical stimulation of M1 in animal brain slice preparations, namely long-term potentiation (LTP) and long-term depression (LTD) (Castro-Alamancos et al. 1995; Hess and Donoghue 1996). In human M1, iTBS may therefore facilitate motor cortical responses through LTP-like plasticity, whereas cTBS could depress them through LTD-like plasticity (Huang et al. 2005).

Animal experiments suggest that motor training engages a number of neurophysiological mechanisms including LTP phenomenon in trained motor cortex (Monfils and Teskey 2004; Rioult-Pedotti et al. 2000). In humans, the effects produced by rapid movements and motor practice on LTP/LTD-like plasticity induction have been investigated with TMS techniques using paired-associative stimulation (PAS) (Stefan et al. 2000; Wolters et al. 2003). Studies using the PAS technique have shown that motor learning influences LTP/LTD aftereffects (Rosenkranz et al. 2007; Stefan et al. 2006; Ziemann et al. 2004).

Using the TBS technique, Huang et al. (2008) reported that if the target muscle is tonically contracted, while TBS is applied, the facilitatory/inhibitory effects elicited by TBS on M1 excitability are abolished. Conversely, if the muscle is voluntarily contracted immediately after TBS, the excitatory aftereffects evoked by iTBS increase and the inhibitory aftereffects of cTBS turn into a facilitation. More recently Gentner et al. (2007) extended these observations and reported that a prior tonic voluntary muscle contraction determined a change in the direction of the aftereffects induced by subsequent cTBS. Whereas ample information is available on changes in the TBS aftereffects induced by a tonic contraction, none is available on the interactions between TBS and phasic voluntary movements. Knowing more about these interactions is a prerequisite for studies investigating new ways to modulate cortical plasticity.

In this study, we investigated whether and if so how repeated phasic voluntary movements preceding TBS influence the aftereffects of TBS on human motor cortical excitability. To do so, in healthy subjects, we delivered single TMS pulses before, immediately after index finger movements, and at three time-points after TBS. We determined M1 excitability from TBS-

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induced changes in motor-evoked potentials (MEPs) size. To evaluate motor task performance, we also measured the kinematic variables mean amplitude and mean peak velocity of the finger movements.

METHODS

Subjects

Ten right-handed healthy subjects (6 men, 4 women; mean age: 35 ± 3 yr) participated in the study. Informed consent was signed by all participants, and the experiments were approved by the local ethical committee.

TMS

Subjects were comfortably seated in an armchair. Single TMS pulses and TBS were delivered with a Super Rapid Magstim 200 magnetic stimulator (Magstim, Whitland, Dyfed, UK), delivering biphasic waveform pulses. The stimulator was connected to a figure-eight coil (external wing 9 cm in diameter) placed tangentially over the scalp with the handle pointing back and away from the midline at ~45° in the optimal position for eliciting MEPs in the contralateral target muscles [1st dorsal interosseous (FDI) or abductor pollicis brevis (APB)]. Motor thresholds were calculated at rest (RMT), as the lowest stimulus intensity able to evoke an MEP of ≥50 µV in 5 of 10 consecutive trials and during a slight voluntary contraction (AMT) of the target muscle (20–30% of the maximum voluntary contraction), as the lowest intensity able to evoke an MEP of ≥200 µV in 5 of 10 consecutive trials. Intensity of single-pulse TMS was set to obtain a mean MEP size of ~1 mV at the baseline.

TBS was applied according to a previously described technique (Huang et al. 2005). The basic TBS paradigm consisted of bursts containing three pulses at 50-Hz frequency repeated every 200 ms (i.e., at 5 Hz). For iTBS, the basic paradigm was given in short trains lasting 2 s (i.e., 10 bursts) repeated every 10 s for a total number of 600 pulses (i.e., 20 trains), whereas for cTBS, it was given in a continuous train lasting 20 s for a total number of 300 pulses. We chose iTBS with 600 pulses and cTBS with 300 pulses because their aftereffects have similar duration (Huang et al. 2005). iTBS and cTBS were delivered at 80% of AMT intensity.

Peripheral nerve electrical stimulation

Electrical mixed nerve stimulation was delivered with a Digitimer DS7 device (Digitimer, Welwyn Garden City, Hertfordshire, UK) through a bipolar electrode (cathode distal). Constant square-waves pulses (duration, 200 µs) were delivered to the median nerve at the wrist.

Electromyographic recording and MEP measurement

The electromyographic (EMG) activity was recorded through a pair of Ag/AgCl electrodes placed over the FDI or APB muscles in a belly-tendon fashion. Raw EMG signal was amplified and filtered (bandwidth: 20 Hz to1 kHz) with a Digitimer D360 amplifier (Digitimer), and sampled at 5 kHz (CED 1401 A/D laboratory interface, Cambridge Electronic Design, Cambridge, UK). Data were stored on a laboratory computer for on-line visual display and further off-line analysis (Signal software, Cambridge Electronic Design). Throughout the experimental sessions EMG activity of the target muscle was continuously monitored with audio and high-gain visual feedback to ensure complete relaxation of the target muscles.

Baseline MEP size was measured peak-to-peak. MEP size after movements and after TBS was expressed as a percentage of the baseline MEP. Trials in which background EMG activity was present were discarded on-line.

Motor task and movement recording

Subjects were comfortably seated beside a table. For the index finger motor task, we used a previously described protocol (Agostino et al. 2007). Subjects abducted the arm at the shoulder by ~45–50°, and the forearm and the palm of the hand at the level of the metacarpo-phalangeal joints were firmly placed on the table. The elbow was flexed at ~90°, the wrist was kept in a neutral position, the thumb was abducted and the other fingers were adducted, flexed at the metacarpo-phalangeal joints by ~70–80° and extended at the interphalangeal joints. After a verbal "ready" signal, subjects were asked to extend their index finger until they reached the neutral position at the metacarpo-phalangeal joints, after a "go" signal, they abducted the index finger and soon after a "stop" signal they returned to the starting position. For the thumb abduction motor task, subjects had their arm adducted at the shoulder with the elbow flexed at 90°. The pronated forearm and the palm of the hand at the level of the metacarpo-phalangeal joints were kept firmly on the table. After three to four practice movements, 30 index finger or thumb abductions were tested in blocks of five movements each. An interval of ~3–5 s elapsed between movements and each block was separated by a 5-s rest. Subjects were instructed to perform movements "as widely and as fast as possible" after the verbal command and continuously encouraged throughout the motor task.

Index finger movements in the three-dimensional space were recorded with the SMART analyzer motion system (BTS Engineering, Milan, Italy) which comprises three infrared cameras (sampling rate, 120 Hz) able to follow the displacement of a passive marker taped on the distal phalanx of the right index finger. A dedicated software reconstructed off-line the displacement of the passive marker and automatically determined the kinematic features of each movement.

Experimental paradigm

EFFECTS OF INDEX FINGER MOVEMENTS ON THE ITBS-INDUCED AFTEREFFECTS IN THE FDI MOTOR AREA. This experiment consisted of two sessions (Fig. 1), taking place ≥10 days apart. In the first session (10 subjects), we tested the effects of iTBS on motor cortex excitability with single TMS pulses given at rest over the FDI motor cortical hot spot before (baseline, T0) and 5 (T1), 15 (T2), and 30 min (T3) after iTBS. In the second session, in the same 10 subjects, we investigated the effects of index finger movements on the iTBS-induced aftereffects. Single TMS pulses were delivered before (T0), immediately after index finger movements and 5 (T1), 15 (T2), and 30 min (T3) after iTBS. In 6 of the 10 subjects, we also evaluated motor performance of the task using a kinematic analysis to measure the mean amplitude and mean peak velocity of the five movements in each block. At each time point, we evaluated RMT and the average size of 15 MEPs.

EFFECTS OF INDEX FINGER MOVEMENTS ON THE CTBS-INDUCED AFTEREFFECTS IN THE FDI MOTOR AREA. This experiment consisted of two sessions (Fig. 1), taking place ≥10 days apart. In the first session (10 subjects), we studied the effects of cTBS with single TMS pulses given at rest over the FDI "hot spot" before (baseline, T0) and 5 (T1), 15 (T2) and 30 min (T3) after cTBS. In the second session, in the same 10 subjects, we evaluated the effects of index finger movements on the aftereffects induced by cTBS. In 6 of the 10 subjects, we also analyzed the mean amplitude and the mean peak velocity of five movements in each block. For the second session of both experiments, TBS was delivered 2 min after subjects did the motor task. At each time point, we evaluated RMT and the average size of 15 MEPs.

Control experiments

EFFECTS OF INDEX FINGER MOVEMENTS ON FDI MOTOR AREA EXCITABILITY TESTED WITH SINGLE-PULSE TMS. To test whether the motor task we used elicited changes in cortical excitability, in five
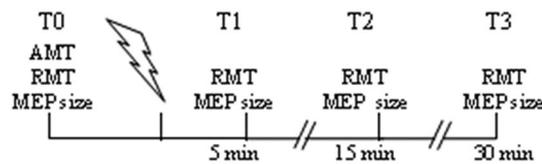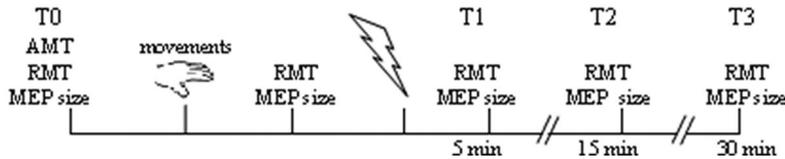
FIG. 1. Experimental paradigm.

subjects we delivered single TMS pulses before (T0) and 5 (T1), 15 (T2), and 30 (T3) minutes after they did the motor task. At each time point, we evaluated RMT and the average size of 15 MEPs.

EFFECTS OF THE MUSCLE TWITCH PRODUCED BY PERIPHERAL NERVE STIMULATION ON THE iTBS. In five subjects, we investigated the possible contribution of peripheral feed-back produced by the muscle twitch on iTBS-induced changes in cortical excitability. Because the FDI muscle twitch elicited by ulnar nerve stimulation at the wrist and by voluntary contraction could not be compared, for this experiment, we recorded the muscle activity from the APB. In this muscle, median nerve stimulation and voluntary contraction induced a similar muscle twitch. The experiment consisted of three sessions, held ≥10 days apart. In the first session, single TMS pulses were delivered at rest over the APB motor cortical hot spot before (T0) and 5 (T1), 15 (T2), and 30 min (T3) after iTBS. In the second session, single TMS pulses were delivered at rest before (T0), immediately after voluntary thumb movements, and at T1, T2, and T3. iTBS was applied ~2 min after subjects did the motor task. In the third session, single TMS pulses were delivered at rest before (T0), immediately after peripheral nerve stimulation, and at T1, T2, and T3. A total of 30 electric pulses were given to the median nerve at the wrist in six blocks at intervals similar to those elapsing between single movements and blocks during the voluntary motor task. The intensity of electrical stimulation was set to obtain a muscle twitch mimicking the APB voluntary motor task. iTBS was delivered ~2 min after peripheral nerve stimulation. In all sessions, at each time point we evaluated RMT and the average size of 15 MEPs. For all the experimental sessions, 20 test TMS pulses were delivered at each time point with an interstimulus interval of ~5 s.

Statistical analysis

A two-way repeated-measures ANOVA with main factors intervention (iTBS/cTBS alone and iTBS/cTBS preceded by voluntary movements) and time (T0, T1, T2, T3) was used to analyze MEP size and RMT after TBS alone and after TBS preceded by the voluntary motor task.

One-way ANOVA with factor time as main factor was used to test MEPs at the baseline and immediately after movements.

For kinematic analysis, the mean amplitude and the mean peak velocity of five index finger movements executed in six blocks were analyzed with a repeated-measures ANOVA with blocks of movements as main factor.

A two-way repeated ANOVA with main factors muscle twitch (APB voluntary movement and APB movements elicited by electrical stimulation) and time (T0, T1, T2, T3) was used to analyze TBS-induced aftereffects when the APB voluntary motor task and median nerve electrical stimulation preceded iTBS.

Tukey's honest significance test was used for post hoc analysis. Values are expressed as mean ± SE. *P* values ≤0.05 were considered to indicate statistical significance.

RESULTS

Effects of index finger movements on the iTBS-induced aftereffects in the FDI motor area

ANOVA for MEP size after iTBS showed a significant effect of factor intervention [$F(1,9) = 40.99$; $P = 0.0001$] and a significant interaction of factor time and intervention [$F(3,27) = 22.00$; $P = 0.00001$]. Post hoc analysis showed that the MEP increased in size after iTBS and that the increase was significant at all intervals tested, with the most significant effect at T2 (T1: $P = 0.008$; T2: $P = 0.00001$ and T3: $P = 0.004$). When finger movements preceded iTBS, the ensuing MEP decreased significantly in size at all intervals tested. The most significant decrease was at T2 (T1: $P = 0.01$; T2: $P = 0.0001$ and T3: $P = 0.05$; Table 1 and Fig. 2).

The baseline MEP size did not significantly differ between the experimental paradigm in which iTBS was delivered alone or after finger movements [$F(1,9) = 0.26$; $P = 0.6$]. Nor did the baseline RMT and MEP size differ significantly from those measured immediately after finger movements ($P = 0.22$ and $P = 0.34$, respectively). ANOVA showed that RMT remained unchanged throughout the experimental session at all the time intervals tested [factor time: $F(3,27) = 0.16$; $P = 0.9$; interaction between factor intervention and factor time: $F(3,27) = 1.54$; $P = 0.22$].

ANOVA for kinematic variables showed a significant effect for factor block [mean amplitude: $F(5,25) = 4.08$; $P = 0.008$; mean peak velocity: $F(5,25) = 6.67$; $P = 0.0004$]. Post hoc analysis showed that movement amplitude significantly increased at the fifth and sixth blocks ($P = 0.03$ and $P = 0.004$, respectively). Post hoc analysis also showed that the increase in peak velocity was significant from the third block of movements with the most significant increase at the sixth block (block III vs. I: $P = 0.01$; block IV vs. I: $P = 0.01$; block V vs. I: $P = 0.0008$; block VI vs. I: $P = 0.0007$; Fig. 3).

Effects of index finger movements on the cTBS-induced aftereffects in the FDI motor area

ANOVA for MEP size after cTBS showed a significant effect of factor intervention [$F(1,9) = 33.1$; $P = 0.0001$] and

TABLE 1. *Analytic results for resting motor threshold (RMT) and motor-evoked potential (MEP) size at each time point for each experimental session*

	T0	Immediately After Movements	T1	T2	T3
Main experiment					
Experiment 1					
Session 1	0.8 ± 0.13	—	1 ± 0.21	1.1 ± 0.19	1 ± 0.12
	51.1 ± 9.65	—	51.1 ± 9.89	51.3 ± 10.04	51.2 ± 9.67
Session 2	0.9 ± 0.10	0.9 ± 0.13	0.7 ± 0.19	0.5 ± 0.17	0.7 ± 0.23
	50.5 ± 7.02	50.9 ± 6.98	50.6 ± 7.18	50.5 ± 7.19	50.6 ± 6.91
Experiment 2					
Session 1	0.95 ± 0.11	—	0.83 ± 0.14	0.6 ± 0.12	0.74 ± 0.12
	50.4 ± 9.25	—	50.5 ± 9.13	50.8 ± 8.91	50.7 ± 8.76
Session 2	0.94 ± 0.10	0.96 ± 0.11	1.13 ± 0.25	1.29 ± 0.22	1.3 ± 0.25
	49.7 ± 6.25	49.8 ± 6.51	49.9 ± 6.52	50 ± 6.84	50 ± 6.04
Control experiments					
Experiment 1	1 ± 0.18	—	0.9 ± 0.19	0.9 ± 0.12	1 ± 0.11
	52.8 ± 10.47	—	52.8 ± 10.42	52.8 ± 10.52	52.6 ± 10.18
Experiment 2					
Session 1	0.86 ± 0.20	—	0.96 ± 0.19	1.24 ± 0.20	1.26 ± 0.24
	52.2 ± 13.68	—	52.2 ± 13.91	52.2 ± 13.90	52.2 ± 14.00
Session 2	0.82 ± 0.25	—	0.62 ± 0.23	0.48 ± 0.17	0.62 ± 0.25
	52.4 ± 13.44	—	52.2 ± 13.73	52.6 ± 13.16	52.8 ± 13.49
Session 3	0.86 ± 0.20	0.96 ± 0.16	0.96 ± 0.19	1.3 ± 0.14	1.1 ± 0.08
	53.4 ± 12.09	53 ± 11.42	53.6 ± 12.36	53.8 ± 12.63	53.6 ± 12.97

Values are means ± SD. MEP (mV) is listed first with RMT (%) values underneath.

a significant interaction of factor time and intervention [$F(3,27) = 23.2$; $P = 0.0001$]. Post hoc analysis showed that the MEP decreased in size after cTBS and that the decrease was significant at all intervals, with the most significant effect at T2 (T1: $P = 0.005$; T2: $P = 0.0001$ and T3: $P = 0.001$). When finger movements preceded cTBS, the ensuing MEP increased in size and the MEP increase was significant at all intervals with the most significant effect at T2 (T1: $P = 0.02$; T2: $P = 0.001$ and T3: $P = 0.002$; Table 1 and Fig. 4).

The baseline MEP size did not significantly differ between the experimental paradigm in which cTBS was given alone or after finger movements ($P = 0.83$). Nor did the baseline RMT and MEP size differ significantly from those obtained soon after finger movements ($P = 0.67$ and $P = 0.16$, respectively). ANOVA showed that RMT remained unchanged throughout the experimental session at all time points tested [factor time: $F(3,27) = 2.25$; $P = 0.1$; interaction between factor intervention and factor time: $F(3,27) = 0.12$; $P = 0.94$].
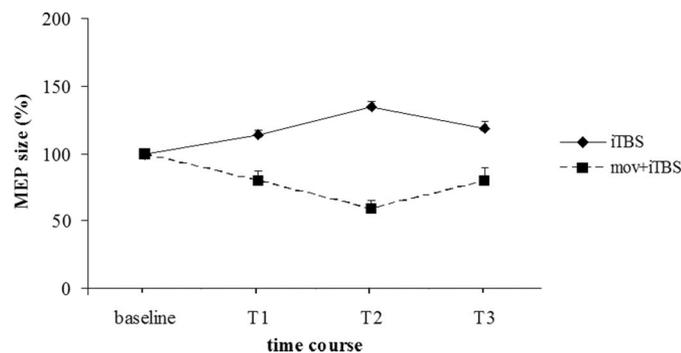

FIG. 2. Effects of index finger movements on the intermittent theta-burst stimulation (iTBS)-induced aftereffects in the 1st dorsal interosseous muscle (FDI) motor area. *x* axis: time course (baseline, T1 = 5 min, T2 = 15 min, T3 = 30 min). *y* axis: motor-evoked potential (MEP) size expressed in percentage of the MEP at the baseline. ◆, MEPs after iTBS; ■, MEPs after iTBS preceded by voluntary movements. Bars represent SE.

ANOVA for kinematic variables showed a significant effect for factor block [mean amplitude: $F(5,25) = 7.60$; $P = 0.0001$; mean peak velocity: $F(5,25) = 6.20$; $P = 0.0007$]. Post hoc analysis showed that the increase in movement amplitude was significant from the fourth block (IV vs. I: $P = 0.04$; V vs. I: $P = 0.03$; VI vs. I: $P = 0.02$). Post hoc analysis also showed that the increase in peak velocity was significant from the third block of movements (III vs. I: $P = 0.02$; IV vs. I: $P = 0.001$; V vs. I: $P = 0.005$; VI vs. I: $P = 0.002$; Fig. 3).

Control experiments

EFFECTS OF INDEX FINGER MOVEMENTS ON FDI MOTOR AREA EXCITABILITY TESTED WITH SINGLE-PULSE TMS. Repeating index finger movements had no significant influence on either the MEP size [$F(3,12) = 1.28$; $P = 0.32$] or the RMT [$F(3,12) = 0.19$; $P = 0.89$; Table 1].

EFFECTS OF MUSCLE TWITCH PRODUCED BY PERIPHERAL NERVE STIMULATION ON THE AFTEREFFECTS INDUCED BY iTBS. ANOVA for MEP size after iTBS on APB cortical motor area showed a significant effect of factor intervention [$F(1,4) = 43.17$; $P = 0.002$] and a significant interaction of factor time and intervention [$F(3,12) = 15.58$; $P = 0.0002$]. Post hoc analysis showed that the MEP increased in size after iTBS and that the increase was significant at all intervals tested (T1: $P = 0.03$; T2: $P = 0.01$ and T3: $P = 0.008$).

When finger movements preceded iTBS, the ensuing MEP decreased in size and the MEP decrease was significant at all intervals tested (T1: $P = 0.04$; T2: $P = 0.048$ and T3: $P = 0.047$). ANOVA for MEP size performed to compare the effect of voluntary muscle twitch versus the electrically induced muscle twitch showed a significant interaction of factor muscle twitch and factor time [$F(6,24) = 14.75$; $P = 0.0001$]. Post hoc analysis showed that median nerve electrical stimulation, mimicking the APB voluntary motor task, left the excitatory aftereffects induced by iTBS unchanged [$F(1,4) = 0.37$; $P = 0.57$;
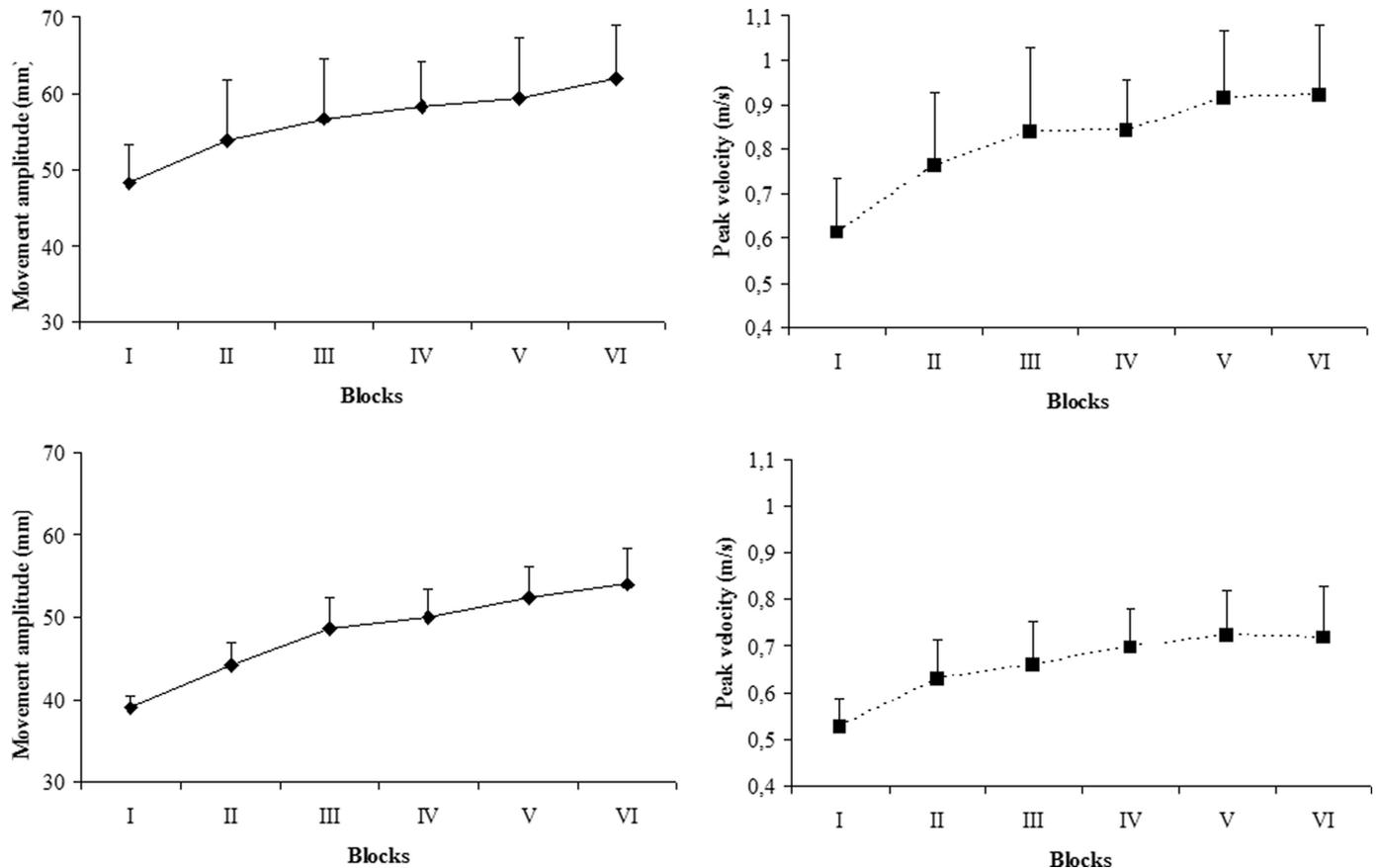
FIG. 3. Mean movement amplitude and peak of velocity of the motor task during the 6 blocks of 5 movements each. *Top*: movement amplitude (*left*) and peak of velocity (*right*) before iTBS protocol. *Bottom*: movement amplitude (*left*) and peak of velocity (*right*) before continuous theta-burst stimulation (cTBS) protocol. x axis: number of blocks; y axis: values expressed in mm for the movement amplitude and m/s for the peak of velocity. Bars represent SE.

Fig. 5]. RMT remained unchanged at all time points tested in the three sessions [iTBS alone: $F(3,12) = 0$; $P = 1.00$; iTBS preceded by voluntary movements: $F(3,12) = 1.6$; $P = 0.24$; iTBS preceded by peripheral electrical stimulation: $F(3,12) = 0.51$; $P = 0.67$]. When iTBS was preceded by electrically induced muscle twitches, RMT and MEP size at baseline did not significantly differ from those measured immediately after electrical peripheral nerve stimulation ($P = 0.29$ and $P = 0.37$, respectively; Table 1).

DISCUSSION

In line with previous published data we found that when no phasic voluntary movements preceded TBS, iTBS produced facilitatory aftereffects and cTBS produced inhibitory aftereffects on MEP size (Huang et al. 2005). The new finding is that in healthy subjects, when phasic voluntary movements preceded TBS, the TBS-induced aftereffects changed direction: iTBS inhibited instead of facilitating and vice versa cTBS
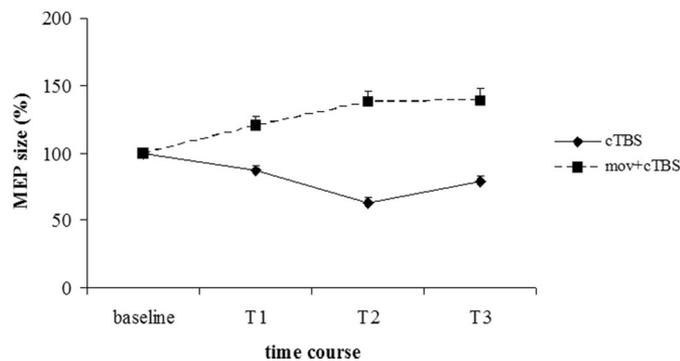
FIG. 4. Effects of index finger movements on the cTBS-induced aftereffects in the 1st dorsal interosseous muscle (FDI) motor area. x axis: time course (baseline, T1 = 5 min, T2 = 15 min, T3 = 30 min). y axis: MEP size expressed in percentage of the MEP at the baseline. ◆, MEPs after cTBS. ■, MEPs after cTBS preceded by voluntary movements. Bars represent SE.
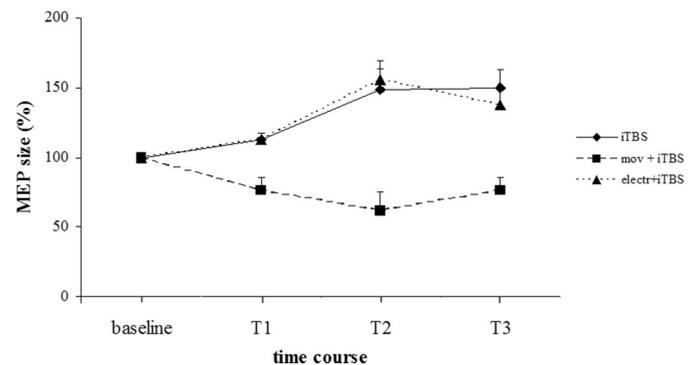
FIG. 5. Effects of thumb movements on the iTBS-induced aftereffects in the abductor pollicis brevis muscle (APB) motor area. x axis: time course (baseline, T1 = 5 min, T2 = 15 min, T3 = 30 min). y axis: MEP size expressed in percentage of the MEP at the baseline. ◆, MEPs after iTBS; ■, MEPs after iTBS preceded by voluntary movements; ▲, MEPs after iTBS preceded by electrical median nerve stimulation mimicking the APB voluntary movements. Bars represent SE.

facilitated instead of inhibiting motor cortex excitability. The motor task alone had no influence on motor cortical excitability as measured by MEP size and when median nerve stimulation preceded iTBS, the direction of the iTBS-induced aftereffects remained unchanged.

The finding that MEPs remained unchanged after the motor task apparently contrasts with the data published by Muellbacher et al. (2001), who showed that practice-related changes in motor performance are associated with an increased MEP size in the muscle involved in training. This difference is probably due to the different duration of the training procedure used in the experimental paradigms: whereas our subjects practiced the task for only 4 min, the subjects studied by Muellbacher et al. (2001) practiced the task twice for 30 min. Moreover, Classen et al. (1998) showed that M1 plasticity induced by practicing a thumb movement depends on training duration and that all the subjects studied showed consistent M1 plasticity after only 30 min training.

In evaluating the possible changes induced by motor performance on the TBS-induced aftereffects, we excluded confounding factors. To avoid muscle contraction that might have altered the expected MEP size changes induced by TBS, we asked the subjects to relax completely, and we continuously monitored the EMG activity in the target muscles with visual and auditory feedback. We also excluded possible overlapping effects of the different TBS sessions and the ensuing motor learning induced by repeating the motor task because ≥10 days elapsed between the various experimental sessions. Moreover subjects entered the experimental protocol after being randomly assigned to cTBS or iTBS as the first interventional procedure. In the control experiments aimed to evaluate the possible changes in motor cortical excitability induced by repeated finger movements, the finding that finger movement left RMT and MEPs size unchanged suggests that the motor task we used has no influence per se on motor cortex excitability. The longer interval elapsing between AMT measurements and TBS in session 2 than in session 1 is unlikely to explain the TBS-induced aftereffects in session 2. In the control experiments, when we evaluated the contribution of peripheral feed-back on iTBS-induced changes in cortical excitability, we observed no changes in the iTBS-induced aftereffects. Because control experiments showed that the interval elapsing between AMT measurement and TBS was similar to that in session 2, the longer interval is unlikely to be responsible for reversing the aftereffects of both iTBS and cTBS.

Our experiments showing that peripheral electrical stimulation—mimicking the voluntary motor task—had no effect on MEPs, excluded the possibility that movement-related muscle twitches could be responsible for the changes in the TBS-induced aftereffects. Hence we conjecture that an interaction took place between the voluntary intent in producing the motor task and the TBS-induced aftereffects. This hypothesis is supported by the finding that voluntary training is more effective than passive training in improving motor performance and eliciting phenomena of cortical plasticity (Kaelin-Lang et al. 2005; Lotze et al. 2003). Accordingly, Huang et al. (2008) found that a tonic voluntary contraction performed immediately after cTBS turned the aftereffects produced by cTBS from inhibition to facilitation.

The explanation we favor hinges on the principles of metaplasticity, defined as the plasticity of synaptic plasticity (Abra-

ham and Bear 1996). In vitro studies show that the history of synaptic activity is a variable that influences the subsequent synaptic state and the degree of LTP/LTD produced by a given experimental protocol (Abraham and Bear 1996). Metaplasticity plays an important role in keeping synaptic strengths within a dynamic range optimal for the learning process (Abraham and Bear 1996). Synaptic plasticity needs to be constrained by homeostatic regulatory mechanisms to maintain overall excitability at stable levels (Abbott and Nelson 2000; Abraham and Tate 1997; Davis 2006; Turrigiano and Nelson 2004). Homeostatic synaptic plasticity relies on the Bienenstock-Cooper-Munro theory (Bienenstock et al. 1982) proposing that integrated postsynaptic activity modulates the threshold for LTP/LTD induction after a stimulation protocol. Hence low levels of postsynaptic activity increase the probability for LTP induction, whereas high levels increase the probability for LTD induction. Recent observations suggested that similar homeostatic mechanisms contribute to regulate plasticity in the human M1. For example, Siebner et al. (2004) showed that the normally induced inhibitory aftereffects elicited by 1-Hz rTMS (Chen et al. 1997) could be reversed to facilitation when preceded by another inhibitory protocol such as cathodal transcranial DC stimulation (Nitsche et al. 2003). Studies investigating how motor tasks interfere with PAS-induced plasticity showed that fast and dynamic prolonged motor practice occludes LTP and enhances LTD induction in human M1 (Rosenkranz et al. 2007; Stefan et al. 2006; Ziemann et al. 2004), suggesting that LTP-like mechanisms probably participate in motor learning. When we analyzed the kinematic features during the fast repetitive finger motor task, we found that during the motor performance, movement amplitude and velocity progressively improved. If the motor task we studied elicited LTP-like mechanisms, as suggested by the practice-related changes in motor performance, the normal homeostatic regulatory processes should have reduced iTBS-like plasticity and facilitated cTBS LTD-like plasticity. Yet they apparently did not because our motor task inverted TBS-induced LTP into TBS-induced LTD and vice versa. Hence we suggest that our motor task, a brief sequence of repeated phasic voluntary movements, engages nonhomeostatic metaplastic mechanisms. We propose that phasic voluntary movements preceding TBS probably reversed the direction of TBS-induced aftereffects through mechanisms of polarity-reversing metaplasticity. The term "polarity-reversing metaplasticity" has been recently used by Gentner et al. (2007) in their experiments examining the influence of previous voluntary muscle contraction on the effects induced by cTBS. They found that without a prior tonic muscle activation, cTBS facilitated MEP size. Conversely, when a voluntary target muscle contraction preceded cTBS, the MEP facilitation turned into inhibition. The investigators suggested that the rapid change in excitability from facilitation to depression produced by prior neuronal activation reflects mechanisms of polarity-reversing metaplasticity (Gentner et al. 2007). The reversed polarity of cortical excitability due to polarity-reversing metaplasticity may arise through several physiological mechanisms including activity-dependent changes in Ca^{2+} signaling that are able to produce rapid and long-lasting changes (Davis et al. 2006; Gentner et al. 2007). The experiments we describe here differ from those of Gentner et al. (2007) in the type of motor task. Whereas we used a phasic movement, Gentner et al. (2007) used a tonic contraction.

Phasic movements affect the motor cortex differently from tonic contraction. Animal experiments have shown that M1 neurons discharge at higher frequencies during the execution of phasic movements than during voluntary tonic contraction (Evarts 1968, 1969). In humans, studies with functional magnetic resonance imaging have shown a higher blood-oxygen-level-dependent (BOLD) signal in M1 during phasic movements than during tonic contraction (Rodriguez et al. 2004). Even though our motor task differs from that of Gentner et al. (2007) phasic and tonic movements are both able to engage mechanisms of polarity-reversing metaplasticity. Finally, in this study, we also provide evidence that polarity-reversing metaplasticity phenomena can affect protocols designed to facilitate as well as those depressing excitability.

In conclusion, our experiments show that repeated phasic voluntary movements preceding TBS influence the aftereffects of iTBS and cTBS and probably do so through mechanisms of polarity-reversing metaplasticity. Finally, our findings also suggest that studies using plasticity protocols for investigating physiological mechanisms or pathological conditions should take into account ongoing activity in the motor cortex.

REFERENCES

Abbott LF, Nelson SB. Synaptic plasticity: taming the beast. *Nat Neurosci* 3: 1178–1183, 2000.

Abraham WC, Bear MF. Metaplasticity: the plasticity of synaptic plasticity. *Trends Neurosci* 19: 126–130, 1996.

Abraham WC, Tate WP. Metaplasticity: a new vista across the field of synaptic plasticity. *Prog Neurobiol* 52: 303–323, 1997.

Agostino R, Iezzi E, Dinapoli L, Gilio F, Conte A, Mari F, Berardelli A. Effects of 5 Hz subthreshold magnetic stimulation of primary motor cortex on fast finger movements in normal subjects. *Exp Brain Res* 180: 105–111, 2007.

Bienenstock EL, Cooper LN, Munro PW. Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. *J Neurosci* 2: 32–48, 1982.

Castro-Alamancos MA, Donoghue JP, Connors BW. Different forms of synaptic plasticity in somatosensory and motor areas of the neocortex. *J Neurosci* 15: 5324–5333, 1995.

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* 48: 1398–1403, 1997.

Classen J, Liepert J, Wise SP, Hallett M, Cohen LG. Rapid plasticity of human cortical movement representation induced by practice. *J Neurophysiol* 79: 1117–1123, 1998.

Davis GW. Homeostatic control of neural activity: from phenomenology to molecular design. *Annu Rev Neurosci* 29: 307–323, 2006.

Di Lazzaro V, Pilato F, Saturno E, Oliviero A, Dileone M, Mazzone P, Insola A, Tonali PA, Ranieri F, Huang YZ, Rothwell JC. Theta-burst repetitive transcranial magnetic stimulation suppresses specific excitatory circuits in the human motor cortex. *J Physiol* 565: 945–950, 2005.

Evarts EV. Relationship of pyramidal cells activity to force exerted during voluntary movements. *J Neurophysiol* 31: 14–28, 1968.

Evarts EV. Activity of pyramidal tract neurons during postural fixation. *J Neurophysiol* 32: 375–385, 1969.

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. *Cereb Cortex* (December 28, 2007). doi:10.1093/cercor/bhm239.

Hess G, Donoghue JP. Long-term potentiation and long-term depression of horizontal connections in rat motor cortex. *Acta Neurobiol Exp* 56: 397–405, 1996.

Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. *Neuron* 45: 201–206, 2005.

Huang YZ, Rothwell JC, Edwards MJ, Chen RS. Effect of physiological activity on an NMDA-dependent form of cortical plasticity in human. *Cereb Cortex* 18: 563–570, 2008.

Kaelin-Lang A, Sawaki L, Cohen LG. Role of voluntary drive in encoding an elementary motor memory. *J Neurophysiol* 93: 1099–1103, 2005.

Lotze M, Braun C, Birbaumer N, Anders S, Cohen LG. Motor learning elicited by voluntary drive. *Brain* 126: 866–872, 2003.

Monfils MH, Teskey GC. Skilled-learning-induced potentiation in rat sensorimotor cortex: a transient form of behavioral long-term potentiation. *Neuroscience* 125: 329–336, 2004.

Muellbacher W, Ziemann U, Boroojerdi B, Cohen L, Hallett M. Role of the human motor cortex in rapid motor learning. *Exp Brain Res* 136: 431–438, 2001.

Nitsche MA, Nitsche MS, Klein CC, Tergau F, Rothwell JC, Paulus W. Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. *Clin Neurophysiol* 114: 600–604, 2003.

Rioult-Pedotti MS, Friedman D, Donoghue JP. Learning-induced LTP in neocortex. *Science* 290: 533–536, 2000.

Rodriguez M, Muniz R, Gonzalez B, Sabaté M. Hand movement distribution in the motor cortex: the influence of a concurrent task and motor imagery. *Neuroimage* 22: 1480–1491, 2004.

Rosenkranz K, Kacar A, Rothwell JC. Differential modulation of motor cortical plasticity and excitability in early and late phases of human motor learning. *J Neurosci* 27: 12058–12066, 2007.

Siebner HR, Lang N, Rizzo V, Nitsche MA, Paulus W, Lemon RN, Rothwell JC. Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. *J Neurosci* 24: 3379–3385, 2004.

Stefan K, Kunesch E, Cohen LG, Benecke R, Classen J. Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain* 123: 572–584, 2000.

Stefan K, Wycislo M, Gentner R, Schramm A, Naumann M, Reiners K, Classen J. Temporary occlusion of associative motor cortical plasticity by prior dynamic motor training. *Cereb Cortex* 16: 376–385, 2006.

Turrigiano GG, Nelson SB. Homeostatic plasticity in the developing nervous system. *Nat Rev Neurosci* 5: 97–107, 2004.

Wolters A, Sandbrink F, Schlottmann A, Kunesch E, Stefan K, Cohen LG, Benecke R, Classen J. A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex. *J Neurophysiol* 89: 2339–2345, 2003.

Ziemann U, Ilić TV, Pauli C, Meintzschel F, Ruge D. Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. *J Neurosci* 24: 1666–1672, 2004.