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Depression of Human Corticospinal Excitability Induced by Magnetic Theta-burst Stimulation: Evidence of Rapid Polarity-Reversing Metaplasticity

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Metaplasticity refers to the activity-dependent modification of the ability of synapses to undergo subsequent potentiation or depression, and is thought to maintain homeostasis of cortical excitability. Continuous magnetic theta-burst stimulation (cTBS; 50 Hz-bursts of 3 subthreshold magnetic stimuli repeated at 5 Hz) is a novel repetitive magnetic stimulation protocol used to model changes of synaptic efficacy in human motor cortex. Here we examined the influence of prior activity on the effects induced by cTBS. Without prior voluntary motor activation, application of cTBS for a duration of 20 s (cTBS300) facilitated subsequently evoked motor potentials (MEP) recorded from APB muscle. In contrast, MEP-size was depressed, when cTBS300 was preceded by voluntary activity of sufficient duration. Remarkably, even without prior voluntary activation, depression of MEP-size was induced when cTBS was extended over 40 s. These findings provide in vivo evidence for extremely rapid metaplasticity reversing potentiation of corticospinal excitability to depression. Polarity-reversing metaplasticity adds considerable complexity to the brain's response toward new experiences. Conditional dependence of cTBS-induced depression of corticospinal excitability on prior neuronal activation suggests that the TBS-model of synaptic plasticity may be closer to synaptic mechanisms than previously thought.

Keywords: human, long-term potentiation, metaplasticity, motor cortex, theta-burst stimulation, transcranial magnetic stimulation

Introduction

Activity-dependent modifications of synaptic efficacy such as long-term potentiation (LTP) or long-term depression (LTD) are believed by many to be important physiological phenomena involved in normal learning and memory formation. Human models of synaptic plasticity are important tools to elucidate mechanisms of normal behavior and neuropsychiatric disorders. It has been proposed that the after-effects induced by theta-burst stimulation (TBS), a novel repetitive transcranial magnetic stimulation (TMS) technique, may be 1 such model (Huang et al. 2005). In TBS, several high-frequency (50 Hz) bursts of 3 TMS pulses are applied over a short period of time. TBS has been shown to modulate corticospinal excitability for several ten minutes after a conditioning train over the primary motor cortex (Huang et al. 2005). Because TBS requires stimulus intensities subthreshold for activating corticospinal descending projections, TBS-induced effects are likely to originate cortically (Huang et al. 2005).

Continuous theta-burst stimulation (cTBS), where TMS bursts are applied at a frequency of 5 Hz over a period of 20 or 40 s, led to a profound depression of motor evoked potentials (MEP) amplitudes of some 50% of the baseline amplitude (Huang et al. 2005). This depression is thought to be related to LTD at terminal excitatory glutamatergic synapses of afferent fibers impinging on pyramidal output cells (Di Lazzaro et al. 2005). This view is supported by recent findings (Huang, Chen, et al. 2007) that TBS-induced depression of corticospinal excitability depends on the activation of *N*-methyl-D-aspartate (NMDA)-receptors, a characteristic of many forms of LTD (Massey and Bashir 2007). Interestingly, the individual burst components of TBS, and intermittent TBS (iTBS, a protocol where a train of bursts is applied over 2 s, and then repeated every 10 s for 190 s) do not lead to depression of corticospinal excitability, but produce short-lasting (Huang and Rothwell 2004) or long-lasting (Huang et al. 2005) enhancing after-effects, respectively. Moreover, in in vitro animal experiments, short-lasting high-frequency stimulation protocols similar to the structure of the TBS protocol often are used to induce LTP (Malenka and Bear 2004), whereas induction of LTD typically involves extended low-frequency stimulation (Massey and Bashir 2007). In view of these observations, it remains unclear how the ability of cTBS to induce depression of corticospinal excitability can be reconciled with results obtained in vitro.

In just 2 years after its publication, cTBS has become the preferred method for inducing "virtual brain lesions" (c.f., Pascual-Leone et al. 1999) to study brain–behaviour relationships in motor and cognitive studies (Mochizuki et al. 2005; Nyffeler et al. 2006; Ragert et al. 2007; Silvanto et al. 2007; Talelli et al. 2007; Vallesi et al. 2007). Exactly by which mechanism cTBS interferes with the processing capacity of the targeted brain region remains unknown, although it is possible that this involves LTD-like phenomena. In addition to this research application, the potential utility of TBS to enhance motor recovery (Talelli et al. 2007) is being explored in clinical studies (http://clinicaltrials.gov/ct/show/NCT00366184;jsessionid=CB82363B25AB99E3478162AEFFDE143B?order=22).

In vitro studies have revealed that LTD depends on the neuronal activation history (Mizuno et al. 2001; Mockett et al. 2002; Ngezahayo et al. 2000), a phenomenon termed metaplasticity (Abraham and Bear 1996). Against this physiological and clinical background, in the present study we characterized the cTBS-induced effect on corticospinal excitability by studying its modifiability by prior activation. We 1st demonstrate that the direction of cTBS-induced modulation of corticospinal excitability is reversed from facilitation to depression, by prior activity and, secondly, that this activity-dependent polarity-reversal may occur with extreme rapidity.

Methods

Subjects

The study was approved by the Ethics committee of the University of Wuerzburg and written informed consent was obtained from all

participants. Experiments were performed on 36 healthy volunteers (14 men, 22 women) aged 20–56 years (mean 26.6 ± 7.4 years). Thirty-five volunteers were right handed.

Stimulation and Recording

TMS was performed using a figure-eight shaped magnetic coil (C-B60 Medtronic) connected to a MagPro X100 magnetic stimulator (Medtronic A/S 2740 Skovlunde, Denmark). The pulse shape was either monophasic or biphasic, as indicated below. The coil was held tangentially to the skull with the handle pointing backward and laterally at a 45° angle to the sagittal plane. With monophasic pulse shape, the direction of the current induced in the brain was posterior to anterior. With biphasic pulse configuration, the initial current direction induced in the brain was anterior to posterior.

cTBS consisted of bursts containing 3 biphasic TMS pulses of 50 Hz repeated at 200 ms for a duration of 20 s (cTBS300) or 40 s (cTBS600) (Huang et al. 2005). The TBS intensity was subthreshold for eliciting a response in the contralateral APB (see below). A neuronavigational device (Brainsight, Rogue Research, Montreal, Canada) was used to increase the fidelity of positioning the magnetic coil over the course of an experiment. Electromyographic (EMG) activity was recorded from the right abductor pollicis brevis (APB) muscle using Ag–AgCl surface electrodes (Fischer Medizintechnik, Nürnberg, Germany). Raw signals were amplified using a 1902 signal conditioner (Cambridge Electronics Design, Cambridge, UK) and bandpass filtered between 1 Hz and 2 kHz. EMG signals were digitized at 5 kHz by an A/D converter (model 1401 plus, Cambridge Electronics Design, Cambridge, UK).

Hot-spot, SI1mV, Resting and Active Motor Threshold, and Timed Isometric Contraction

The optimal position ("hot spot") of the magnetic coil for eliciting MEPs in the resting APB was assessed over the motor cortex at a moderately suprathreshold stimulation intensity using a monophasic pulse configuration, and then was registered by the neuronavigation system. Thereafter, the stimulus intensity producing MEP-amplitudes of ~1 mV at rest (SI1mV) was established.

Using a biphasic pulse configuration, the resting motor threshold (RMT) was determined as the minimum stimulator intensity needed to produce a response of at least 50 μV in the relaxed APB in at least 5 of 10 consecutive trials.

In some experiments timed continuous isometric contractions were required. Subjects were instructed to perform isometric abductions with their right thumb against a force transducer (Grass CP122A, Grass Instruments CO, W. Warwick, RI) for a duration of 1.5 min (ACT1.5), or 5 min (ACT5) at ~25% of the maximal individual force. Thereby, the strength of voluntary contraction matched closely that used by Huang et al. (2005) to assess the active motor threshold. The force signal was fed back to the subject on a computer screen.

Interventions

The effect of 6 interventions on corticospinal excitability was tested in different experiments. The order of the experiments was pseudorandomized with a minimum of 48 h between 2 experiments, and balanced between subjects. Sixteen subjects each participated in intervention 1–3, whereas 9 subjects each participated in intervention 4–6. A schematic overview of all 6 experiments is provided in Figure 1.

Intervention 1 (ACT0 + cTBS300): After assessment of RMT subjects paused for 2 min (no isometric voluntary activation, ACT0). The pre-cTBS corticospinal excitability of APB muscle representation was then established by collecting 30 responses at SI1mV using a stimulation rate of 0.2 ± 10% Hz with the target muscle at rest. Immediately after establishing the pre-cTBS corticospinal excitability, cTBS300 was applied at 70% of the RMT. The relative intensity of cTBS300 was based on previous studies reporting that 70% of the RMT is equivalent to 80% of the AMT (Chen et al. 1998). Following cTBS300, subjects paused for 2 min.

Interventions 2 and 3 (ACT1.5 + cTBS300 and ACT5 + cTBS300): Following isometric thumb abduction for a duration of 1.5 min (ACT1.5, Expt. 2) or 5 min (ACT5, Expt. 3), subjects paused for 2 min. The pre-cTBS corticospinal excitability of APB muscle representation was established as described above. Immediately after establishing the pre-cTBS corticospinal excitability, cTBS300 was applied at 70% of the RMT. Following cTBS300, subjects paused for 2 min.

Intervention 4 (ACT0 + cTBS600): The protocol was similar to intervention 1, except for the fact that cTBS was performed for a duration of 40 s, instead of 20 s.

Intervention 5 (ACT5 + cTBS0): After determining the RMT, the corticospinal excitability of APB muscle representation was established by collecting 30 MEP responses at SI1mV with the target muscle at rest. Following isometric thumb abduction for a duration of 5 min (ACT5), subjects paused for 2 min. The "pre-cTBS" corticospinal excitability of APB muscle representation was then established as described above. Immediately after establishing the "pre-cTBS" corticospinal excitability, subjects paused for 2 min. The pause was extended for another 20 s to account for the fact that no cTBS was applied ("cTBS0").

Intervention 6 (ACT0 + cTBS_SHAM): The protocol was similar to intervention 1, except for the fact that cTBS was performed by a different unconnected coil. To simulate the characteristic TMS noise during cTBS the original coil was positioned nearby.

We also attempted to replicate the findings of Huang et al. (2005). We used the same protocol in 13 subjects of whom 7 had also participated in interventions 1 and 6 had also participated in intervention 3. The protocol was similar to intervention 1, except for

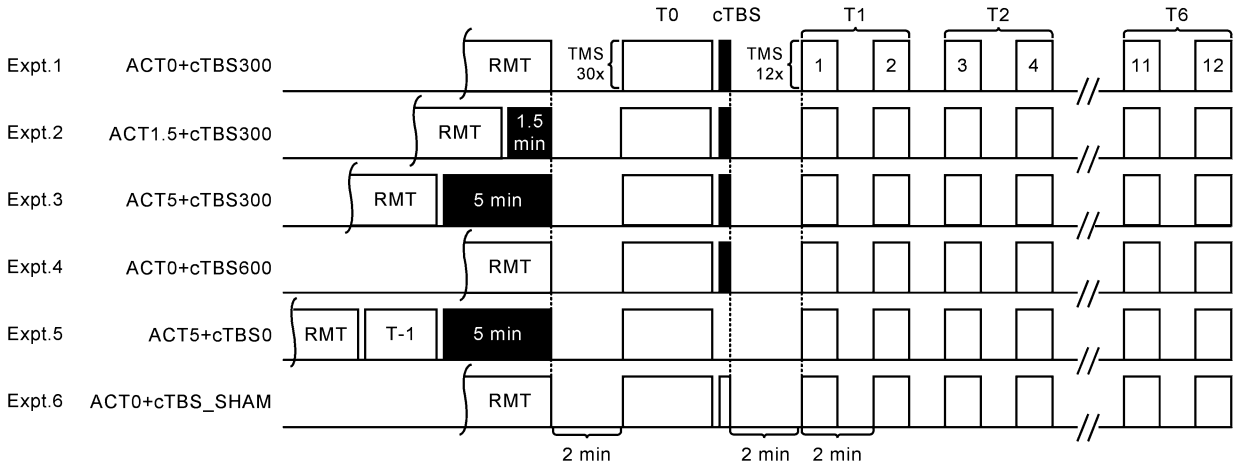

Figure 1. Schematic overview of the 6 principal interventions. After determining the stimulation intensity for evoking MEP amplitudes of ~1 mV (SI1mV) and the resting MEP threshold subjects paused for 2 min, either directly or after an isometric voluntary contraction (black filled blocks) (for 1.5 or 5 min). The pre-cTBS corticospinal excitability (T0) was then established, followed by the application of cTBS. Postintervention measurements were performed in 12 blocks consisting of 12 MEP responses at SI1mV followed by 1 min of rest, up to 25-min postintervention. Two successive time points were then binned.

the following modifications: The stimulus intensity was set at 80% of active motor threshold as established beforehand; and instead of the APB muscle, the 1st dorsal interosseus muscle was studied (Huang et al. 2005). The active motor threshold was defined as the minimum single pulse intensity required to produce a MEP of greater than 200 μV on more than 5 out of 10 trials from the contralateral 1st dorsal interosseus muscle while the subject was maintaining an active voluntary contraction. Finally, intervention 1 (ACT0 + cTBS300) was also applied in 3 subjects, while recording MEP responses from 1st dorsal interosseus muscle instead of APB.

Following all interventions, corticospinal excitability was probed by collecting 12 MEP responses at a stimulation rate of 0.2 ± 10% Hz beginning after completion of the 2nd minute post-cTBS intervention every 2 min until completion of the 25th minute postintervention.

Complete muscle relaxation during the resting periods and during the assessment of MEP responses was monitored by audiovisual feedback. Subjects were instructed to maintain attention to the task throughout the entire session. Identical stimulus intensities were used before and after intervention.

Data Analysis

MEP amplitudes were measured peak-to-peak in each individual trial. For each subject MEP amplitudes were averaged before TBS (cTBS300, cTBS600, cTBS0, or cTBS_SHAM) intervention (30 responses, T0). In Expt. 5 (ACT5 + cTBS0), T-1 was designated the time at which MEPs were sampled before ACT5. Post-cTBS intervention, values were binned at 2 successive time points (T1, 3 and 5 min; T2, 7 and 9 min, T3, 11 and 13 min, T4, 15 and 17 min; T5, 19 and 21 min; T6, 23 and 25 min; 24 responses each) to reduce variability of results. To compare the effect of interventions on cortical excitability, all averaged MEP amplitudes were normalized to "pre-cTBS" assessed at T0 before cTBS-intervention.

The effect of cTBS on corticospinal excitability was evaluated by a 2-way repeated measures analysis of variance ($ANOVA_{RM}$), with "PROTOCOL" as between-subjects factor with 6 levels (ACT0 + cTBS300, ACT1.5 + cTBS300, ACT5 + cTBS300, ACT0 + cTBS600, ACT5 + cTBS0, ACT0 + cTBS_SHAM) and "TIME" as within-subject factor with 7 levels (T0, T1, . . ., T6). Because the experiment replicating the original protocol by Huang et al. (2005) used a different target muscle, this series was evaluated separately by a $ANOVA_{RM}$, using "TIME" as within-subject factor with 7 levels (T0, T1, . . ., T6). Two-tailed Student's *t*-tests were performed for post hoc testing. The false discovery rate correction (FDRC) procedure was used for correction for multiple comparisons (Curran-Everett 2000). Effects were considered significant, if $P < 0.05$. All data are presented as means ± SD unless indicated otherwise.

Results

None of the subjects reported any adverse effect, including fatigue, after any of the interventions.

Age and baseline physiological data (Table 1) did not differ significantly between different interventions. Figure 2*A* shows the effect of the 6 main interventions on corticospinal excitability.

cTBS300 modulated the magnitude of MEP-amplitudes recorded from the right APB muscle depending on the presence of prior isometric activation (Fig. 2). If cTBS300 was applied on its own (ACT0 + cTBS300), MEP size increased steadily. A similar facilitation was noted, if cTBS300 was preceded by short (1.5 min) isometric contraction (ACT1.5 + cTBS300). However, if cTBS300 was applied after an isometric contraction of 5-min duration (ACT5 + cTBS300), MEP amplitudes decreased. The decrease was 1st present at T1 (3–5 min) and lasted up to T5 (19–21 min). cTBS600 without prior voluntary isometric contraction (ACT0 + cTBS600) decreased MEP size. The decrease was present at all time epochs between, and including, T2 (7–9 min) to T4 (15–17 min). Isometric contraction alone, in the absence of subsequent cTBS (ACT5 + cTBS0), did not lead to suppression of MEP amplitudes, but led to a delayed increase of MEP-amplitudes reaching a maximum at T6 (23–25 min). In ACT5 + cTBS0, MEP-amplitudes obtained at T-1 (before voluntary activation; 0.77 ± 0.17 mV; SD) were not significantly different from those obtained at T0 (after 5 min of voluntary isometric contraction plus 2 min of rest; 0.75 ± 0.09 mV; $P = 0.674$; paired *t*-test). Sham cTBS in the absence of voluntary isometric contraction (ACT0 + cTBS_SHAM) did not change MEP amplitudes.

Table 1
Pre-cTBS physiological parameters

	MT (biphasic) (% of MSO)	Stimulation intensity (probing TMS, % of MSO)	Stimulation intensity (cTBS, % of MSO)	Pre-cTBS MEP at T0 (mV)
ACT0 + cTBS300	34 ± 7	61 ± 15	24 ± 5	0.8 ± 0.2
ACT1.5 + cTBS300	32 ± 6	59 ± 15	23 ± 5	0.9 ± 0.3
ACT5 + cTBS300	32 ± 6	57 ± 15	22 ± 4	0.9 ± 0.4
ACT0 + cTBS600	29 ± 3	52 ± 6	21 ± 1	1.0 ± 0.1
ACT5 + cTBS0	34 ± 5	63 ± 14	24 ± 4	0.7 ± 0.1
ACT0 + cTBS_SHAM	32 ± 6	59 ± 13	23 ± 4	1.0 ± 0.2
cTBS_Huang	25 ± 3	55 ± 10	20 ± 3	0.9 ± 0.3

Note: MT (biphasic), motor threshold established with a biphasic magnetic pulse configuration; % of maximal stimulator output (MSO), percent of maximal stimulator output. cTBS_Huang, replication of the original TBS-protocol used by Huang et al. (2005). In this case, cTBS300 was applied at 80% of the active motor threshold (AMT), as determined immediately beforehand and recordings were from 1st dorsal interosseus muscle.

$ANOVA_{RM}$ revealed a significant effect of TIME ($F_{(6,64)} = 2.533$, $P = 0.020$). Of greater interest, the PROTOCOL*TIME interaction was significant ($F_{(30,340)} = 2.168$; $P < 0.001$) suggesting that the effect of TIME (by which the efficacy of the interventions is implicated) was dependent on the type of intervention. Subsequently, for each intervention a 1-way $ANOVA_{RM}$ was performed separately with TIME as within-subject factor. Except for ACT5 + cTBS0 and ACT0 + cTBS_SHAM, $ANOVA_{RM}$ revealed significant effects of TIME for all protocols 1–6. The results of this statistical analysis are summarized in Table 2. Conditional on significant effects of TIME, post hoc *t*-testing was performed.

To facilitate comparison between protocols, the effects of the interventions are displayed at 2 different time periods, T2 (7–9 min), and T6 (23–25 min). Examples from individual subjects are displayed in Figure 2*B*, group results in Figure 2*C,D*. This illustrates suppression of MEP amplitudes at T2 (7–9 min) by ACT5 + cTBS300 and ACT0 + cTBS600. At T6 (23–25 min), MEP amplitudes were enhanced following ACT0 + cTBS300.

To address the concern that usage of partially overlapping populations of volunteers may have distorted the results, results obtained in experiments 1 (ACT0 + cTBS300) and 4 (ACT0 + cTBS600) were compared between subjects participating in both, or only 1 of these experiments. In experiment 1 (ACT0 + cTBS300), MEP amplitudes increase at T6 (23–25 min) was not statistically different between 2-time participants (36.8 ± 19.6%) and 1-time participants (21.4 ± 9.9%, $P = 0.446$; unpaired 2-tailed *t*-test). In experiment 4 (ACT0 + cTBS600), MEP amplitudes decreased at T2 (7–9 min) to a similar degree in 2-times participants (29.4 ± 5.0%) and in 1-time participants (26.2 ± 7.2%), again with the difference between 2 groups of participants being not significant ($P = 0.876$; unpaired 2-tailed *t*-test).

To compare the findings to those obtained previously by Huang et al. (2005) we replicated their experiment in all possible details. This included, in particular, the usage of the

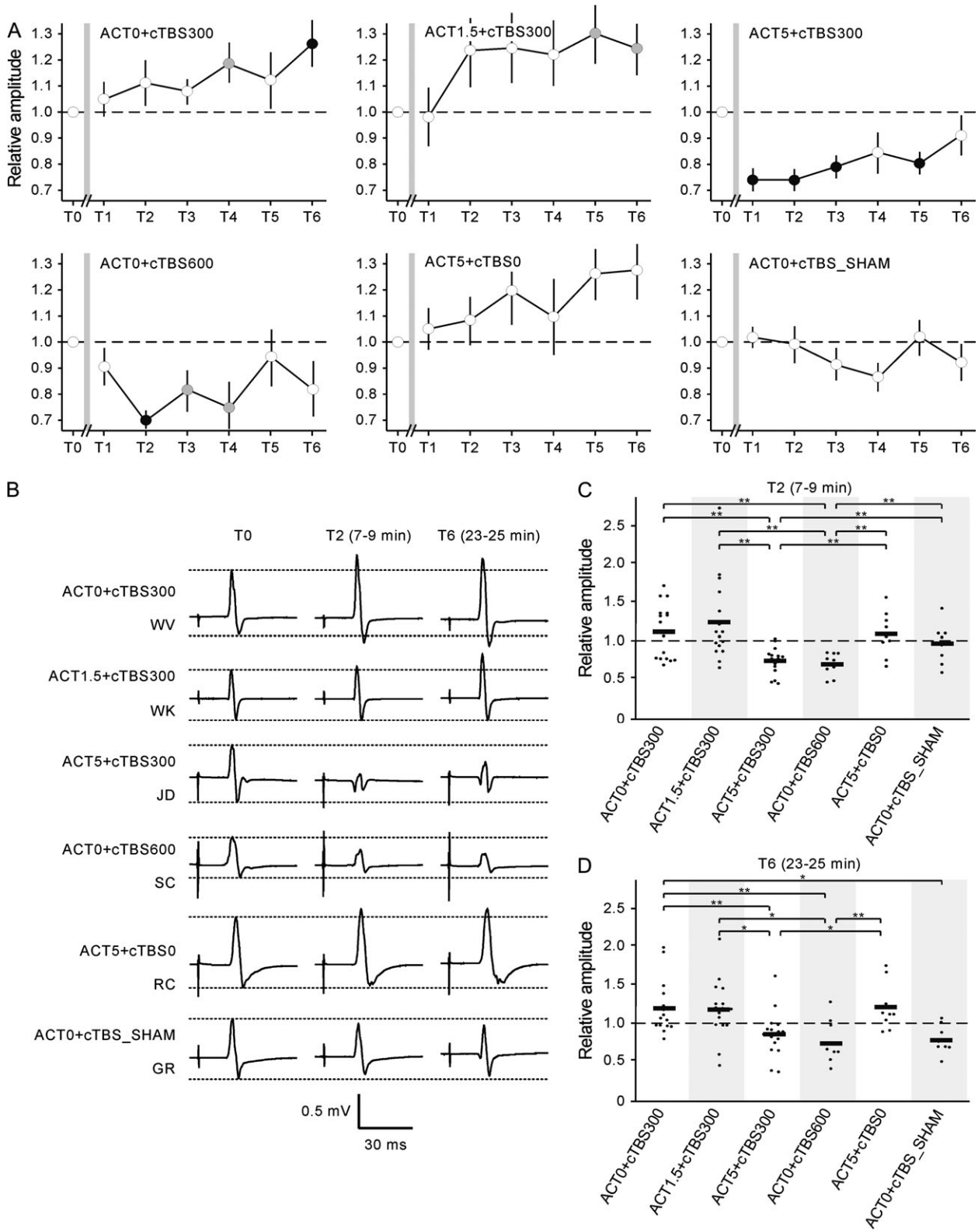

Figure 2. (*A*) Time course of changes in MEP responses of all 6 principal interventions normalized to pre-cTBS (T0). Gray filled symbols, significant differences from pre-cTBS (T0) MEP amplitudes ($P < 0.05$, 2-tailed *t*-test). Black filled symbols, significant after FDRC. Error bars, SEM. (*B*) Examples of averaged MEP responses of individual subjects obtained before (T0) and 7–9 min (T2) and 23–25 min (T6) postintervention. (*C*) Results (horizontal line, group mean) obtained at 7–9 min (T2). (*D*) Results (horizontal line, group mean) obtained at 23–25 min (T6). Asterisks denote significant differences after FDRC.

Table 2
Results of the 1-way ANOVAs conducted for experiments 1–6

Intervention	*F* value	*P* value
ACT0 + cTBS300	2.413	**0.033**
ACT1.5 + cTBS300	2.562	**0.025**
ACT5 + cTBS300	4.863	**<0.001**
ACT0 + cTBS600	3.219	**0.010**
ACT5 + cTBS0	2.038	0.079
ACT0 + cTBS_SHAM	1.450	0.216

Note: For all experiments, $ANOVA_{RM}$ were performed with TIME (T0, T1, . . ., T6) as within-subject factor. Significant *P* values in bold.

active motor threshold for determination of the interventional stimulation intensity. Approximately 3–5 min of contraction was needed to assess the active motor threshold. cTBS using the active motor threshold decreased the size of MEPs recorded from the 1st dorsal interosseus muscle. $ANOVA_{RM}$ revealed a significant effect of TIME (F (6,72) = 2.272, P = 0.046). Using FDRC for multiple comparisons, post hoc *t*-testing revealed significant excitability depressing effects at T2 (–20.0 ± 5.3%; $P < 0.001$); T3 (–24.5 ± 6.9%; $P = 0.002$) and T4 (–19.0 ± 6.1%; $P = 0.005$). In contrast, application of cTBS300 without prior activation led to a slight facilitation of MEP amplitudes in 1st dorsal interosseus ($N = 3$ subjects; maximal at T2: 25.6 ± 12.7%), suggesting that the representation of 1st dorsal interosseus was qualitatively similarly responsive toward cTBS300 as that of the APB muscle.

Discussion

The present findings show that the application of high-frequency bursts of subthreshold magnetic stimuli over a period of 20 s led to an increase of corticospinal excitability as indexed by the magnitude of MEPs evoked after the conditioning train. The increase of corticospinal excitability was turned into depression, when the TBS-intervention was preceded by isometric voluntary contraction or when the TBS-train was extended to 40 s.

Enhancement of Corticospinal Excitability by cTBS

Because sham TBS did not alter MEP size significantly, the increase of corticospinal excitability following cTBS300 cannot be attributed to the lack of voluntary or afferent input to the motor cortex (c.f., Todd et al. 2006), and thus results from the application of cTBS300 itself. The individual burst components of TBS are followed by a short-lasting enhancement of MEP-responses (Huang and Rothwell 2004). iTBS, a protocol where trains of bursts are applied intermittently for 190 s, markedly prolongs this effect (Huang et al. 2005). Because pharmacological experiments implicate activation of NMDA-receptors in this process (Huang, Chen, et al. 2007) and because protocols similar to TBS are particularly powerful to induce LTP in animal preparations (Malenka and Bear 2004), Huang et al. (2005) have proposed that LTP underlies the iTBS-induced effects. Although the nature of the increase of cTBS300-induced corticospinal excitability is presently unclear, it seems possible that a similar or identical LTP-like mechanism is involved.

Enhancing → Depressing, Rapidly Evolving Metaplasticity Revealed by cTBS

The increase of MEP-amplitudes induced by cTBS300 differs from previous observations (Huang et al. 2005) where application of cTBS for 20 s induced depression of MEP amplitudes. This apparent discrepancy may, however, be resolved when considering that Huang et al. (2005) used the active, rather than the RMT to establish the magnetic stimulation intensity during TBS. Determination of the active motor threshold requires the subject to contract the target muscle, usually for cumulatively some 3–5 min. Indeed, we confirmed a decrease of MEP size recorded from 1st dorsal interosseus muscle when the intensity of TBS was calibrated using the active motor threshold according to the original protocol used by Huang et al. (2005). In the present study, MEP amplitudes decreased when cTBS300 was applied after 5 min of voluntary APB activation. Additional experiments ruled out that depression of MEP amplitudes relative to pre-cTBS was a consequence of prior voluntary activation alone: Preceding isometric contraction (ACT5 + cTBS0) did not change corticospinal excitability at the time of the cTBS-intervention, but led to a moderate (yet statistically nonsignificant) delayed enhancement of corticospinal excitability. Together, these findings strongly suggest that prior cortical activation likely has substantially contributed to the cTBS300-induced effects reported before (Huang et al. 2005).

Modulation of the stimulation-induced effects by prior activity has been observed in several previous studies utilizing different TMS protocols. One set of experiments indicated that the scale of stimulation-induced plasticity can be altered by preconditioning. For example, a paired associative stimulation protocol (Wolkers et al. 2003) which induces depression of corticospinal excitability, led to stronger suppression if the intervention was preceded by a ballistic motor training task (Ziemann et al. 2004). Similarly, the inhibitory effect of 1-Hz repetitive TMS of the motor cortex was enhanced by preconditioning with 6-Hz TMS (Iyer et al. 2003) or anodal direct current transcranial stimulation (Siebner et al. 2004). Quite different from these scaling effects, Siebner et al. (2004) demonstrated that 1-Hz repetitive TMS, when it was conditioned by cathodal direct current transcranial stimulation, induced facilitation rather than the usual depression, which normally follows 1-Hz repetitive TMS application to naive motor cortex (Chen et al. 1997). This phenomenon may be termed depressing → enhancing metaplasticity to indicate that a natively excitability depressing protocol (in this case 1-Hz repetitive TMS) was turned into 1 inducing enhancement of excitability by virtue of metaplasticity. In 1 previous study, a statistically nonsignificant depression of motor cortex excitability was induced when facilitating paired associative stimulation was applied after training ballistic thumb movements (Ziemann et al. 2004). The present study appears to provide the 1st unequivocal evidence that a natively excitability-enhancing protocol (ACT0 + cTBS300) was turned into 1 which depressed excitability, by prior neuronal activation ("enhancing → depressing metaplasticity").

Intriguingly, cTBS600, which comprised application of TMS bursts for twice as long as cTBS300, induced depression of corticospinal excitability in the absence of voluntary contraction. Because cTBS300 alone induced facilitation, the cTBS600-induced depression cannot be understood by simple addition of 2 successive cTBS300-induced effects. Rather, it appears likely that the initial 300 pulses in the stimulation train primed neuronal elements to undergo depression by subsequent cTBS300. In this way, the cTBS600-induced depression represents a metaplasticity effect, much in the same way as preceding voluntary contraction reversed the polarity of

cTBS300-induced effects. Similarity of the effects of the initial 300 pulses in the cTBS600 protocol on 1 hand, and 5 min of voluntary activation on the other hand, is also suggested by the observation that corticospinal excitability tended to progressively increase after 5 min of voluntary contraction (Expt. 5). Such similarity between the physiological effects of voluntary contraction and cTBS may also underlie the recent observation that sustained MEP size enhancement (but not suppression) was induced, if voluntary muscle contraction was performed immediately *after* cTBS300 (using 80% of active motor threshold) (Huang, Rothwell, et al. 2007). Because this protocol involved a triple intervention (voluntary activation, TBS and again voluntary activation), it is difficult to speculate about its underlying mechanisms. However, conceivably, the combined effect of assessment of active motor threshold plus cTBS may have acted as a conditioning intervention, augmenting the facilitating effect of the 2nd voluntary activation on synaptic transmission.

Physiological Mechanism of cTBS-Induced Suppression of Corticospinal Excitability

The present results have implications relevant for the understanding of the mechanism of action of cTBS. TMS operates on neuronal circuits involving many synapses. To explain opposite changes of corticospinal excitability induced by cTBS and iTBS, respectively, Huang et al. (2005) suggested that the effects of both protocols differ in the relative weight of facilitating and depressing effects induced simultaneously on 2 different sets of synapses, of which both contribute to the generation of MEPs. According to this hypothesis, both cTBS and iTBS may induce facilitatory effects on 1 set of synpases, whereas depressing effects on synaptic efficacy, sufficient to outweigh and dominate, or outlast, the facilitatory ones, are induced only by cTBS. This hypothesis appears to imply that TBS may be able to primarily induce strong LTD if only on a specific set of synapses. If we accept this conclusion, the present study has identified neuronal activation by voluntary contraction or immediately preceding cTBS as necessary priming intervention for the depressing effect of the cTBS300 on 1 set of synapses. In experimental studies on synaptic plasticity, priming activity is usually implemented some time before the application of the induction protocol. However, there is also evidence that priming and subsequent LTD-inducing activity need not be separated by a quiescent time interval (Mizuno et al. 2001; Mockett et al. 2002). Rather, activity early in a stimulus train may activate metaplasticity that is permissive for LTD induction by stimuli late in the train (Mizuno et al. 2001; Mockett et al. 2002).

However, to the best of our knowledge, with few notable exceptions (Gall et al. 2005; Lien et al. 2006; Yoshimura et al. 2003) TBS protocols have not primarily induced LTD in any brain preparation in animal studies. Alternatively, therefore, it may be worthwhile to consider the possibility that cTBS induces primarily synaptic facilitation in the naive motor cortex, whereas cTBS-induced LTD-like phenomena arise due to activity-dependent metaplasticity. Although this hypothesis does not exclude more complex scenarios, it appears to parsimoniously bridge system-level phenomena with in vitro phenomena and obviates the need for a dual-compartment model.

Interestingly, a one- or dual-compartment hypothesis puts different constraints on the mechanism of activity-dependent metaplasticity. Whereas priming may occur in the absence of any modification of synaptic efficacy (Christie and Abraham 1992; Mizuno et al. 2001; Wagner and Alger 1995), long-term potentiation (Wagner and Alger 1995), as well as short-term potentiation (Wexler and Stanton 1993) may facilitate subsequent LTD induction. Both voluntary contraction and unconditioned cTBS300 led to delayed enhancement of corticospinal excitability. However, with neither of these priming treatments was enhancement present at the time of the application of the depression-inducing cTBS300-protocol. Therefore, if a one-compartment model is accepted, synaptic potentiation as a necessary element of the priming mechanism appears highly unlikely. On the other hand, within the framework of a dual-compartment model, the effects of LTP and LTD occurring at different sets of synapses may have canceled each other at the time of the application of the depression-inducing cTBS300-intervention.

Several candidate mechanisms of metaplasticity may scale the magnitude or reverse the polarity of subsequently evoked modifications of synaptic efficacy (Abraham and Tate 1997; Turrigiano 2007). The initial 20 s of the cTBS600 protocol set an upper time limit to the evolution of the metaplasticity mechanism rendering gene-based mechanisms unlikely. Activity-dependent changes in Ca^{2+}-signaling, which involve neurons (Davis 2006) as well as glial cells (Wang et al. 2006), may occur sufficiently fast and last sufficiently long to underlie the present rapid metaplasticity. The magnitude of activity-dependent rise in postsynaptic Ca^{2+}-concentration is believed to determine the polarity of changes of synaptic efficacy (LTP or LTD) (Artola et al. 1990, 1996; Lisman 1989; Kemp and Bashir 2001; Massey and Bashir 2007) including those induced by TBS (Kimura et al. 1990; Yoshimura et al. 1991; Yasuda and Tsumoto 1996).

The evidence concerning the synaptic nature of cTBS-induced phenomena is indirect and solely based on analogies with animal experiments. Alternative mechanisms underlying alterations in corticospinal excitability may include changes of intrinsic neuronal excitability (Turrigiano and Nelson 2000; Kim and Linden 2007). Independent of the physiological nature of the homeostatic mechanism, the present observations suggest that activity-dependent polarity-reversal of corticospinal excitability may occur without changes in the input pattern and may evolve with surprising rapidity. We note that, if identical input patterns differing only in duration indeed induced synaptic changes of opposite polarity, the present findings may pose significant constraints on the in vivo operation of LTP/LTD as a universal memory-coding system. Rapid polarity-reversing metaplasticity adds considerable flexibility and complexity to the brain's response toward new experiences.

Relevance for Induction of "Virtual Brain Lesions" and Therapeutic Modulation by cTBS

cTBS fulfills several important properties of an ideal tool allowing to study structure–function relationships in humans. Indeed, specific behavioral changes have been observed after cTBS directed to several distinct brain regions (Mochizuki et al. 2005; Nyffeler et al. 2006; Ragert et al. 2007; Silvanto et al. 2007; Talelli et al. 2007; Vallesi et al. 2007). Our findings suggest that, at least in the primary motor cortex, cTBS300 does not induce depression of neuronal excitability unless conditioned by prior activity. Variable TBS-responsiveness of different cortical regions (Martin et al. 2006) may indicate that the amount of conditioning prior activity required for sufficient priming

differs between cortical regions. However, in view of the proven efficacy of cTBS300 as a tool revealing regional motor area function by interference, it is possible that behavioral disruption by cTBS is independent of its excitability depressing effects. This view appears to be supported by a recent study by Silvanto et al. (2007). Using a variant of cTBS, offline TMS perceptually suppressed attributes encoded by the neural populations least active during interference, whereas attributes encoded by the presumably more active neuronal populations remained unchanged (Silvanto et al. 2007). In addition to their impact on models how TMS may lead to behavioral disruption, the present findings may need to be considered when therapeutic depression of cortical excitability is intended.

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Notes

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