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Homeostatic metaplasticity of corticospinal excitatory and intracortical inhibitory neural circuits in human motor cortex

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Key points

- Homeostatic metaplasticity is an important mechanism for maintaining overall synaptic weight of a neuronal network in the physiological range.
- Homeostatic metaplasticity has been demonstrated, so far largely exclusively, for excitatory synaptic neurotransmission.
- New non-invasive transcranial magnetic theta burst stimulation (TBS) experiments at the systems level of human motor cortex demonstrate for the first time that homeostatic metaplasticity is also present in inhibitory intracortical circuits.
- In addition, manipulation of intracortical inhibition by priming TBS contributes to the homeostatic regulation of metaplasticity in the corticospinal excitatory pathway.
- Findings are important for therapeutic applications of non-invasive brain stimulation that aim at correcting excitatory or inhibitory neurotransmission outside the physiological range in humans with neuropsychiatric disorders.

Abstract Homeostatic metaplasticity, a fundamental principle for maintaining overall synaptic weight in the physiological range in neuronal networks, was demonstrated at the cellular and systems level predominantly for excitatory synaptic neurotransmission. Although inhibitory networks are crucial for regulating excitability, it is largely unknown to what extent homeostatic metaplasticity of inhibition also exists. Here, we employed intermittent and continuous transcranial magnetic theta burst stimulation (iTBS, cTBS) of the primary motor cortex in healthy subjects for induction of long-term potentiation (LTP)-like and long-term depression (LTD)-like plasticity. We studied metaplasticity by testing the interactions of priming TBS with LTP/LTD-like plasticity induced by subsequent test TBS. Changes in excitatory neurotransmission were measured by the input–output curve of motor-evoked potentials (IO-MEP), and changes in $GABA_A$ergic inhibitory neurotransmission by the IO of short-interval intracortical inhibition (IO-SICI, four conditioning stimulus intensities of 70–100% active motor threshold, interstimulus interval 2.0 ms). Non-primed iTBS increased IO-MEP, while non-primed cTBS decreased IO-MEP. Pairing of identical protocols (iTBS→iTBS, cTBS→cTBS) resulted in suppression of the non-primed TBS effects on IO-MEP, and pairing of different protocols (cTBS→iTBS, iTBS→cTBS) enhanced the test TBS effects on IO-MEP. While non-primed TBS did not result in significant changes of IO-SICI, iTBS→iTBS resulted in IO-SICI decrease, and cTBS→cTBS in IO-SICI increase compared with the non-primed conditions. The changes in SICI induced by priming TBS correlated with the changes in MEP induced by subsequent test

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TBS. Findings demonstrate that plasticity in both excitatory and inhibitory circuits in the human motor cortex are regulated by homeostatic metaplasticity, and that priming effects on inhibition contribute to the homeostatic regulation of metaplasticity in excitatory circuits.

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Abbreviations AMT, active motor threshold; cTBS, continuous theta burst stimulation; DSI, depolarization-induced suppression of inhibition; EMG, electromyography; FDI, first dorsal interosseous muscle; iLTD, inhibitory long-term depression; IO, input–output curve; ISI, interstimulus interval; iTBS, intermittent theta burst stimulation; LTD, long-term depression; LTP, long-term potentiation; M1, primary motor cortex; MEP, motor-evoked potential; PAS, paired associative stimulation; rTMS, repetitive transcranial magnetic stimulation; SICI, short-interval intracortical inhibition; TBS, theta burst stimulation.

Introduction

Maintaining the balance between excitation and inhibition is essential for long-term stability and function of dynamic neural networks (Abbott & Nelson, 2000; Abraham, 2008). As plasticity of excitatory and inhibitory synaptic input to a neuron will change its overall excitability in opposite directions, alterations in synaptic strength need to be tightly controlled to keep neuronal excitability within the physiological range. Homeostatic metaplasticity, as formalized in the Bienenstock–Cooper–Munro theory of bidirectional synaptic plasticity (Bienenstock *et al.* 1982), provides one influential conceptual framework to solve this problem. It states that plasticity at a given synapse is bidirectional, resulting in either long-term potentiation (LTP) or long-term depression (LTD), and that the threshold for induction of LTP *versus* LTD of synapses is not stable but varies as a function of the history of postsynaptic activity: the synaptic modification threshold decreases at low level of previous integrated postsynaptic activity, favouring induction of LTP over LTD. Conversely, the threshold increases at a high level of recent postsynaptic activity, thereby favouring the probability of subsequent LTD over LTP (Bienenstock *et al.* 1982). Similar to homeostatic metaplasticity in animal experiments (Abraham & Tate, 1997; Abraham, 2008), studies in human primary motor cortex (M1) demonstrated homeostatic metaplasticity for corticospinal excitatory neural circuits, as indexed by changes in motor-evoked potential (MEP) amplitude (Iyer *et al.* 2003; Lang *et al.* 2004; Siebner *et al.* 2004; Ziemann *et al.* 2004; Müller *et al.* 2007; Hamada *et al.* 2008; Todd *et al.* 2009; Fricke *et al.* 2011; Gamboa *et al.* 2011). In contrast, very little is known to what extent a similar homeostatic regulation of plasticity also exists in inhibitory cortical circuits in humans. To the best of our knowledge, this has only been sparsely addressed: Siebner and colleagues (2004) found homeostatic regulation of 1 Hz repetitive transcranial magnetic stimulation (rTMS) after-effects on MEP amplitude by priming with anodal *versus* cathodal transcranial direct current stimulation, but they did not find consistent changes in short-interval intracortical inhibition (SICI), an index of $GABA_A$ergic synaptic neurotransmission in M1 (Kujirai *et al.* 1993; Ziemann *et al.* 1996*a*; Di Lazzaro *et al.* 2000; Ilic *et al.* 2002). Those authors concluded: '. . .results favour a direct homeostatic effect in the corticospinal output neurons rather than a homeostatic mechanism within intracortical interneuronal circuits' (Siebner *et al.* 2004). Another study investigated the interactions of two transcranial magnetic theta burst stimulation (TBS) protocols and also demonstrated homeostatic regulation of MEP amplitude, but found no SICI metaplasticity (Doeltgen & Ridding, 2011*b*). Also in basic experiments, the issue of metaplasticity at inhibitory synapses has only been sparsely studied (Fischer *et al.* 1997; Edwards *et al.* 2008).

To address the issue of metaplasticity of excitatory and inhibitory neural circuits, and their interrelation, at the systems level of the human M1, we applied two consecutive sessions of TBS in healthy subjects. We selected TBS because it is capable of inducing LTP-/LTD-like plasticity in both corticospinal excitatory and intracortical inhibitory neural circuits of the stimulated cortex (Di Lazzaro *et al.* 2005, 2008*a*; Huang *et al.* 2005, 2007; Murakami *et al.* 2008; Suppa *et al.* 2008; Ziemann *et al.* 2008; McAllister *et al.* 2009). We studied the effects of intermittent (iTBS) and continuous (cTBS) TBS alone, and the interactions between priming TBS and test TBS applied 15 min later, on input–output curves (IOs) of MEP amplitude and SICI. We decided to measure IO-MEP rather than intracortial facilitation or short-interval intracortical facilitation, putative paired-pulse TMS markers of intracortical excitatory circuitry (Ziemann *et al.* 1996*c*, 1998*b*) because MEP amplitude is the best studied excitability marker in TBS studies, and because TBS had only weak or no effects on intracortical facilitation (Huang *et al.* 2005; Hasan *et al.* 2012) and short-interval intracortical facilitation (Doeltgen & Ridding, 2011*b*).

Our results show that homeostatic metaplasticity is a general principle in both corticospinal excitatory and intracortical inhibitory neural circuits in human M1, and

that metaplasticity in corticospinal excitatory circuits can be explained, at least in part, by priming modulation of inhibitory control of these circuits.

Methods

Subjects

We studied 14 strictly right-handed healthy volunteers (four women; 21–42 years old; mean age, 27.6 ± 6.0 years). Handedness was assessed by the Edinburgh Handedness Inventory (Oldfield, 1971), and the mean laterality score was 95.3 ± 8.3%. Written informed consent was obtained from all subjects prior to participation. None of the participants had a history of neuropsychiatric disease or was on CNS-active drugs at the time of the experiments. The study was approved by the ethics committee of the Medical Faculty of the Goethe-University Frankfurt, and conformed to the latest version of the Declaration of Helsinki.

Electromyography (EMG) recordings

Participants were seated on a comfortable reclining chair. MEPs were recorded from the right first dorsal interosseous muscle (FDI) by surface EMG. Pairs of Ag–AgCl surface electrodes were placed on the muscle belly and the metacarpophalangeal joint of the index finger. The EMG raw signal was amplified, band-pass filtered (20 Hz–2 kHz; Counterpoint Mk2 electromyograph, Dantec, Denmark), then digitized with an analog-to-digital converter (micro1401, CED, Cambridge, UK) at a sampling rate of 5 kHz and stored on a laboratory computer. Later, these data were transferred to a personal computer for offline analysis.

TMS

TMS was applied by using two Magstim 200 magnetic stimulators (Magstim Co., Whitland, Dyfed, UK) connected through a BiStim Module (Magstim Co.) with a figure-of-eight coil (external loop diameters, 70 mm). The current waveform was monophasic. The stimulating coil was held tangentially to the skull with the coil handle pointing backwards and laterally 45 deg away from the anterior–posterior axis. The centre of the coil junction was placed over the M1 hand area of the left hemisphere. The 'motor hot spot' was determined as the site where TMS consistently elicited the largest MEPs from the right FDI. This coil position was marked on the scalp by a felt pen to ensure constant coil placement throughout the experiment. At the hot spot, the intensity to produce MEPs of, on average, 1 mV peak-to-peak amplitude ($SI_{1\,mV}$), was determined. Thereafter, the active motor threshold (AMT) was determined, which was defined as the minimum stimulus intensity that elicited small MEP amplitudes $\geq$200 μV in at least 5 out of 10 consecutive trials during a weak voluntary contraction of the right FDI (Rossini *et al.* 1999). Stimulus intensities are indicated as a percentage of the maximum stimulator output.

IO-MEP were measured by stimulation at six intensity levels, ranging from 90% to 140% of $SI_{1\,mV}$ in steps of 10% $SI_{1\,mV}$. Ten stimuli were recorded at each intensity level in a randomized order of stimulus intensities. The inter-trial interval for these and all other measurements varied between 4.5 s and 5.5 s to limit anticipation of the next trial.

IO-SICI were measured using an established paired-pulse TMS protocol (Kujirai *et al.* 1993; Ziemann *et al.* 1996*b*). SICI was tested at an interstimulus interval (ISI) of 2.0 ms to study true $GABA_A$ergic synaptic neurotransmission and limit contamination by short-interval intracortical facilitation (Ziemann *et al.* 1996*b*; Fisher *et al.* 2002; Peurala *et al.* 2008). The intensity of the conditioning stimulus was set to four intensity levels, ranging from 70% to 100% AMT, while test stimulus intensity was adjusted throughout the experiment to maintain unconditioned MEPs of on average 1 mV in peak-to-peak amplitude. This is important because variation in unconditioned test MEP amplitude may result in SICI change (Sanger *et al.* 2001; Müller-Dahlhaus *et al.* 2008). These adjustments in test stimulus intensity were in general small, and are presented in Table 1 together with the mean unconditioned MEP amplitudes.

Ten trials of paired-pulses at each conditioning stimulus intensity level and test stimulus alone conditions were recorded in a random order. SICI was calculated as the ratio of the mean conditioned MEP over the mean unconditioned test MEP (Kujirai *et al.* 1993; Ziemann *et al.* 1996*b*).

TBS

TBS over the left M1 hand area was applied using a MagPro X100 magnetic stimulator connected to a 75 mm figure-of-eight coil (MagVenture, Farum, Denmark). The current had a biphasic waveform with a pulse width of 280 μs. The stimulating coil was held tangentially to the skull with the coil handle pointing backwards and laterally 45 deg away from the anterior–posterior axis, and the second phase of the biphasic current was directed from medial–anterior to lateral–posterior in M1. TBS was applied largely in accord with previous studies (Huang *et al.* 2005; Ziemann *et al.* 2008). In brief, TBS consisted of a burst of three pulses at 50 Hz (20 ms inter-pulse interval), which was repeated at 240 ms intervals (i.e. repetition rate, 4.2 Hz). This is a slight deviation from the original TBS protocol in which the 50 Hz bursts were

Table 1. Unconditioned test MEP amplitudes (in mV) and test stimulus intensity (SI, in % max. stimulator output) in the IO-SICI measurements (mean ± SEM)

		B0	B1	P1	P2	F	p
Experiment 1 ($n = 9$)							
Condition 1	MEP		1.11 ± 0.10	1.14 ± 0.18	1.29 ± 0.23	0.64	0.54
	SI		55.1 ± 2.6	54.4 ± 2.7	54.7 ± 2.7	1.93	0.18
Condition 2	MEP		1.21 ± 0.13	1.17 ± 0.14	1.26 ± 0.21	0.24	0.79
	SI		55.0 ± 2.6	55.0 ± 2.6	54.8 ± 2.5	1	0.39
Condition 3	MEP	1.26 ± 0.18	1.14 ± 0.15	1.46 ± 0.21	1.27 ± 0.15	0.96	0.43
	SI	55.2 ± 2.7	55.2 ± 2.7	54.8 ± 2.6	54.8 ± 2.7	1.16	0.34
Condition 4	MEP	1.18 ± 0.15	1.25 ± 0.19	1.11 ± 0.17	1.23 ± 0.23	0.34	0.80
	SI	55.4 ± 2.5	55.2 ± 2.4	55.4 ± 2.5	55.4 ± 2.5	1	0.41
Condition 5	MEP	1.04 ± 0.10	1.07 ± 0.12	0.99 ± 0.12	1.23 ± 0.25	0.63	0.60
	SI	54.9 ± 2.7	54.4 ± 2.8	54.6 ± 2.9	54.7 ± 2.8	1.35	0.28
Condition 6	MEP	1.20 ± 0.14	1.18 ± 0.14	1.08 ± 0.13	1.09 ± 0.08	0.54	0.66
	SI	54.2 ± 2.6	54.2 ± 2.6	54.4 ± 2.7	54.4 ± 2.7	0.18	0.91
Experiment 2 ($n = 8$)							
Condition 1	MEP		1.06 ± 0.09	0.99 ± 0.12	1.02 ± 0.10	0.37	0.67
	SI		52.4 ± 3.7	52.1 ± 3.8	52.4 ± 3.7	1	0.39
Condition 2	MEP		1.23 ± 0.09	1.19 ± 0.07	1.15 ± 0.07	0.62	0.55
	SI		52.4 ± 3.7	52.4 ± 3.7	52.4 ± 3.7	0	1
Condition 3	MEP	1.30 ± 0.10	1.32 ± 0.11	1.44 ± 0.13	1.36 ± 0.16	0.33	0.80
	SI	52.4 ± 3.7	51.9 ± 3.9	51.5 ± 4.0	51.9 ± 3.9	1	0.41
Condition 4	MEP	1.21 ± 0.11	1.33 ± 0.13	1.33 ± 0.09	1.23 ± 0.08	0.21	0.89
	SI	52.3 ± 3.8	51.0 ± 3.4	51.6 ± 3.7	51.9 ± 3.7	0.85	0.49
Condition 5	MEP	1.11 ± 0.16	1.09 ± 0.09	1.06 ± 0.07	1.13 ± 0.10	1,05	0.39
	SI	52.3 ± 3.7	52.6 ± 3.8	52.4 ± 3.8	52.9 ± 3.7	0.46	0.77
Condition 6	MEP	1.05 ± 0.11	0.96 ± 0.07	1.18 ± 0.09	1.20 ± 0.09	2,35	0.10
	SI	51.4 ± 3.5	51.4 ± 3.3	51.1 ± 3.2	50.9 ± 3.6	0.83	0.49

For experimental conditions 1–6 and time points (B0, B1, P1, P2), see Fig. 1. Note, that the unconditioned test MEP amplitudes did not change post-TBS in any of the experimental conditions (all $P > 0.1$) due to small adjustments of test stimulus intensity. MEP, motor-evoked potential; SI, stimulus intensity.

repeated at 200 ms intervals (i.e. repetition rate, 5 Hz; Huang *et al.* 2005). However, the repetition rate still falls well within the theta range (4–7 Hz), and the non-primed iTBS and cTBS effects on MEP amplitude closely replicated previous findings (see Results). iTBS consisted of 10 bursts of TBS train (30 pulses) repeated every 10 s for a total of 600 pulses. In cTBS, the TBS train was applied without interruption for a total of 600 pulses. Stimulus intensity was set at 70% ($TBS_{70\%AMT}$) or 80% ($TBS_{80\%AMT}$) AMT.

Experiment 1

Nine subjects (two women, seven men; mean age, 29.2 ± 6.9 years) participated in this experiment. We studied the effects of non-primed $iTBS_{80\%AMT}$ and $cTBS_{80\%AMT}$, and examined the priming effects of $iTBS_{80\%AMT}$ and $cTBS_{80\%AMT}$ on a subsequent $iTBS_{80\%AMT}$ or $cTBS_{80\%AMT}$ protocol on IO-MEP and IO-SICI (Fig. 1*A*). In the conditions of non-primed TBS protocols (Conditions 1 and 2, Fig. 1*A*), baseline IO-MEP amplitudes and IO-SICI were recorded (Baseline 1, B1). Then iTBS or cTBS was applied, and IO-MEP amplitudes and IO-SICI recordings were performed immediately (Post 1, P1) and 15 min (P2) after the TBS application. In the conditions of TBS-primed TBS protocols (Conditions 3–6, Fig. 1*A*), baseline IO-MEP amplitudes and IO-SICI were additionally recorded before priming TBS (Baseline 0, B0). We examined the interactions of opposite TBS–TBS protocols ($cTBS_{80\%AMT}$-primed $iTBS_{80\%AMT}$ and $iTBS_{80\%AMT}$-primed $cTBS_{80\%AMT}$, Conditions 3 and 4) and of identical TBS–TBS protocols ($iTBS_{80\%AMT}$-primed $iTBS_{80\%AMT}$ and $cTBS_{80\%AMT}$-primed $cTBS_{80\%AMT}$, Conditions 5 and 6). The interval between priming TBS and test TBS was set at 15 min to allow for the examination of priming TBS effects on IO-MEP and IO-SICI at time point B1 (cf. Fig. 1*A*), and because previous studies using transcranial direct current stimulation indicated strong interactions between successive protocols at intervals of 10–20 min (Monte-Silva *et al.* 2010; Fricke *et al.* 2011). The six experimental conditions were run in a balanced pseudo-randomized crossover design with at least 1 week between successive sessions. All experiments in a given individual were performed at the same time of day in

order to avoid inter-session variability due to diurnal effects (Sale *et al.* 2007; Ridding & Ziemann, 2010)

Experiment 2

Eight subjects (three women, five men; mean age, 27.4 ± 4.7 years) participated in this experiment (three subjects had also participated in Experiment 1). The aim of this experiment was to investigate to what extent priming TBS that by itself does not induce overt change in corticospinal excitability has an influence on subsequent TBS-induced plasticity. The procedures and time lines of Experiment 2 were the same as in Experiment 1, except that a lower stimulus intensity of 70% AMT was used for the priming TBS protocols (Fig. 1*B*). This intensity was chosen because neither $iTBS_{70\%AMT}$ nor $cTBS_{70\%AMT}$ produced any change in MEP amplitude in a previous study (McAllister *et al.* 2009). We examined the interaction of opposite TBS–TBS protocols ($cTBS_{70\%AMT}$-primed $iTBS_{80\%AMT}$ and $iTBS_{70\%AMT}$-primed $cTBS_{80\%AMT}$, Conditions 3 and 4) and of identical TBS–TBS protocols ($iTBS_{70\%AMT}$-primed $iTBS_{80\%AMT}$ and $cTBS_{70\%AMT}$-primed $cTBS_{80\%AMT}$, Conditions 5 and 6). In addition, the effects of non-primed $TBS_{80\%AMT}$ protocols (Conditions 1 and 2) were studied to compare the effects of the $TBS_{70\%AMT}$-primed $TBS_{80\%AMT}$ protocols with those of non-primed ones. The interval between the priming and the second TBS was again set at 15 min, and the six experimental conditions were run in a balanced pseudo-randomized crossover design with at least 1 week between successive sessions.

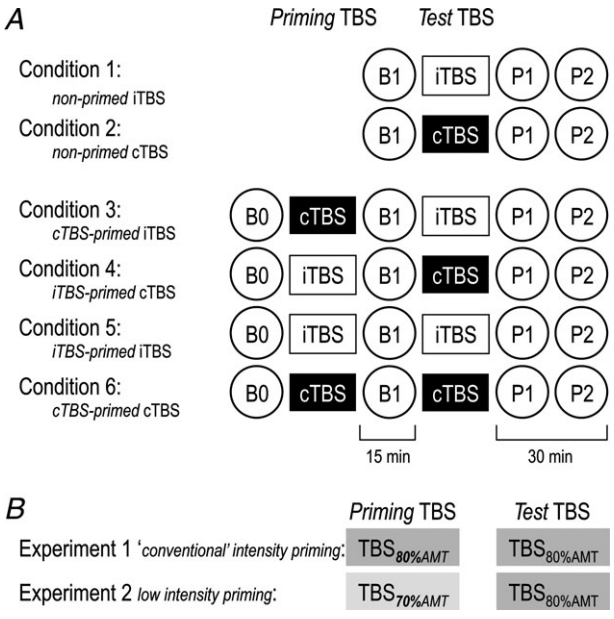

Figure 1. Experimental design
A, Experiments 1 and 2 each consisted of six experimental conditions: two non-primed theta burst stimulation (TBS) protocols (Conditions 1 and 2), and four TBS-primed TBS protocols (Conditions 3–6: white rectangles, intermittent TBS (iTBS); black rectangles, continuous TBS (cTBS)). IO-MEP amplitudes, a measure of corticospinal excitability, and IO-SICI, a measure of excitability of $GABA_A$ergic inhibitory neurons in motor cortex were recorded at each time point (circles, B0, B1, P1, P2). *B*, in Experiment 1, 'conventional' (Huang *et al.* 2005) priming and test TBS protocols (600 pulses at intensity of 80% AMT: $TBS_{80\%AMT}$) were used. In Experiment 2, low-intensity priming TBS (600 pulses at 70% AMT: $TBS_{70\%AMT}$) was followed by conventional test TBS (600 pulses at 80% AMT: $TBS_{80\%AMT}$).

Statistical analysis

Data were analysed using SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL 60606, USA). Generally, separate analyses were performed for the two different test TBS protocols ($iTBS_{80\%AMT}$, i.e. Conditions 1, 3 and 5 in Fig. 1*A*; $cTBS_{80\%AMT}$, i.e. Conditions 2, 4 and 6 in Fig. 1*A*) because we were interested in the interactions of a given test TBS protocol with different preceding priming TBS protocols.

To examine the effects of non-primed $TBS_{80\%AMT}$ on IO-MEP and IO-SICI, absolute MEP amplitudes were analysed using two-way repeated-measures (rm)ANOVAs with Time (B1, P1, P2: 3 levels) and Intensity (IO-MEP: 6 levels; IO-SICI: 4 levels) as the within-subject factors.

To compare the primed with the non-primed $TBS_{80\%AMT}$ effects on IO-MEP and IO-SICI, the individual MEP and SICI data at each level of stimulus intensity at time points P1 and P2 were normalized to those at B1 by calculating the ratios P1/B1 and P2/B1. The normalized data were analysed using three-way rmANOVAs with Experimental condition (Conditions 1, 3 and 5 for $iTBS_{80\%AMT}$, Conditions 2, 4 and 6 for $cTBS_{80\%AMT}$: 3 levels), Time (P1, P2: 2 levels) and Intensity (IO-MEP: 6 levels; IO-SICI: 4 levels) as the within-subject factors.

In case of significant main effects or interactions, *post hoc* testing was performed using Fisher's PLSD test.

Metaplasticity was assessed further as follows: changes in SICI by priming TBS were indexed by averaging IO-SICI across the four intensities of the conditioning stimulus to get one SICI value per time point, experiment and subject, and then calculating the increment between time points B1 (after priming TBS) and B0 (before priming TBS): $(SICI_{B1} - SICI_{B0})/SICI_{B0}$. Similarly, changes in MEP were indexed by averaging IO-MEP across the six stimulation intensities to get one MEP value per time point, experiment and subject, and then calculating the increment between time points P1, P2 (after test TBS) and B1 (before test TBS): $(MEP_{P1,P2} - MEP_{B1})/MEP_{B1}$. Finally, to reveal the specific effects of priming TBS, ΔSICI and ΔMEP were calculated, i.e. the increments $(SICI_{P1,P2} - SICI_{B1})/SICI_{B1}$ and $(MEP_{P1,P2} - MEP_{B1})/MEP_{B1}$ induced by primed TBS

minus non-primed TBS. The relation between ΔSICI and ΔMEP was tested by linear regression analyses. For this analysis, Conditions 3–6 in Fig. 1 were pooled.

IO-MEP amplitudes and IO-SICI at B1 (i.e. before test $TBS_{80\%AMT}$) were compared by two-way rmANOVAs with Experimental condition (Conditions 1, 3 and 5 for $iTBS_{80\%AMT}$, Conditions 2, 4 and 6 for $cTBS_{80\%AMT}$: 3 levels) and Intensity (IO-MEP: 6 levels, IO-SICI: 4 levels) as the within-subject effects, because differences in baseline excitability may contribute to any observed differential effects of priming TBS.

To study the effects of the low-intensity priming TBS protocols in Experiment 2, IO-MEP amplitudes and IO-SICI at B0 and B1 were also compared using three-way rmANOVAs with Time (B0, B1: 2 levels), Priming protocol (Conditions 4 and 5 in Fig. 1*A* for priming $iTBS_{70\%AMT}$, Conditions 3 and 6 in Fig. 1*A* for priming $cTBS_{70\%AMT}$: 2 levels) and Intensity (IO-MEP: 6 levels; IO-SICI: 4 levels) as the within-subject factors.

Furthermore, the unconditioned test MEP amplitudes in the IO-SICI measurements at B0, B1, P1 and P2 were compared using one-way rmANOVAs with Time as within-subject factor to ensure stable unconditioned MEPs throughout an experiment.

Finally, we investigated the influence of the priming TBS-induced changes in SICI on the test TBS effects on MEP amplitude by using correlation analyses to clarify the interrelation between intracortical inhibitory and corticospinal excitatory neural circuits. For this analysis, Conditions 3–6 in Fig. 1 were pooled. Changes in SICI by priming TBS were indexed by $(SICI_{B1} - SICI_{B0})/SICI_{B0}$, and changes in MEP by primed TBS were indexed by ΔMEP. Linear regression analyses were calculated for $(SICI_{B1} - SICI_{B0})/SICI_{B0}$ *versus* ΔMEP. Additional linear regression analyses were performed for $(MEP_{B1} - MEP_{B0})/MEP_{B0}$ *versus* ΔMEP to reveal any dependence of changes in MEP amplitude induced by primed TBS on those induced by priming TBS, and for $(MEP_{B1} - MEP_{B0})/MEP_{B0}$ *versus* $(SICI_{B1} - SICI_{B0})/SICI_{B0}$ to reveal concomitant changes in MEP amplitude and SICI induced by priming TBS. Indeed, we found significant correlations in these additional linear regression analyses (see Results). Therefore, a *partial* correlation was finally computed that removed the effects of $(MEP_{B1} - MEP_{B0})/MEP_{B0}$ from the correlation between $(SICI_{B1} - SICI_{B0})/SICI_{B0}$ and ΔMEP.

Mauchly's test was used to check for violation of sphericity in the rmANOVAs, and whenever Mauchly's $W < 0.05$ the degrees of freedom were adjusted using the Greenhouse–Geisser correction. In all tests, a value of $P \leq 0.05$ was considered to be statistically significant. All data were expressed as means ± standard error of the mean.

Results

No participant reported any adverse effects during or after the experiments.

Experiment 1 ($TBS_{80\%AMT}$-primed $TBS_{80\%AMT}$ *versus* non-primed $TBS_{80\%AMT}$)

Baseline IO-MEP amplitudes at B1. A two-way rmANOVA of absolute IO-MEP amplitudes at B1 (iTBS Conditions 1, 3 and 5 in Fig. 1*A*) showed significant effects of Experimental condition ($F_{2,16} = 5.73$, $P = 0.013$; grand average MEP amplitude over all intensities, non-primed $iTBS_{80\%AMT} = 2.08 \pm 0.21$ mV; $cTBS_{80\%AMT}$-primed $iTBS_{80\%AMT} = 1.92 \pm 0.20$ mV; $iTBS_{80\%AMT}$-primed $iTBS_{80\%AMT} = 2.60 \pm 0.23$ mV), but no interaction between Experimental condition and Intensity. *Post hoc* testing revealed significant differences between $iTBS_{80\%AMT}$-primed $iTBS_{80\%AMT}$ and non-primed $iTBS_{80\%AMT}$ ($P = 0.023$), but not between $cTBS_{80\%AMT}$-primed $iTBS_{80\%AMT}$ and non-primed $iTBS_{80\%AMT}$ ($P = 0.48$). Another two-way rmANOVA of absolute IO-MEP amplitudes at B1 (cTBS Conditions 2, 4 and 6 in Fig. 1*A*; grand average MEP amplitude over all intensities: non-primed $cTBS_{80\%AMT} = 2.39 \pm 0.22$ mV; $iTBS_{80\%AMT}$-primed $cTBS_{80\%AMT} = 2.76 \pm 0.25$ mV; $cTBS_{80\%AMT}$-primed $cTBS_{80\%AMT} = 1.94 \pm 0.23$ mV) did not show significant effects of Experimental condition ($F_{2,16} = 2.90$, $P = 0.08$) or its interaction with Intensity ($F_{3.11,24.88} = 1.48$, $P = 0.24$).

These data provided part of the rationale for Experiment 2 (see below), which was designed to use low-intensity priming TBS in order to avoid significant changes in IO-MEP at B1 as a possible contaminating source for the priming TBS effects on the subsequent test TBS protocol.

Non-primed TBS effects on IO-MEP. A two-way rmANOVA of the non-primed $iTBS_{80\%AMT}$ effects on IO-MEP showed a significant effect of Time ($F_{2,16} = 5.58$, $P = 0.015$). *Post hoc* comparisons revealed increased IO-MEP at both time points after non-primed $iTBS_{80\%AMT}$ (P1 *versus* B1: $P = 0.039$; P2 *versus* B1: $P = 0.005$; Fig. 2*A*).

Similarly, a two-way rmANOVA of the non-primed $cTBS_{80\%AMT}$ effects on IO-MEP revealed a significant effect of Time ($F_{2,16} = 11.85$, $P < 0.001$), and *post hoc* comparisons showed decreased IO-MEP at both time points after non-primed $cTBS_{80\%AMT}$ (P1 *versus* B1: $P < 0.001$; P2 *versus* B1: $P < 0.001$; Fig. 2*B*).

These data replicate previous findings on the bidirectional change of MEP amplitude by iTBS *versus* cTBS (Huang *et al.* 2005; Suppa *et al.* 2008).

TBS-primed TBS effects on IO-MEP. A three-way rmANOVA on the comparison between TBS-primed iTBS effects and non-primed iTBS effects on IO-MEP demonstrated a significant effect of Experimental condition ($F_{2,16} = 14.15$, $P < 0.001$) and Intensity ($F_{2.28,11.4} = 11.50$, $P < 0.001$), and a significant interaction of Experimental condition with Intensity ($F_{4.10,32.8} = 5.40$, $P = 0.002$), while Time or any of its interactions were not significant (Fig. 3*A*). *Post hoc* comparisons revealed that $cTBS_{80\%AMT}$-primed iTBS resulted in significantly enhanced IO-MEP compared with non-primed iTBS ($F_{1,8} = 8.84$, $P = 0.018$), while $iTBS_{80\%AMT}$-primed iTBS resulted in IO-MEP depression when compared with non-primed iTBS ($F_{1,8} = 9.62$, $P = 0.015$). These differences were significant at several single stimulation intensities (indicated by asterisks in Fig. 3*A*).

A three-way rmANOVA on the comparison between TBS-primed cTBS effects and non-primed cTBS effects on IO-MEP demonstrated a significant effect of Experimental condition ($F_{2,16} = 21.36$, $P < 0.001$), while Intensity ($F_{1.22,9.76} = 2.72$, $P = 0.13$), the interaction of Experimental condition with Intensity ($F_{1.62,12.96} = 2.05$, $P = 0.17$) and Time or any of its interactions were not significant (Fig. 3*B*). $cTBS_{80\%AMT}$-primed cTBS *versus* non-primed cTBS resulted in a significant effect of Experimental condition ($F_{1,8} = 25.88$, $P < 0.001$), which was explained by a shift from MEP depression with non-primed cTBS to MEP potentiation with $cTBS_{80\%AMT}$-primed cTBS (Fig. 3*B*). In summary, the IO-MEP data are in accordance with homeostatic regulation of MEP amplitude in subsequent TBS protocols.

Baseline IO-SICI at B1. A two-way rmANOVA of IO-SICI at B1 (iTBS Conditions 1, 3 and 5 in Fig. 1*A*) did not show a significant effect of Experimental condition ($F_{2,16} = 2.60$, $P = 0.11$; grand average SICI over all intensities, non-primed $iTBS_{80\%AMT} = 0.42 \pm 0.04$; $cTBS_{80\%AMT}$-primed $iTBS_{80\%AMT} = 0.45 \pm 0.06$; $iTBS_{80\%AMT}$-primed $iTBS_{80\%AMT} = 0.36 \pm 0.05$), or the interaction between Experimental condition and Intensity ($F_{3.49,27.92} = 0.87$, $P = 0.48$). In contrast, the other two-way rmANOVA of IO-SICI at B1 (cTBS Conditions 2, 4 and 6 in Fig. 1*A*; grand average SICI over all intensities: non-primed $cTBS_{80\%AMT} = 0.33 \pm 0.05$; $iTBS_{80\%AMT}$-primed $cTBS_{80\%AMT} = 0.28 \pm 0.04$; $cTBS_{80\%AMT}$-primed $cTBS_{80\%AMT} = 0.49 \pm 0.07$) showed a significant effect of Experimental condition ($F_{2,16} = 6.84$, $P = 0.007$) but not of its interaction with Intensity ($F_{1.86,14.88} = 0.99$, $P = 0.39$). *Post hoc* testing revealed a significant difference between non-primed $cTBS_{80\%AMT}$ and $cTBS_{80\%AMT}$-primed $cTBS_{80\%AMT}$

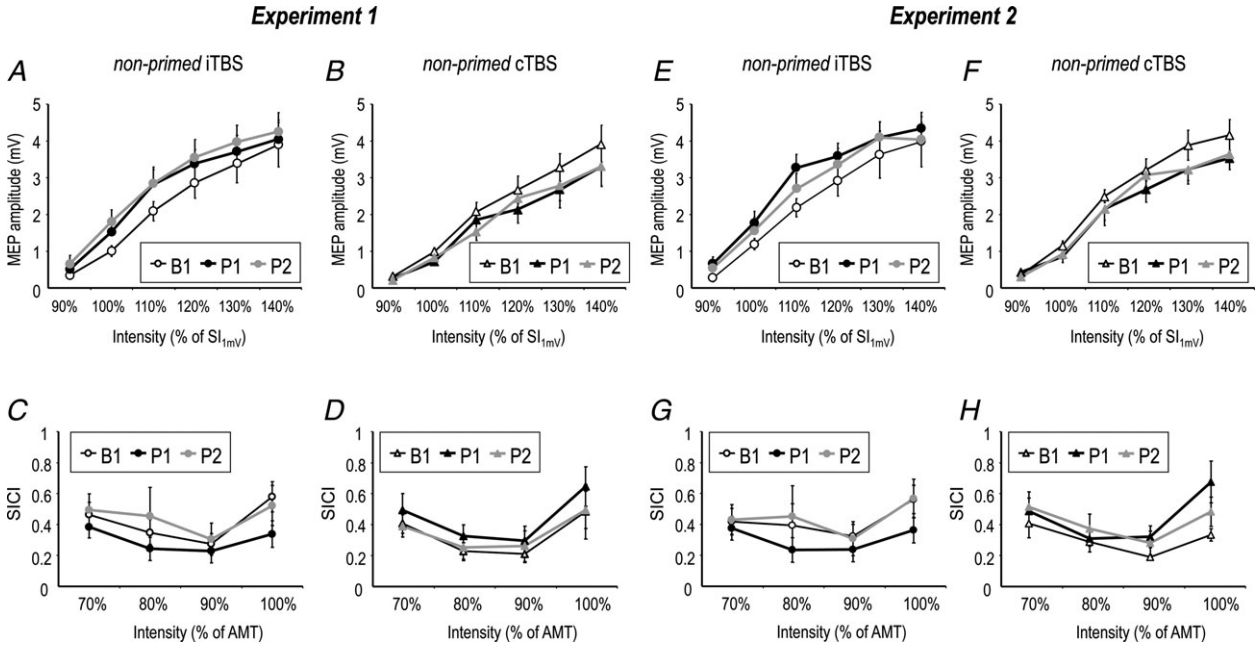

Figure 2. Effects of non-primed theta burst stimulation (TBS) on IO-motor-evoked potential (MEP; *A* and *B*, *E* and *F*) and IO-short-interval intracortical inhibition (SICI; *C* and *D*, *G* and *H*) in Experiment 1 (*A–D*) and Experiment 2 (*E–H*) at post-TBS time points P1 (black symbols) and P2 (grey symbols) *versus* baseline B1 (white symbols)

Note increase of IO-MEP after non-primed intermittent (i)TBS (*A* and *E*), decrease of IO-MEP after non-primed continuous (c)TBS (*B* and *F*), but no significant change of IO-SICI in any of the conditions (*C* and *D*, *G* and *H*). All data are means ± SEM. AMT. active motor threshold.

($P = 0.019$), but not between non-primed $cTBS_{80\%AMT}$ and $iTBS_{80\%AMT}$-primed $cTBS_{80\%AMT}$ ($P = 0.35$).

Non-primed TBS effects on IO-SICI. A two-way rmANOVA of the effects of non-primed $iTBS_{80\%AMT}$ on IO-SICI did not reveal significant effects of Time ($F_{2,16} = 1.99$, $P = 0.17$) or its interaction with Intensity ($F_{2.21,17.68} = 1.26$, $P = 0.31$; Fig. 2*C*). Similarly, non-primed $cTBS_{80\%AMT}$ showed no effects of Time ($F_{2,16} = 2.03$, $P = 0.16$) or its interaction with Intensity ($F_{1.75,14} = 0.20$, $P = 0.79$; Fig. 2*D*).

TBS-primed TBS effects on IO-SICI. A three-way rmANOVA on TBS-primed iTBS *versus* non-primed iTBS effects (Conditions 1, 3 and 5 in Fig. 1*A*) on IO-SICI demonstrated a significant effect of Experimental condition ($F_{2,16} = 5.71$, $P = 0.013$), but not of its interactions with Time or Intensity (Fig. 4*A*). *Post hoc*

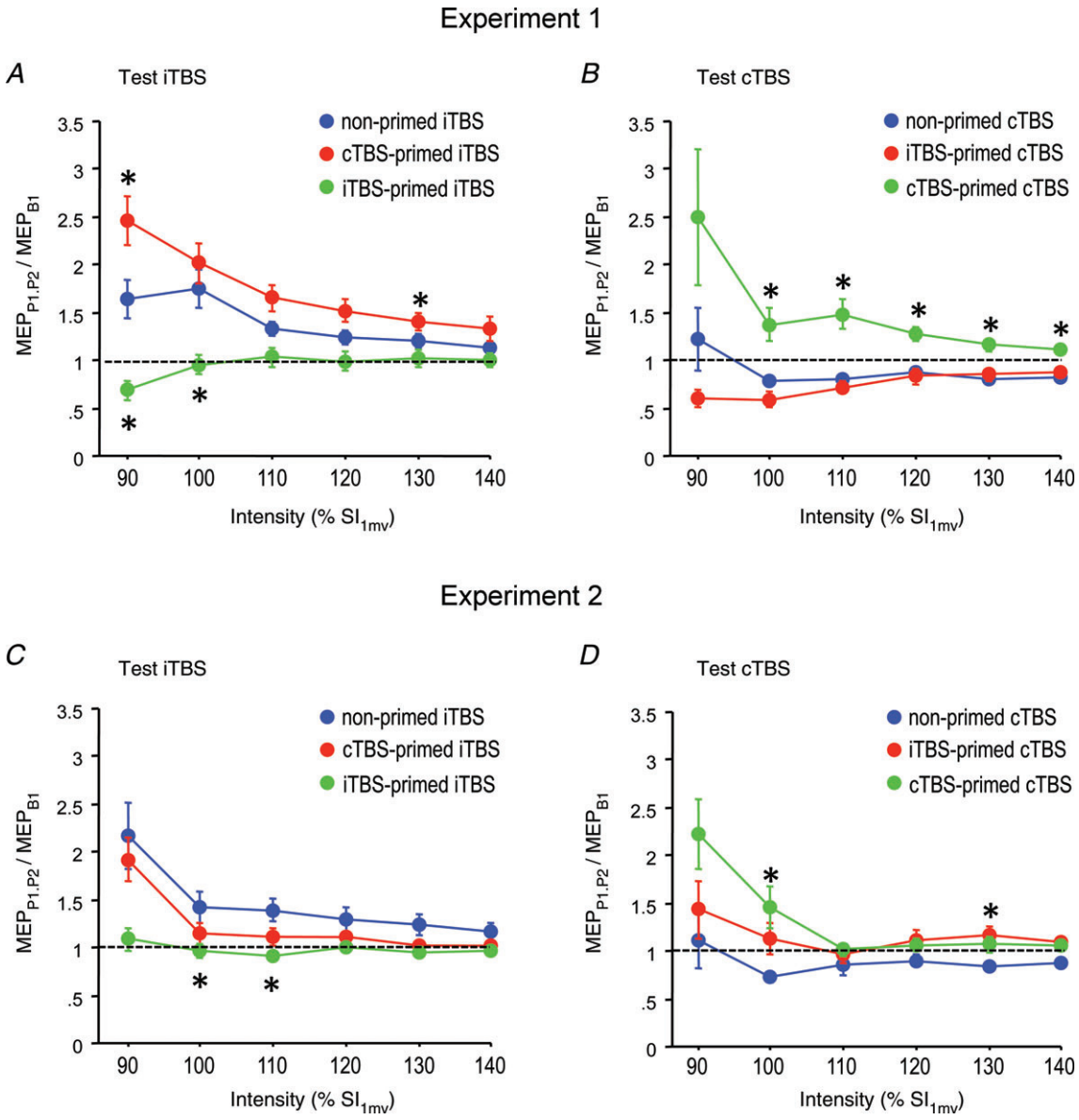

Figure 3. Theta burst stimulation (TBS)-primed intermittent (i)TBS effects (*A* and *C*) and TBS-primed continuous (c)TBS effects (*B* and *D*) on IO-motor-evoked potential (MEP) in Experiment 1 (*A* and *B*) and Experiment 2 (*C* and *D*) expressed as IO-MEP averaged across time points P1 and P2 normalized to B1
Red curves: opposite priming TBS and test TBS; green curves: identical priming and test TBS; blue curves: non-primed TBS (for comparison). Note homeostatic interactions with enhancement of non-primed TBS effects by opposite priming in Experiment 1, and suppression of non-primed TBS effects by identical priming in Experiments 1 and 2. All data are means ± SEM. Asterisks: $P < 0.05$ (comparison of TBS-primed TBS effects with non-primed TBS effects).

comparisons revealed that $iTBS_{80\%AMT}$-primed iTBS resulted in significantly decreased IO-SICI compared with non-primed iTBS ($P = 0.034$), while $cTBS_{80\%AMT}$-primed iTBS was not significantly different from non-primed iTBS ($P = 0.35$). The other rmANOVA (Conditions 2, 4 and 6 in Fig. 1*A*) on IO-SICI also revealed a significant effect of Experimental condition ($F_{2,16} = 6.54$, $P = 0.008$), but not of its interactions with Time or Intensity (Fig. 4*B*). However, the *post hoc* tests did not show significant differences of IO-SICI after $TBS_{80\%AMT}$-primed cTBS compared with non-primed cTBS (cTBS-primed cTBS: $P = 0.07$; iTBS-primed cTBS: $P = 0.10$; Fig 4*B*).

Unconditioned MEPs in IO-SICI measurements of Experiments 1 and 2. Table 1 summarizes the unconditioned MEP amplitudes in all Experimental conditions of Experiments 1 and 2. There were no effects

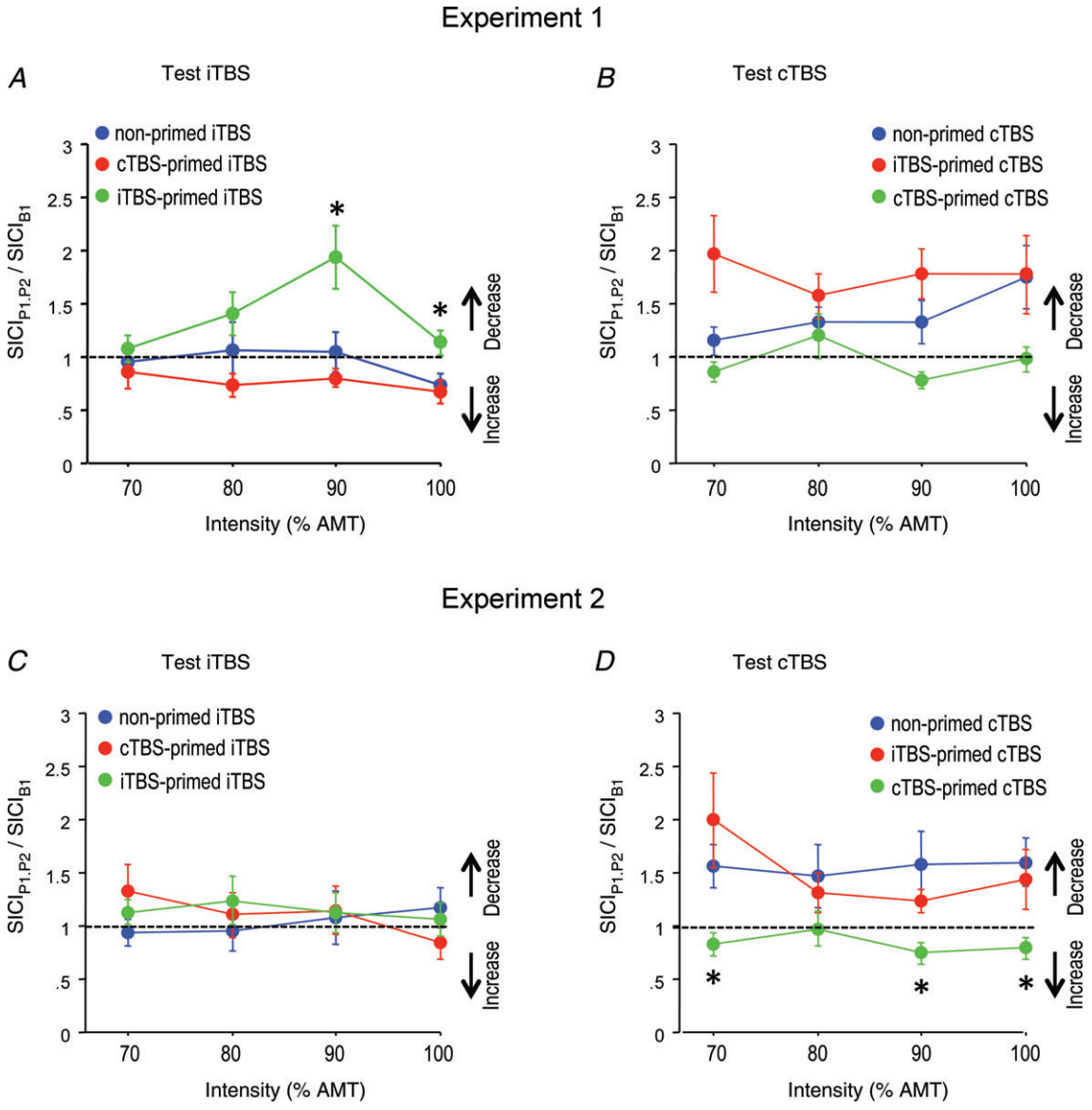

Figure 4. Theta burst stimulation (TBS)-primed intermittent (i)TBS effects (*A* and *C*) and TBS-primed continuous (c)TBS effects (*B* and *D*) on IO-short-interval intracortical inhibition (SICI) in Experiment 1 (*A* and *B*) and Experiment 2 (*C* and *D*) expressed as IO-SICI averaged across time points P1 and P2 normalized to B1

Red curves: opposite priming TBS and test TBS; green curves: identical priming and test TBS; blue curves: non-primed TBS (for comparison). Note homeostatic interactions with iTBS-primed iTBS leading to a decrease of IO-SICI when compared with non-primed iTBS in Experiment 1, and cTBS-primed cTBS leading to an increase in IO-SICI when compared with non-primed cTBS in Experiment 2. All data are means ± SEM. Asterisks: $P < 0.05$ (comparison of TBS-primed TBS effects with non-primed TBS effects).

of Time in any of these experiments (all $P > 0.10$), and the targeted peak-to-peak amplitude of 1 mV was overall closely achieved. Thus, there was no variation of unconditioned MEP amplitude that could have contributed to the TBS-primed TBS effects on IO-SICI described above.

Correlation between ΔSICI and ΔMEP. Regression analysis revealed a significant negative linear correlation between ΔSICI and ΔMEP (Fig. 5; $r = -0.46$, $P = 0.005$), i.e. increases (decreases) in SICI (reflected by negative and positive ΔSICI, respectively) were associated with increases (decreases) in MEP. This provides additional evidence that there is parallel homeostatic regulation of excitability in a $GABA_A$ergic inhibitory intracortical circuit and in the excitatory corticospinal projection.

Correlation of SICI change induced by priming TBS with ΔMEP induced by primed TBS. Linear regression of $(SICI_{B1} - SICI_{B0})/SICI_{B0}$ showed a significant positive correlation with ΔMEP (Fig. 6*A*; $r = 0.61$, $P < 0.0001$), i.e. a decrease (increase) in SICI by priming $TBS_{80\%AMT}$ was associated with a positive (negative) ΔMEP.

Linear regression analyses of $(MEP_{B1} - MEP_{B0})/MEP_{B0}$ *versus* ΔMEP revealed a significant negative correlation ($r = -0.64$, $P < 0.0001$; data not shown). Similarly, linear regression analysis of $(MEP_{B1} - MEP_{B0})/MEP_{B0}$ *versus* $(SICI_{B1} - SICI_{B0})/SICI_{B0}$ also showed a significant negative correlation ($r = -0.60$, $P < 0.0001$; data not shown).

Finally, the partial correlation analysis that removed the effects of $(MEP_{B1} - MEP_{B0})/MEP_{B0}$ in the correlation of $(SICI_{B1} - SICI_{B0})/SICI_{B0}$ with ΔMEP still showed a significant positive correlation ($r = 0.37$, $P = 0.027$), strongly suggesting that changes in intracortical inhibitory circuits induced by priming TBS contribute to the control of homeostatic metaplasticity in the corticospinal excitatory pathway.

In contrast, the partial correlation analysis that removed the effects of $(SICI_{B1} - SICI_{B0})/SICI_{B0}$ in the correlation of $(MEP_{B1} - MEP_{B0})/MEP_{B0}$ with ΔSICI was not significant ($P = 0.67$). This nil result suggests that homeostatic metaplasticity of SICI is not a simple consequence of the changes in MEP amplitude induced by priming TBS.

Experiment 2 ($TBS_{70\%AMT}$-primed $TBS_{80\%AMT}$ *versus* non-primed $TBS_{80\%AMT}$)

Baseline IO-MEP amplitudes at B1. A two-way rmANOVA of absolute IO-MEP amplitudes at B1 (iTBS Conditions 1, 3 and 5 in Fig. 1*A*; grand average MEP amplitudes across all intensities: non-primed $iTBS_{80\%AMT} = 2.12 \pm 0.23$ mV; $cTBS_{70\%AMT}$-primed $iTBS_{80\%AMT} = 2.61 \pm 0.26$ mV; $iTBS_{70\%AMT}$-primed $iTBS_{80\%AMT} = 2.40 \pm 0.23$ mV) did not show significant main effects of Experimental condition ($F_{2,14} = 0.94$, $P = 0.41$) or its interaction with Intensity ($F_{2.09,14.63} = 0.49$, $P = 0.63$). Similarly, IO-MEP amplitudes at B1 (cTBS Conditions 2, 4 and 6 in Fig. 1*A*; grand average MEP amplitudes across all intensities: non-primed $cTBS_{80\%AMT} = 2.57 \pm 0.24$ mV; $iTBS_{70\%AMT}$-primed $cTBS_{80\%AMT} = 2.20 \pm 0.20$ mV; $cTBS_{70\%AMT}$-primed $cTBS_{80\%AMT} = 2.24 \pm 0.21$ mV) did not show a significant effect of Experimental condition ($F_{2,14} = 1.30$, $P = 0.30$). The interaction of Experimental condition with Intensity was significant ($F_{4.39,30.73} = 3.42$, $P = 0.017$), but there were no differences in MEP amplitude between Experimental conditions at any single Intensity (all $P > 0.05$). Therefore, Experiment 2 achieved the goal to avoid the significant IO-MEP baseline differences between experimental conditions in Experiment 1.

Effects of priming $TBS_{70\%AMT}$ on IO-MEP (comparison of B1 with B0). A three-way rmANOVA of priming $iTBS_{70\%AMT}$ did not reveal an effect of Time (B1 *versus* B0; grand average MEP amplitude over protocol and intensity, $B1 = 2.30 \pm 0.15$ mV; $B0 = 2.17 \pm 0.14$ mV), or the interactions of Time with Protocol ($iTBS_{70\%AMT}$-primed $cTBS_{80\%AMT}$ *versus* $iTBS_{70\%AMT}$-primed $iTBS_{80\%AMT}$) or Intensity (all $P > 0.3$). Similarly, a three-way rmANOVA of priming $cTBS_{70\%AMT}$ did not reveal an effect of Time (B1 *versus* B0; grand average MEP amplitude over protocol and intensity, $B1 = 2.42 \pm 0.17$ mV; $B0 = 2.35 \pm 0.16$ mV), or the interactions of Time with Protocol ($cTBS_{70\%AMT}$-primed $iTBS_{80\%AMT}$ *versus*

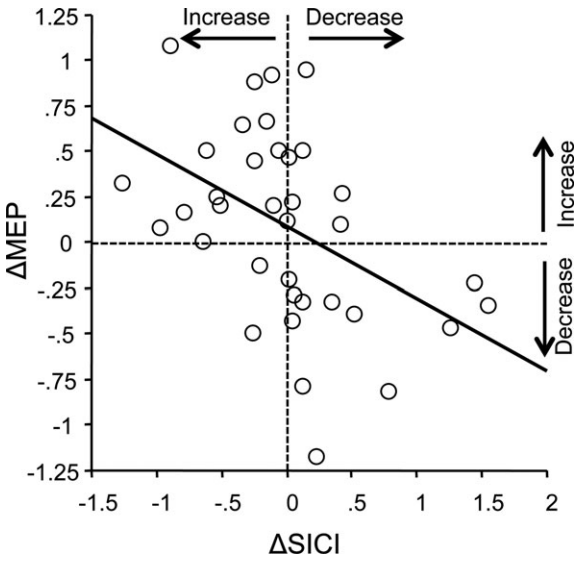

Figure 5. Negative linear correlation between Δshort-interval intracortical inhibition (SICI; *x*-axis) and Δmotor-evoked potential (MEP; *y*-axis) induced by primed TBS minus non-primed TBS in Experiment 1
The thick line indicates the regression line.

$cTBS_{70\%AMT}$-primed $cTBS_{80\%AMT}$) or Intensity (all $P > 0.4$).

These nil findings confirm the data from one previous study that priming $TBS_{70\%AMT}$ does not produce significant effects on MEP amplitude (McAllister *et al.* 2009) and indicate, in addition to the lack of difference of IO-MEP between Experimental conditions at B1, that the observed effects of priming $TBS_{70\%AMT}$ on subsequent TBS (see below) occurred in the absence of significant changes in corticospinal excitability induced by the priming protocol itself.

Non-primed TBS effects on IO-MEP. A two-way rmANOVA of the non-primed $iTBS_{80\%AMT}$ effects on IO-MEP showed a significant effect of Time ($F_{2,14} = 3.72$, $P = 0.05$), and *post hoc* comparisons revealed increased IO-MEP at P1 after non-primed $iTBS_{80\%AMT}$ (P1 *versus* B1: $P = 0.017$; P2 *versus* B1: $P = 0.17$; Fig. 2*E*).

Similarly, a two-way rmANOVA of the non-primed $cTBS_{80\%AMT}$ effects on IO-MEP revealed a significant effect of Time ($F_{2,14} = 4.02$, $P = 0.042$), and *post hoc* comparisons showed decreased IO-MEP at both time points after non-primed $cTBS_{80\%AMT}$ (P1 *versus* B1: $P = 0.019$; P2 *versus* B1: $P = 0.047$; Fig. 2*F*).

These data largely replicate the findings from Experiment 1.

TBS-primed TBS effects on IO-MEP. A three-way rmANOVA for the test iTBS protocols (Conditions 1, 3 and 5 in Fig. 1*A*) demonstrated significant effects of Experimental condition ($F_{2,14} = 6.22$, $P = 0.012$), Intensity ($F_{2.02,15.4} = 11.81$, $P = 0.001$), and the interaction between Experimental condition, Time and Intensity ($F_{3.58,25.03} = 3.38$, $P = 0.043$). *Post hoc* testing showed that IO-MEP between non-primed $iTBS_{80\%AMT}$ and $iTBS_{70\%AMT}$-primed $iTBS_{80\%AMT}$ were different ($P = 0.003$), while the difference between non-primed $iTBS_{80\%AMT}$ and $cTBS_{70\%AMT}$-primed $iTBS_{80\%AMT}$ was not significant ($P = 0.11$; Fig. 3*C*).

Another three-way rmANOVA for the test cTBS protocols (Conditions 2, 4 and 6 in Fig. 1*A*) demonstrated a significant effect of Experimental condition ($F_{2,14} = 3.64$, $P = 0.05$), but not of the interaction between Experimental condition and Intensity ($F_{1.49,10.43} = 2.31$, $P = 0.15$). *Post hoc* testing showed that IO-MEP between non-primed $cTBS_{80\%AMT}$ and $cTBS_{70\%AMT}$-primed $cTBS_{80\%AMT}$ were different ($P = 0.018$), while the difference between non-primed $cTBS_{80\%AMT}$ and $iTBS_{70\%AMT}$-primed $cTBS_{80\%AMT}$ was not significant ($P = 0.12$; Fig. 3*D*).

In summary, these data are qualitatively different from Experiment 1 because the weaker priming TBS resulted only in suppression of the effects of a subsequent *identical* TBS protocol ($iTBS_{70\%AMT}$-primed $iTBS_{80\%AMT}$, $cTBS_{70\%AMT}$-primed $cTBS_{80\%AMT}$), but no longer in enhancement of the effects of a subsequent *opposite* TBS protocol ($cTBS_{70\%AMT}$-primed $iTBS_{80\%AMT}$, $iTBS_{70\%AMT}$-primed $cTBS_{80\%AMT}$).

Baseline IO-SICI at B1. A two-way rmANOVA of IO-SICI at B1 (iTBS Conditions 1, 3 and 5 in Fig. 1*A*) did not show a significant effect of Experimental condition ($F_{2,14} = 0.27$, $P = 0.77$; grand average SICI over all intensities, non-primed $iTBS_{80\%AMT} = 0.43 \pm$

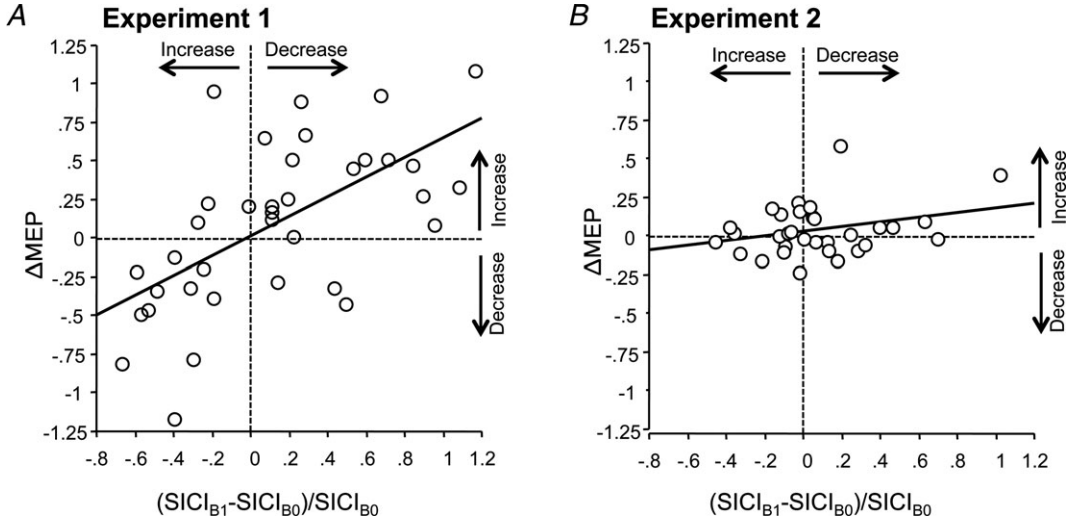

Figure 6. Regression plots between changes in short-interval intracortical inhibition (SICI; *x*-axes) induced by priming TBS and changes in MEP amplitude (Δmotor-evoked potential (MEP), *y*-axes) induced by primed TBS minus non-primed TBS in Experiment 1 (*A*) and Experiment 2 (*B*)
The thick lines indicate regression lines. Note the significant linear correlation between changes in SICI and ΔMEP in Experiment 1.

0.05; $cTBS_{70\%AMT}$-primed $iTBS_{80\%AMT} = 0.38 \pm 0.06$; $iTBS_{70\%AMT}$-primed $iTBS_{80\%AMT} = 0.41 \pm 0.05$), or the interaction between Experimental condition and Intensity ($F_{2.71,18.97} = 1.92$, $P = 0.17$). Similarly, the other two-way rmANOVA of IO-SICI at B1 (cTBS Conditions 2, 4 and 6 in Fig. 1*A*; grand average SICI over all intensities: non-primed $cTBS_{80\%AMT} = 0.32 \pm 0.04$; $iTBS_{70\%AMT}$-primed $cTBS_{80\%AMT} = 0.34 \pm 0.05$; $cTBS_{70\%AMT}$-primed $cTBS_{80\%AMT} = 0.49 \pm 0.08$) did not show a significant effect of Experimental condition ($F_{2,14} = 2.67$, $P = 0.10$) or of its interaction with Intensity ($F_{2.29,16.03} = 0.57$, $P = 0.60$). Therefore, there were no differences in IO-SICI at B1, which could have accounted for the TBS-primed TBS effects on IO-SICI (see below).

Effects of priming $TBS_{70\%AMT}$ on IO-SICI (comparison of B1 with B0). A three-way rmANOVA of priming $iTBS_{70\%AMT}$ did not reveal an effect of Time (B1 *versus* B0; grand average SICI over protocol and intensity, $B1 = 0.38 \pm 0.03$; $B0 = 0.41 \pm 0.04$), or of the interactions of Time with Protocol ($iTBS_{70\%AMT}$-primed $cTBS_{80\%AMT}$ *versus* $iTBS_{70\%AMT}$-primed $iTBS_{80\%AMT}$) or Time with Intensity (all $P > 0.5$). Similarly, a three-way rmANOVA of priming $cTBS_{70\%AMT}$ did not reveal an effect of Time (B1 *versus* B0; grand average SICI over protocol and intensity, $B1 = 0.43 \pm 0.03$; $B0 = 0.37 \pm 0.04$), or of the interactions of Time with Protocol ($cTBS_{70\%AMT}$-primed $iTBS_{80\%AMT}$ *versus* $cTBS_{70\%AMT}$-primed $cTBS_{80\%AMT}$) or Time with Intensity (all $P > 0.15$).

Non-primed TBS effects on IO-SICI. A two-way rmANOVA of the effects of non-primed $iTBS_{80\%AMT}$ on IO-SICI did not reveal significant effects of Time ($F_{2,14} = 3.52$, $P = 0.06$) or its interaction with Intensity ($F_{6,42} = 2.25$, $P = 0.06$; Fig. 2*G*). Similarly, non-primed $cTBS_{80\%AMT}$ showed no effects of Time ($F_{2,14} = 2.53$, $P = 0.11$) or its interaction with Intensity ($F_{3.06,21.42} = 1.49$, $P = 0.25$; Fig. 2*H*).

TBS-primed TBS effects on IO-SICI. A three-way rmANOVA on TBS-primed iTBS *versus* non-primed iTBS effects (Conditions 1, 3 and 5 in Fig. 1*A*) on IO-SICI did not show a significant effect of Experimental condition ($F_{2,14} = 0.16$, $P = 0.86$) or of its interactions with Time or Intensity (Fig. 4*C*). The other rmANOVA on TBS-primed cTBS *versus* non-primed cTBS effects (Conditions 2, 4 and 6 in Fig. 1*A*) on IO-SICI showed a significant effect of Experimental condition ($F_{2,14} = 3.78$, $P = 0.049$), but not of its interactions with Time or Intensity (Fig. 4*D*). *Post hoc* tests showed an increase of IO-SICI after $cTBS_{70\%AMT}$-primed $cTBS_{80\%AMT}$ compared with non-primed $cTBS_{80\%AMT}$ ($P = 0.027$), but no difference of IO-SICI after $iTBS_{70\%AMT}$-primed $cTBS_{80\%AMT}$ compared with non-primed $cTBS_{80\%AMT}$ ($P = 0.85$; Fig 4*D*).

Correlation between ΔSICI and ΔMEP. Regression analysis did not reveal a significant linear correlation between ΔSICI and ΔMEP ($r = -0.14$, $P = 0.44$).

Correlation of SICI change induced by priming TBS with ΔMEP induced by primed TBS. $(SICI_{B1} - SICI_{B0})/SICI_{B0}$ induced by priming $TBS_{70\%AMT}$ did not correlate with ΔMEP induced by primed TBS minus non-primed TBS (Fig. 6*B*; $r = 0.16$, $P = 0.37$).

Discussion

This study provided two important novel findings at the systems level of human cerebral cortex. (1) Homeostatic metaplasticity regulates excitability not only of the excitatory corticospinal pathway but, in parallel, also of inhibitory intracortical neural circuits. Homeostatic metaplasticity could be demonstrated even in the absence of changes in IO-MEP or IO-SICI by priming TBS, suggesting true metaplasticity rather than, for example, depotentiation or de-depression (Abraham, 2008). (2) Changes in excitability of $GABA_A$ergic circuits by priming TBS correlated with the magnitude and direction of homeostatic metaplasticity of MEP amplitude, strongly suggesting a role of $GABA_A$ergic neurotransmission for controlling plasticity in excitatory corticospinal circuits.

Homeostatic regulation of excitatory plasticity (MEP amplitude)

MEP amplitude represents excitability of excitatory neurotransmission in the corticospinal projection (Hallett, 2007; Di Lazzaro *et al.* 2008*b*). Data in Experiment 1 are in full agreement with homeostatic regulation of MEP plasticity in two subsequent TBS protocols. This is in accord with several previous studies that demonstrated homeostatic regulation of MEP plasticity when using different plasticity-inducing non-invasive brain stimulation protocols or practice-dependent plasticity (Iyer *et al.* 2003; Lang *et al.* 2004; Siebner *et al.* 2004; Ziemann *et al.* 2004; Stefan *et al.* 2006; Müller *et al.* 2007; Hamada *et al.* 2008; Fricke *et al.* 2011). Gamboa and colleagues examined interactions of priming TBS – test TBS protocols at different intervals: two identical iTBS protocols at 5 and 20 min intervals resulted in significantly less MEP amplitude increase when compared with non-primed iTBS (Gamboa *et al.* 2011). Likewise, two identical cTBS protocols at 2 and 5 min intervals suppressed the MEP depression induced by non-primed cTBS or even resulted in a switch towards MEP potentiation (Gamboa *et al.* 2011). Another TBS study demonstrated that iTBS-primed cTBS results in a decrease of MEP amplitude, while

non-primed cTBS did not alter MEP amplitude, also suggesting a homeostatic interaction between opposite priming TBS and test TBS protocols (Todd *et al.* 2009). At variance with those studies, one recent cTBS→cTBS study did not show any effect of non-primed $cTBS_{80\%AMT}$ or $cTBS_{80\%AMT}$-primed $cTBS_{80\%AMT}$ on MEP amplitude, and rather a non-homeostatic cTBS→cTBS interaction that resulted in a long-lasting MEP depression, if stimulus intensity was set to 70% resting motor threshold (Goldsworthy *et al.* 2012). That study suggested that voluntary activation of the target muscle for determination of AMT prior to TBS may be important for determining the quality of the TBS→TBS interaction. We always measured AMT prior to TBS and, with this setting, replicated and extended the findings of the majority of previous studies by demonstrating that interactions between identical TBS protocols are suppressive, whereas interactions of opposite TBS protocols enhance the non-primed TBS effects, supporting the idea of a general validity of homeostatic metaplasticity in regulating excitatory neuronal circuits in human M1.

The cellular mechanisms underlying these observations at the systems level naturally remain uncertain. Evidence in basic experiments supports a role for the NR2A/B subunit ratio in NMDA receptors with low ratios induced by low neuronal activity favouring LTP- over LTD-induction and vice versa (Philpot *et al.* 2007). NR2B-containing NMDA receptors carry more calcium charge per unit current than NR2A-containing NMDA receptors (Sobczyk *et al.* 2005). TBS-induced long-term changes in MEP amplitude can be prevented by NMDA receptor antagonists (Huang *et al.* 2007), and TBS-induced MEP potentiation switched to MEP depression under the influence of nimodipine, an L-type voltage-gated Ca^{2+} channel antagonist (Wankerl *et al.* 2010). Therefore, it is conceivable that priming iTBS increases the NR2A/B ratio and reduces calcium charge, leading to an increase of the threshold for induction of LTP-like plasticity (i.e. probability for LTP-induction decreases, probability for LTD-induction increases), while priming cTBS has the opposite effects. This line of thought will require further experimental testing, for example by pharmacological intervention with a Ca^{2+} channel antagonist, which is beyond the scope of this paper.

Homeostatic regulation of plasticity in intracortical inhibitory circuits

SICI at an ISI of 2.0 ms likely reflects excitability of $GABA_A$ergic synaptic neurotransmission (Ziemann *et al.* 1996*b*; Di Lazzaro *et al.* 2000; Peurala *et al.* 2008). Previous TBS studies showed that iTBS increases, whereas cTBS decreases SICI (Huang *et al.* 2005, 2008; Suppa *et al.* 2008), but these effects were not consistently found across all studies (Doeltgen & Ridding, 2011*a*,*b*; Hasan *et al.* 2012). To the best of our knowledge, only one study so far has tested the effects of priming TBS on primed TBS-induced changes in SICI, but did not find a significant interaction of iTBS-primed cTBS when compared with non-primed cTBS or non-primed iTBS (Doeltgen & Ridding, 2011*b*). Therefore, we describe here for the first time homeostatic metaplasticity of an inhibitory $GABA_A$ergic neuronal circuit of the human motor cortex. It should be noted that this homeostatic interaction was less expressed compared with homeostatic metaplasticity of MEP amplitude, as it was found only when testing the interaction of two identical but not two opposite TBS protocols (cf. Fig. 4). It could be argued that we did not describe homeostatic metaplasticity because priming TBS had no significant effect on IO-SICI. However, three important arguments are against this notion. (1) The study of metaplasticity is facilitated when the priming stimulation does not overtly alter the strength of synaptic transmission, but instead changes only the state of readiness of synapses to generate LTP or LTD later on (Abraham, 2008). If the priming resulted in overt change in synaptic strength (e.g. LTP), then it is difficult to sort out whether a lack of further LTP induction by test stimulation is caused by saturated LTP or mechanisms that generate LTP being actively inhibited. (2) Homeostatic metaplasticity of MEP amplitude occurs without effects of priming on MEP amplitude (see below). (3) The majority of previous studies showed an increase in SICI after iTBS, and a decrease by cTBS (Huang *et al.* 2005, 2008; Suppa *et al.* 2008). Therefore, it can be assumed that priming iTBS and cTBS in the present study were subthreshold for inducing a significant increase and decrease in IO-SICI, respectively. This is supported further by non-significant trends towards these results (cf. Fig. 4). In summary, it is very likely that the iTBS→iTBS and cTBS→cTBS interactions on IO-SICI represent homeostatic metaplasticity of $GABA_A$ergic neuronal circuitry in human M1. Finally, the significant correlation of ΔSICI with ΔMEP (Fig. 5) provides evidence that the homeostatic metaplasticity induced by two subsequent TBS protocols operates on excitability in the intracortical inhibitory circuit and the corticospinal projection in a parallel manner. This is consistent with previous findings that SICI and MEP amplitude also change in parallel after a single TBS train (Huang *et al.* 2005, 2008; Suppa *et al.* 2008).

The cellular and molecular mechanisms can only be speculated upon, and priming effects on LTP/LTD in inhibitory interneurons have only sparsely been studied. In rat hippocampus, LTD in inhibitory interneurons (inhibitory LTD, iLTD) was only elicited by high-frequency stimulation, if preceded by depolarization-induced suppression of inhibition (DSI; Edwards *et al.* 2008). The DSI priming effect is mediated by mobilization of the endocannabinoid system in postsynaptic pyramidal

cells and a retrograde signalling mechanism acting on presynaptic CB1 receptors under conditions of sufficient intracellular Ca^{2+} concentration in the postsynaptic cells (Howlett *et al.* 2004). Whether or not such an interaction between excitatory postsynaptic cells and inhibitory presynaptic cells through retrograde signalling was responsible for the present findings is uncertain, but at least the iTBS-primed iTBS interaction on IO-SICI (cf. Fig. 4*A*) would be consistent with such a mechanism, by assuming that a priming iTBS-induced increase of activity in excitatory cells (Benali *et al.* 2011) resulted in enhanced iLTD-like plasticity by subsequent primed iTBS. Another possibility would certainly be homeostatic regulation of LTP-/LTD-like plasticity in inhibitory postsynaptic cells by their previous level of activity according to the Bienenstock–Cooper–Munro theory (Bienenstock *et al.* 1982).

In summary, the present findings strongly suggest for the first time that priming regulates plasticity in inhibitory interneurons in human M1 in accord with the principles of homeostatic metaplasticity.

Mechanisms of low- *versus* high-intensity priming TBS

Fundamental to the concept of metaplasticity is that the priming protocol by itself does not need to induce any changes in synaptic efficacy (Huang *et al.* 1992; Abraham & Tate, 1997; Wang & Wagner, 1999). Consistently, in human M1, plastic changes by the priming protocol are not necessary to induce homeostatic metaplasticity (Iyer *et al.* 2003; Hamada *et al.* 2008; Todd *et al.* 2009; Delvendahl *et al.* 2010). To address the question of metaplastic regulation of corticospinal and inhibitory intracortical circuits in the absence of any cortical excitability changes after the priming protocols, we used low-intensity priming at a stimulation intensity of 70%AMT in Experiment 2, which by itself did not alter the excitability of corticospinal excitatory or intracortical inhibitory circuits. Low-intensity 70%AMT TBS effects have been studied in only one other study, which, consistent with the present data, showed no effects of 70%AMT cTBS or iTBS on MEP amplitude, intracortical facilitation or short-interval intracortical facilitation (McAllister *et al.* 2009). In contrast with the present study, those authors found a rather selective short-lasting decrease of SICI after 70%AMT cTBS (but no change of SICI after 70%AMT iTBS). A lower excitation threshold of intracortical inhibitory circuits compared with the corticospinal system may explain this selective SICI modification (Kujirai *et al.* 1993; Ilic *et al.* 2002). Although the homeostatic interactions appeared more pronounced with the higher-intensity $TBS_{80\%AMT}$ than low-intensity $TBS_{70\%AMT}$ priming, this was not generally true, as the suppressive cTBS-primed cTBS interaction on IO-SICI was significant only with the lower-intensity $TBS_{70\%AMT}$ priming (cf. Fig. 4*B* and *D*).

In addition, we observed that the homeostatic interactions on IO-MEP in Experiment 2 were only present when two identical TBS protocols were applied (cf. Fig. 3*C* and *D*), suggesting that homeostatic metaplasticity had a lower threshold in these conditions. This was not explained by any difference in the effects of priming TBS in experimental conditions with identical *versus* different TBS protocols (mean absolute $(MEP_{B1}\text{-}MEP_{B0})/MEP_{B0}$: 0.20 ± 0.06 *versus* 0.21 ± 0.05, $P = 0.86$). A parsimonious explanation may be given by the observation that iTBS increases the late I-waves but not the I1-wave (Di Lazzaro *et al.* 2008*a*), while cTBS suppresses predominantly the I1-wave (Di Lazzaro *et al.* 2005). If homeostatic metaplasticity as induced by subsequent TBS protocols occurs through homosynaptic mechanisms (Müller *et al.* 2007), then this would result in stronger interaction between identical protocols.

In summary, we provided evidence that homeostatic metaplasticity of corticospinal excitatory and intracortical inhibitory circuits in human cortex can be induced in the absence of any excitability changes in these circuits after priming TBS, thus supporting true metaplasticity, while other interactions between plasticity-inducing protocols, such as depotentiation or de-depression would require induction of LTP or LTD by the priming protocol (Abraham, 2008; Huang *et al.* 2010).

Inhibitory control of corticospinal plasticity

Excitability of inhibitory circuits is of significant importance for regulation of LTP/LTD in M1. In slices of rat motor cortex, LTP was significantly facilitated or could be induced only if the $GABA_A$ergic tone was reduced by local application of a $GABA_A$ receptor antagonist (Hess & Donoghue, 1994; Castro-Alamancos *et al.* 1995; Hess *et al.* 1996). In human M1, LTP-like plasticity induced by non-invasive brain stimulation was facilitated by disinhibition (Ziemann *et al.* 1998*a*), but reduced by $GABA_A$ergic drugs (Heidegger *et al.* 2010).

One previous study investigated the interactions between priming low-frequency (0.1 Hz) rTMS and subsequent paired associative stimulation (PAS; Delvendahl *et al.* 2010). Those authors showed that priming rTMS itself did not alter MEP amplitude but resulted in increased SICI. This effect was associated with occlusion of subsequent PAS-induced LTP-like and LTD-like plasticity and, therefore, provided supportive evidence for a critical role of $GABA_A$-related excitability for controlling direction and amount of plasticity (Delvendahl *et al.* 2010). Our data significantly extend those observations by showing a linear correlation over the full range of changes in

SICI induced by priming $TBS_{80\%AMT}$ and changes in MEP amplitude induced by test TBS, i.e. decreases in SICI were associated with LTP-like increase in MEP amplitude by primed iTBS and even primed cTBS, while increases in SICI were associated with LTD-like decrease in MEP amplitude by primed cTBS and even primed iTBS (cf. Fig. 6*A*). These correlations were no longer significant with priming $TBS_{70\%AMT}$ (Fig. 6*B*). This suggests a critical dependence of the expression of the controlling effect of inhibition on plasticity in the excitatory corticospinal projection on priming TBS intensity.

In conclusion, the present findings extend previous evidence of homeostatic metaplasticity in human M1 by demonstrating for the first time homeostatic metaplasticity of inhibitory intracortical circuits and corroborating the notion of a significant role for inhibitory mechanisms in controlling direction and magnitude of subsequent plasticity of the excitatory corticospinal projection. This opens up the opportunity to purposefully utilize priming to modify inhibitory control in order to direct metaplasticity in excitatory neural circuits to increase or decrease the probability of induction of LTP-/LTD-like plasticity.

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Author contributions

All experiments were performed in the motor cortex laboratory at the Department of Neurology, Goethe-University Frankfurt, Germany. T.M., F.M.D. and U.Z. contributed to conception and design of the experiments; all authors contributed to collection, analysis and interpretation of data, and to drafting the article or revising it critically for important intellectual content.

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