10–20 System. Electrode impedance was maintained below 5 kΩ. EEGs were recorded from C3′–Fz and C4′–Fz using a 0.3–3000 Hz band-pass filter, then digitized with an analogue-to-digital converter (micro1401, CED, Cambridge, UK) at a sampling rate of 20 kHz and stored on a personal computer for further analysis. We used a C3′–Fz and C4′–Fz montage for recording HFOs, because it has been shown from previous studies to be appropriate (Mochizuki et al., 1999; Sakuma et al., 2004). SEPs with an epoch of 50 ms duration were recorded before and immediately after TBS. In total, responses to 5000 stimuli were recorded, which took about 20 min. Responses to each of the first 2500 stimuli (R1) and the second 2500 stimuli (R2), as well as to all 5000 stimuli (R1 + R2), were averaged offline using Spike2 software (CED, Cambridge, UK). For separation of HFOs from the underlying N20, the digitized wide-band signal was band-pass filtered (400–800 Hz) digitally and averaged. In wide-band recordings, amplitudes of the P14 peak to the N20 peak (P14–N20), the N20 peak to the P25 peak (N20–P25), and the P25 peak to the N33 peak (P25–N33) from C3′–Fz and C4′–Fz were measured and analyzed (Fig. 1A). The size of the HFOs was calculated from their root-mean-square amplitude from their onset to their offset. Onset/offset criteria for HFOs were defined as their amplitudes exceeding the averaged background noise level for the subject's control session by three standard deviations. All of these parameters were separated into two parts: (1) early HFOs (onset to N20 peak) and (2) late HFOs (N20 peak to offset), as shown in Fig. 1B.

2.4. Experiment 2: the effects of TBS on motor cortical excitability

Motor cortical excitability was assessed in 12 subjects (6 subjects with iTBS, 6 subjects with cTBS). TMS was performed using a round coil with external diameters of 130 mm (Magstim Co., Dyfed, Wales) connected to a Magstim 200 stimulator (Magstim Co., Whitland, Dyfed, UK). The coil was positioned over the vertex in the optimal scalp position that would elicit motor responses in the right FDI muscle. The resting motor threshold (RMT) was defined as the intensity of stimulation that elicits at least 5 MEPs of 50 μV in 10 trials from the right FDI muscle (Rossini et al., 1994). The MEP amplitude was measured by using the stimulator intensity sufficient to evoke a peak-to-peak amplitude of 1 mV in the relaxed FDI muscle. SICI was measured using the paired-pulse method (Kujirai et al., 1993). In the original work on TBS, the authors suggest that the activity of SICI reflects the function of GABAergic interneurons; therefore, we used a similar experimental design where SICI was evaluated at an ISI of 2 ms (Huang et al., 2005, in press). The conditioning stimulus intensity was set at 80% RMT for SICI. The test stimulus intensity was set at a peak-to-peak amplitude of 1 mV of the MEP in the relaxed FDI muscle. The stimulation rate was about 0.1 Hz, and it took about 5 min to record 30 trials for 2 parameters (15 trials for each parameter were recorded and averaged). The order of presentation of the MEP (test stimulation only) and SICI (ISI = 2 ms) intervals was randomized by a computer program (Spike2, CED, Cambridge, UK). MEPs and SICI were recorded from the FDI muscle by a pair of 2 × 2 cm Ag–AgCl disposable surface electrodes in a belly-tendon montage. The electromyogram was recorded from a pair of electrodes and filtered (50–200 Hz), then digitized with an analogue-to-digital converter (micro1401, CED, Cambridge, UK) at a sampling rate of 10 kHz and stored on a personal computer. Each parameter was measured before, 0–5, 10–15, 20–25, and 30–35 min after the TBS session.

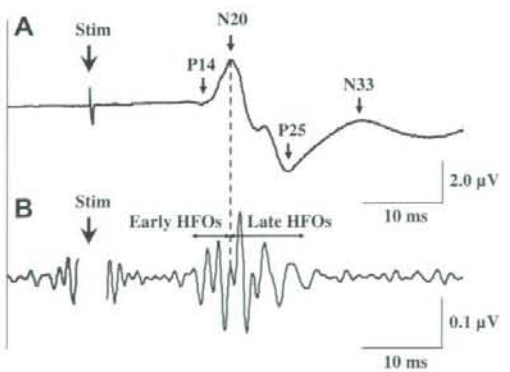

Fig. 1. (A) Typical wide-band (0.3–3000 Hz) and (B) narrow-band (400–800 Hz) somatosensory-evoked potentials (SEPs) from C3′–Fz following right median nerve stimulation in a subject. (B) The narrow-band trace shows a short burst of high-frequency oscillations (HFOs) around N20.

2.5. Statistical analysis

Data were analyzed using SPSS for Windows version 14.0. The individual SEP and HFO values for each subject were evaluated using a three-way analysis of variance (ANOVA) of mixed design with the within-subject factors of Time (before TBS vs. after TBS: R1 and R2, R1 + R2) and Recording Site (C3′ vs. C4′) and the between-subject factor of Intervention (iTBS vs. cTBS). In addition, the effects of each TBS on SEPs and HFOs were evaluated using two-way or one-way ANOVA. Each TBS-induced effect on the MEP and SICI was studied using a one-way, repeated-measures ANOVA with Time (before TBS vs. 0–5, 10–15, 20–25, and 30–35 min after TBS) as the within-subject factor. When the effect was significant, a post hoc Dunnett's paired t test was performed on the data. Statistics for the data in Fig. 4 were performed on normalized data, whereas the statistical analysis of each time course was performed separately on absolute values. A value of $p < 0.05$ was considered to be statistically significant. Data were expressed as means ± standard error of the mean.

3. Results

3.1. Experiment 1: the effects of TBS on SEPs and HFOs

In separate analyses for R1 and R2, a three-way ANOVA of mixed design revealed no significant three-way interactions on SEPs and HFOs. HFOs were revealed to have a significant two-way interaction for Time × Intervention (early HFOs: $F_{(2,72)} = 5.568$, $p = 0.008$; late HFOs: $F_{(2,72)} = 3.261$, $p = 0.044$; total HFOs: $F_{(2,72)} = 4.444$, $p = 0.019$), as shown in Table 1. Separate two-way ANOVAs on HFOs following iTBS and cTBS revealed no significant Time × Site interactions. Late HFOs were revealed to have a significant two-way interaction for Intervention × Site ($F_{(2,72)} = 16.763$, $p < 0.001$), and significant main effects for Intervention ($F_{(2,72)} = 23.039$, $p < 0.001$), Site ($F_{(2,72)} = 14.469$, $p = 0.001$) (Table 1). One-way ANOVA revealed a significant change in late HFOs from C3′ following iTBS ($F_{(2,18)} = 3.66$, $p = 0.046$), but no significant changes in early and total HFOs following iTBS (early HFOs: $F_{(2,18)} = 3.554$, $p = 0.079$; total HFOs: $F_{(2,18)} = 4.132$, $p = 0.059$) and HFOs following cTBS (early HFOs: $F_{(2,18)} = 2.235$, $p = 0.169$; late HFOs: $F_{(2,18)} = 1.872$, $p = 0.215$; total HFOs: $F_{(2,18)} = 1.742$, $p = 0.236$). Post hoc analysis revealed late HFOs in R1 increased significantly ($p = 0.030$) following iTBS, whereas late HFOs in R2 did not ($p = 0.615$), as shown in Figs. 2 and 3A. No significant changes were shown in HFOs from C4′ following TBS. Although there were no significant main and interaction effects on SEPs recorded from both

Table 1
Results of the three-way, repeated-measures ANOVA for the effects of TBS on SEPs and HFOs

	df	Early HFOs		Late HFOs		Total HFOs		P14–N20		N20–P25		P25–N33	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Analyses for R1 and R2													
Time	72	0.562	0.575	0.457	0.637	0.890	0.420	0.822	0.448	0.712	0.498	2.914	0.068
Intervention	1	5.571	0.024*	23.039	<0.001*	3.304	0.077	0.709	0.405	2.882	0.098	0.148	0.703
Recording site	1	0.059	0.810	14.469	0.001*	0.000	0.986	0.074	0.788	0.008	0.929	0.039	0.845
Time × Intervention	72	5.568	0.008*	3.261	0.044*	4.444	0.019*	1.754	0.188	0.050	0.951	0.052	0.949
Intervention × Site	1	0.075	0.786	16.763	<0.001*	0.004	0.949	0.157	0.695	0.087	0.769	0.253	0.618
Site × Time	72	1.316	0.281	0.042	0.959	0.820	0.449	0.186	0.669	1.558	0.225	3.082	0.059
Time × Intervention × Site	72	0.786	0.464	1.617	0.213	1.070	0.354	0.766	0.473	0.232	0.794	0.041	0.960
Analyses for R1 + R2													
Time	36	0.019	0.891	0.024	0.877	0.008	0.931	3.582	0.066	2.007	0.165	1.014	0.321
Intervention	1	6.577	0.015*	1.006	0.322	3.914	0.056	0.547	0.464	2.836	0.101	0.162	0.690
Recording site	1	0.201	0.656	0.029	0.866	0.000	0.991	0.099	0.755	0.006	0.940	0.025	0.874
Time × Intervention	36	2.828	0.101	4.556	0.040*	4.074	0.049*	1.357	0.252	0.122	0.729	0.038	0.847
Intervention × Site	1	0.040	0.844	0.022	0.848	0.002	0.966	0.167	0.685	0.077	0.783	0.257	0.615
Site × Time	36	1.845	0.183	0.248	0.621	0.814	0.373	2.866	0.099	0.878	0.355	1.110	0.299
Time × Intervention × Site	36	0.481	0.492	2.227	0.144	1.048	0.313	0.612	0.439	0.082	0.777	0.014	0.906

df, degrees of freedom; *F*, *F* values; *p*, *p* values.
* $p < 0.05$.

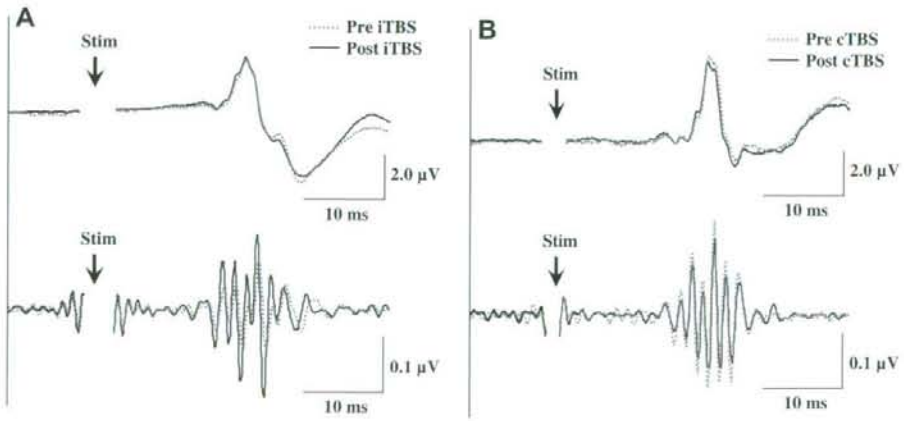

Fig. 2. Representative SEPs and HFOs from C3′–Fz were obtained in a subject before and after theta burst stimulation (TBS) over the motor cortex. SEP (upper trace) and HFO (lower trace) waveforms before and after TBS were superimposed. (A and B) HFO amplitudes were enlarged/reduced significantly after iTBS/cTBS, respectively, whereas there were no statistically significant changes in SEPs.

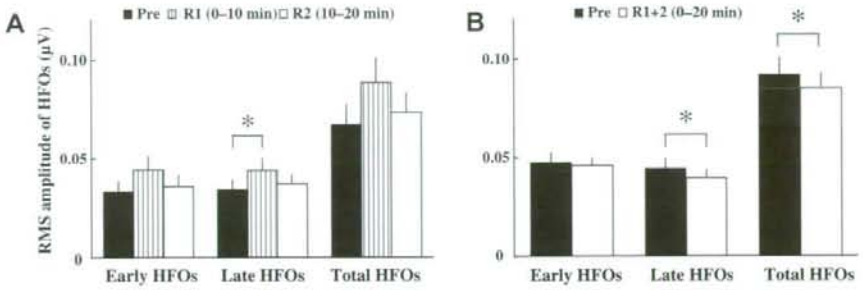

Fig. 3. Effect of TBS on root-mean-square (RMS) amplitudes of HFOs. (A) Late HFO amplitudes recorded from C3′–Fz in the first stimuli for 10 min increased significantly following iTBS, whereas (B) late and total HFO amplitudes decreased notably following cTBS. $^{*}p < 0.05$ by a one-way analysis of variance (ANOVA) with Time (before TBS vs. after TBS). Error bar = standard error of the mean.

sides (Table 1), the P25–N33 amplitude from C3′ revealed a tendency to increase ($F_{(2,18)} = 3.311$, $p = 0.090$).

In analyses for R1 + R2, a three-way ANOVA of mixed design revealed no significant three-way interactions on SEPs and HFOs. Late and total HFOs were revealed to have a significant two-way interaction for Time × Intervention (late HFOs: $F_{(1,36)} = 4.556$, $p = 0.040$; total HFOs: $F_{(1,36)} = 4.074$, $p = 0.049$), whereas there were no significant interactions for Time × Intervention on early HFOs, as shown in Table 1. Separate two-way ANOVAs on late and total HFOs following iTBS and cTBS revealed no significant Time × Site interactions. One-way ANOVA revealed that late and total HFOs from C3′ decreased significantly following cTBS (late HFOs: $F_{(1,9)} = 17.531$, $p = 0.002$; total HFOs: $F_{(1,9)} = 13.684$, $p = 0.005$), but there were no significant effects following iTBS, as shown in Figs. 2 and 3B. No significant changes were shown in HFOs from C4′ following TBS. SEPs recorded from both C3′ and C4′ also showed no significant main and interaction effects, as shown in Table 1.

3.2. Experiment 2: the effects of TBS on motor cortical excitability

One-way, repeated-measures ANOVA revealed significant changes in the MEP and SICI following iTBS (MEP: $F_{(4,20)} = 4.015$, $p = 0.015$; SICI: $F_{(4,20)} = 4.318$, $p = 0.038$) as a factor of Time. Post hoc analysis revealed that MEPs increased significantly for 0–15 min after iTBS (0–5 min: $p = 0.008$; 10–15 min: $p = 0.042$; 20–25 min: $p = 0.937$; 30–35 min: $p = 0.933$) (Fig. 4). There was a significant increment in SICI only for 0–5 min after iTBS (0–5 min: $p = 0.015$; 10–15 min: $p = 0.278$; 20–25 min: $p = 0.578$; 30–35 min: $p = 1.000$) (Fig. 5A).

The MEP and SICI changed notably following cTBS (MEP: $F_{(4,20)} = 2.956$, $p = 0.045$; SICI: $F_{(4,20)} = 5.814$, $p = 0.001$) as a factor of Time. Post hoc analysis revealed that MEPs decreased significantly for 10–35 min after

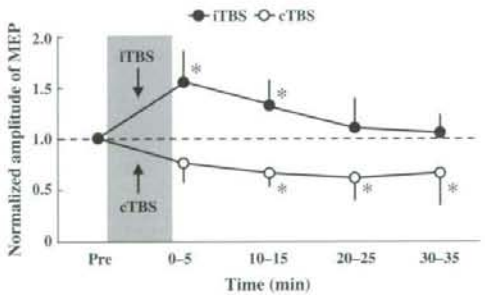

Fig. 4. Effects of TBS on motor-evoked potentials (MEPs). The size ratios (post-TBS/pre-TBS) of the MEPs are shown. The MEPs were enlarged/reduced significantly following iTBS/cTBS, respectively.

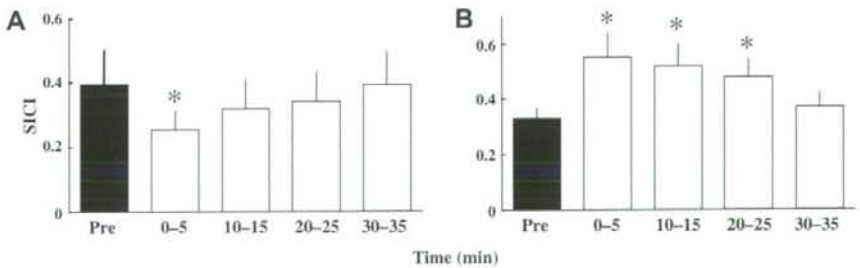

Fig. 5. Effect of TBS on short-interval intracortical inhibition (SICI). (A and B) SICI was increased/decreased significantly after iTBS/cTBS, respectively. The decrement in SICI after cTBS lasted longer than the increment in SICI after iTBS.

cTBS (0–5 min: $p = 0.256$; 10–15 min: $p = 0.038$; 20–25 min: $p = 0.029$; 30–35 min: $p = 0.049$) (Fig. 4). SICI was reduced notably for 0–25 min after cTBS (0–5 min: $p = 0.004$; 10–15 min: $p = 0.012$; 20–25 min: $p = 0.042$; 30–35 min: $p = 0.607$) (Fig. 5B). The decrement in SICI following cTBS lasted longer than the increment in SICI following iTBS.

4. Discussion

In the present study, we investigated how the two types of TBS over the motor cortex influenced the sensorimotor cortices. Late HFO amplitudes increased significantly after iTBS. In contrast, late and total HFO amplitudes decreased notably after cTBS. Wide-band SEP amplitudes did not change after either intervention. On motor cortical excitability, MEPs and SICI were increased/decreased significantly after iTBS/cTBS, respectively. The decrement in SICI after cTBS lasted longer than the increment in SICI after iTBS. Since we obtained the same results about MEPs and SICI as those reported previously (Huang et al., 2005), we concluded that TBS caused plastic changes in the motor cortex appropriately in the present study.

Previous studies revealed that rTMS over the motor cortex changed SEPs. Low-frequency rTMS over the motor cortex reduced the cortical SEP (Enomoto et al., 2001). rTMS over the motor cortex paired with a preceding repetitive motor point stimulation increased the cortical SEP (Tsuji and Rothwell, 2002). In the present study, wide-band SEPs did not change notably, but significant effects were shown in HFOs after TBS over the motor cortex. Since the TBS protocol was based on Huang's report in the present session, the stimulus intensity of TBS was fixed at 80% AMT (mean 41.4 ± 7.3%) (Huang et al., 2005). This stimulus intensity was weaker than that in the previous studies of rTMS over the motor cortex (in Enomoto's study, 110% AMT; in Tsuji and Rothwell's study, 105% RMT). SEPs were unaffected after 90% RMT, 0.9 Hz rTMS over the sensorimotor cortices (Satow et al., 2003). In the case of 80% RMT, 0.5 Hz rTMS over the somatosensory cortex, the increment of HFOs was obtained, but there was no change in SEPs (Ogawa et al., 2004). Because the rTMS paradigm and stimulus site in the present study were different from those in the previous studies, we could not compare our results with the previous results directly. But we supposed that the stimulus intensity was one of the most important factors influencing the cortical excitability. In the present study, the results of SEPs and HFOs following TBS were different. These results implied that the generator mechanisms for HFOs and SEPs are different. Assuming that TBS over the motor cortex does not affect directly the somatosensory cortex, we suggest that some indirect mechanism may exist in the modulation of the HFOs.

Ishikawa and colleagues reported that cTBS over the motor cortex increased the SEPs, whereas cTBS over the somatosensory cortex produced reversed events (Ishikawa et al., 2007). Although the stimulus paradigm and intensity were very similar between the Ishikawa's study and the present study, our SEP results of the P25–N33 amplitude from C3′ did not increase significantly. One major reason for this is the difference in the position of the reference electrode. In the present study, the reference was set to Fz according to IFCN recommendations (Nuwer et al., 1994). Our experiment, as well as previous studies, demonstrated that using the Fz reference for recording HFOs is appropriate (Mochizuki et al., 1999; Sakuma et al., 2004). In Ishikawa's study, SEPs were recorded with a reference to the contralateral earlobe, and the same directional changes not only in the parietal but also in the frontal components of SEPs were obtained after cTBS (Ishikawa et al., 2007). We also consider that large intra-individual variability of SEP amplitudes affected statistical analysis. For example, the P25–N33 amplitude from C3′ in cTBS study was 0.22–3.19 μV (mean 1.65 ± 0.99 μV). However to resolve this discrepancy, further studies using multi-channel SEPs or MEG will be needed.

More than a decade has passed since the first report of HFOs, however, the origin of HFOs is still controversial. There are different hypotheses of HFO generation; thalamocortical afferent fibers (Gobbele et al., 1998; Klostermann et al., 2002), fast inhibitory postsynaptic potentials of pyramidal cells (Jones et al., 2000), GABAergic inhibitory interneurons in area 3*b* (Hashimoto et al., 1996, 1999; Ozaki et al., 2001), and cholinergic neurons in previous studies. But the precise anatomical location of the generator remains unclear. Since HFOs in Parkinson's disease are larger than those in normal subjects (Mochizuki et al., 1999), dopamine may have some role in the changes in HFOs.

In TMS studies, the phenomenon of SICI is generally thought to reflect the activity of GABAergic inhibitory interneurons within the motor cortex (Chen et al., 1998; Kujirai et al., 1993; Ziemann et al., 1998b; Ziemann, 2004). Previous pharmacological studies reveal that the change of SICI does not implicate only GABAergic function. The NMDA antagonist enhances SICI (Ziemann et al., 1998a,c). The dopamine agonists also induce the facilitation of SICI (Ziemann et al., 1996, 1997; Ziemann, 2004), whereas dopamine antagonist and norepinephrine agonist decrease SICI (Ilic et al., 2003; Ziemann, 2004). In Parkinson's disease, the lack of dopamine by degeneration of dopaminergic neurons decreases the function of SICI, but improves after L-dopa administration (Ridding et al., 1995).

In the present study, late HFOs increased significantly after iTBS, whereas late and total HFOs decreased after cTBS. These results were parallel to the changes in SICI after TBS. In addition, the decrement in HFOs following cTBS lasted longer than the increment in HFOs following iTBS, and the time courses of HFO changes following TBS were very similar to those of SICI changes. There are several lines of evidence in the present study which suggest that changes in SICI and HFOs following TBS may share a related mechanism. This includes: (1) The observa-

tion that HFOs in the somatosensory cortex are increased/decreased in response to iTBS/cTBS (similar to SICI in the motor cortex). (2) The time courses of HFO changes following TBS were also similar to those of SICI changes. These parallel changes in SICI and HFOs following TBS led us to speculate that a common neural mechanism is involved in the generation of SICI and HFOs, i.e. the activity of GABAergic inhibitory interneurons and their networks with pyramidal cells. Thus, although indirect, the present results provide an additional piece of evidence supporting the GABAergic inhibitory interneuron hypothesis as the HFO generator mechanism.

In the Huang's study, not only MEP but also SICI increased/decreased after iTBS/cTBS. Intracortical facilitation, where more than one circuit might contribute to, also decreased after cTBS (Hanajima et al., 1998). The authors suggested that iTBS/cTBS increased/decreased the effectiveness of synaptic connections in these parameters (Huang et al., 2005). Our results showed that both MEP and SICI changed in same directions after TBS, in line with Huang's study. Therefore, we speculate that the effectiveness of synaptic connections among interneurons or between pyramidal cells and interneurons may be changed by TBS. Since it has been speculated that both HFOs and SICI may reflect the functions of GABAergic inhibitory interneurons, we consider that the changes in the effectiveness of synaptic connections among GABAergic inhibitory interneurons can be detected following TBS by recording SICI and HFOs.

Low-frequency rTMS over the motor cortex suppressed the excitability not only in the motor (Chen et al., 1997) but also in the somatosensory cortices (Enomoto et al., 2001). It is well known that the robust cortico-cortical connections are present between the motor and somatosensory cortices. Enomoto and colleagues speculated that low-frequency rTMS over the motor cortex produced an inhibitory effect in the somatosensory cortex via the cortico-cortical connections between the motor and somatosensory cortices (Enomoto et al., 2001). SEPs and HFOs represent parallel and partly independent steps in sensory processing. SEPs also represent stable somatosensory input while HFOs are easily influenced by several factors. When the sleep stage became deeper, HFOs got smaller, but SEP amplitudes did not change (Yamada et al., 1988). Low-frequency, weak-intensity rTMS (0.5 Hz, 80% RMT, 50 pulses) over the somatosensory cortex enlarged HFOs, whereas there was no significant change in slow SEPs (Ogawa et al., 2004). Furthermore, the authors described that the contribution of the motor cortex to rTMS could not be excluded completely (Ogawa et al., 2004). In the present study, iTBS/cTBS increased/decreased HFOs in the somatosensory cortex similar to SICI in the motor cortex, and the time courses of HFO changes following TBS were also similar to those of SICI changes. Since HFOs are prone to be influenced by rTMS as compared with SEPs (based on a previous study), we speculate that the changes in the effectiveness of synaptic connections among GABAergic inhibitory interneurons and between the interneurons and pyramidal cells by TBS over the motor cortex might appear not only in the motor cortex but also in the somatosensory cortex via the cortico-cortical connections. As a result, HFOs changed in parallel with SICI.

In conclusion, TBS over the motor cortex changed the cortical excitability in the somatosensory as well as motor cortices by changing the effectiveness of synaptic connections. Late HFOs increased significantly after iTBS, whereas late and total HFOs decreased notably after cTBS. Because these bidirectional TBS effects on HFOs were parallel to those on SICI, TBS might change the effectiveness of synaptic connections among GABAergic inhibitory interneurons and between the interneurons and pyramidal cells in the sensorimotor cortices. Accordingly, this study provided an additional piece of evidence that HFOs reflect the function of GABAergic inhibitory interneurons.

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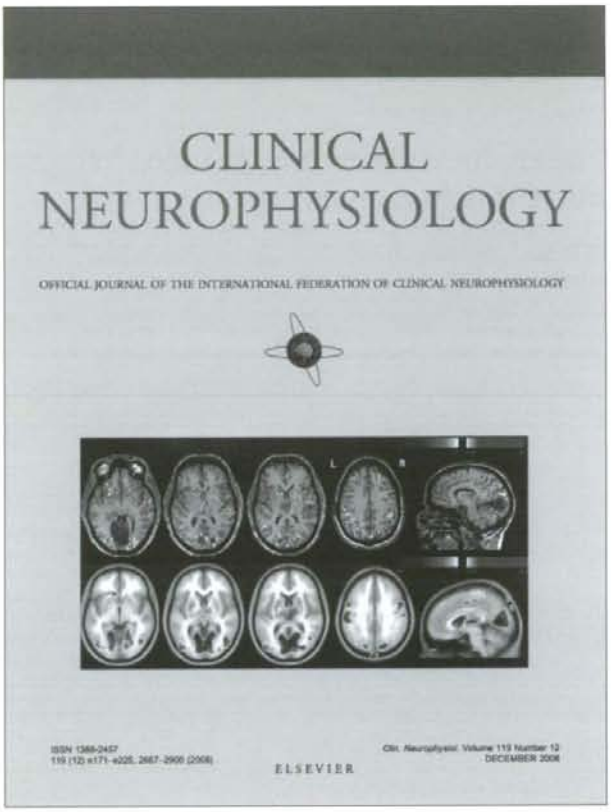

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Parallel inhibition of cortico-muscular synchronization and cortico-spinal excitability by theta burst TMS in humans

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ABSTRACT

Objective: To investigate the after-effects of theta burst TMS (TBS) on cortico-muscular synchronization, and on cortico-spinal excitability, in humans.
Methods: We studied 10 healthy subjects using a continuous paradigm of TBS (cTBS), i.e. 600 pulses in 40 s. Before and after the cTBS, coherence function was computed as a measure of cortico-muscular synchronization by recording electroencephalogram (EEG) from 19 scalp sites and electromyogram (EMG) from right first dorsal interosseous (FDI) muscle during the isometric contraction. In a separate experiment, motor-evoked potentials (MEPs) in response to single TMS pulses were recorded from the FDI muscle before and after the cTBS, to measure cortico-spinal excitability.
Results: When the cTBS was applied over the left primary motor cortex (M1), the beta-band cortico-muscular coherence for the C3 scalp site, as well as the MEP amplitude significantly decreased in 30–60 min, and then recovered to the original levels in 90–120 min. Neither sham stimulation nor cTBS applied over 2 cm posterior to M1 produced significant effects.
Conclusions: cTBS-over-M1 can inhibit the cortico-muscular synchronization in parallel with the decline of cortico-spinal excitability.
Significance: Our results provide the first evidence that TBS can efficiently alter the functional cortico-muscular coupling in humans.

1. Introduction

Previous studies demonstrated the functional and anatomical neural circuits in the human motor cortex, which realize the dynamic communication with the corresponding peripheral muscles via the ascending and descending spinal tracts. It has been suggested that the synaptic connections in those cortical circuits can be potentiated or depressed by means of repetitive transcranial magnetic stimulation (rTMS) (Fitzgerald et al., 2006) by adjusting the stimulation parameters, e.g. intensity, duration, total number, and frequency of TMS pulses applied. For instance, low-frequency rTMS applied on the primary motor cortex (M1) can produce long-lasting suppression of motor-evoked potentials (MEPs) (0.9 Hz rTMS: Chen et al., 1997; 1 Hz rTMS: Touge et al., 2001), and on the other hand, high-frequency rTMS facilitates MEPs (5 Hz rTMS: Pascual-Leone et al., 1994; Berardelli et al., 1998; Peinemann et al., 2004). A well-known stimulation pattern called "theta burst stimulation" (TBS) can efficiently induce long-lasting synaptic modifications in the motor areas of rodent cortices (Hess and Donoghue, 1999). And lately, TBS has been adopted as a novel rTMS paradigm for clinical studies on humans (Huang et al., 2005). A few studies suggested that TBS significantly improves the efficiency of the rTMS applications by shortening stimulation duration, decreasing the number of pulses applied and yet prolonging the after-effects in the cortical plasticity: The 1-Hz rTMS given for 25 min with 1500 pulses had a 30-min-long suppressive effect on MEP amplitudes (Touge et al., 2001), while the continuous paradigm of TBS (cTBS) applied for only 40 s with 600 pulses induced the 60-min-long suppression (Huang et al., 2005; Ishikawa et al., 2007). Moreover, a recent study showed that cTBS can induce such long-lasting after-effects not only in MEP but also in the somatosensory-evoked potentials (SEPs) (Ishikawa et al., 2007). Although MEP and SEP are quantitative measures widely employed for examining cortico-spinal and sensory cortical excitability, respectively, both measures necessarily use exogenous stimulations for eliciting the response potentials, e.g. TMS for MEP. By contrast, the functional coupling between cortices and the corresponding

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muscles can be assessed by means of the coherence function in the frequency domain, or of the cumulant density function in the time domain (Halliday et al., 1995). Since these measures require no exogenous stimuli, but recordings of cortical and muscular activities, e.g. electroencephalographic (EEG) and electromyographic (EMG) signals, one can quantitatively examine the cortico-muscular synchronization during voluntary muscle contractions in rather near-natural conditions. In the previous studies, the cortico-muscular coherence during isometric muscle contractions has been found specifically in the beta frequency band (13–30 Hz), and in spatially localized cortical areas (e.g. Salenius et al., 1997; Halliday et al., 1998; Mima and Hallett, 1999a; Murayama et al., 2001). Cortico-muscular coherence analysis may also have some diagnostic potential in neurological diseases arising from abnormal cortical oscillations, such as epilepsy. Previous studies showed that those rhythmic abnormalities could be assessed by examining cortico-muscular coherence while subjects were maintaining voluntary isometric contraction (Silén et al., 2002) or a posture (e.g. wrist extension and thumb adduction) (Grosse et al., 2003). However, there is a lack of evidence if such a functional coupling between the cortex and the corresponding muscle during a natural motor task can be modulated by rTMS. Therefore, in this study, we examined how cTBS applied over either M1 or S1 (2-cm posterior to M1) can affect the beta-band cortico-muscular coherence during voluntary isometric contraction. In addition, to assess the link between cortico-muscular synchronization and cortico-spinal excitability, we examined the after-effects of cTBS on MEP amplitudes in a separate experiment.

2. Methods

2.1. Subjects

Two different sets of experiments were made in this study, "Experiment 1" and "Experiment 2". In total, 10 healthy right-handed volunteers (9 men, 1 woman; 27.5 ± 2.6 years of age (means ± SE)) participated in the experiments. Eight (7 men, 1 woman; 25.6 ± 2.2 years) and seven (7 men; 29.5 ± 3.5 years) out of the 10 subjects participated in the Experiment 1 and 2, respectively. Five individuals out of the 10 subjects (5 men, 27.4 ± 3.4 years) participated in both experiments. All subjects gave written informed consents for the experiments which were approved by the local ethical committee due to the requirements of the Declaration of Helsinki.

2.2. Experimental procedures

In the first experiment (Experiment 1), in order to assess the effects of cTBS on cortico-muscular synchronization, EEG and EMG signals were simultaneously recorded before and after cTBS. cTBS was applied either over a scalp location of M1or S1, or as sham. The location of M1 was defined as the "motor hot spot" for the right FDI muscle by scanning with single-pulse TMS (Ishikawa et al., 2007). The location 2 cm posterior to the M1 location (M1 + 2 cm) presumably lies over the crown of the postcentral gyrus, which was previously referred to as sensorimotor area (Bäumer et al., 2007). Therefore, we referred to this location as S1 in this study.

In the second experiment (Experiment 2), to assess the effects of cTBS on cortico-spinal excitability, MEPs were recorded before and after cTBS. cTBS was applied either over the scalp location of M1 or as sham.

For each subject, "active motor threshold (AMT)" over the M1 for the right FDI muscle was defined as the lowest TMS intensity at which 5 out of 10 consecutive stimuli could elicit reliable amplitude of MEPs (~200 μV) during slight tonic contraction of the target muscle (~20% of the maximum voluntary contraction).

2.2.1. Experiment 1: Assessment of the after-effects of cTBS on EEG–EMG coherence

First, to obtain baseline coherence values (as the magnitudes and the frequency bands) for each subject, two recording sessions were made before cTBS application, as shown in Fig. 1 ("pre30" and "pre0" in the panel of "Experiment 1"). The location of "motor hot spot" and the stimulation intensity were searched between the pre30 and pre0 sessions (Fig. 1, "Search M1 and Intensity"), so that we could confirm that single-pulse TMS did not alter the coherence. Three minutes after the pre0 session, we delivered 40-sec-long cTBS, and then 2 min later, a post-hoc recording session was made. Afterwards, a recording session was repeated every 30 min (i.e. 30, 60, 90, and 120 min after cTBS). Thus, there were seven recording sessions in total.

Motor task in the present experiments was similar to that described in the previous studies (Safri et al., 2006, 2007). Briefly, in each recording session, subjects were asked to maintain weak isometric contraction of their right FDI muscle with ~15% force level of the maximum voluntary contraction (MVC). A force sensor was placed between the thumb and the index finger for monitoring the force level during the EEG–EMG recordings. The sensor output was shown also to the subject for her/him to adjust the force level of muscle contraction, but only prior to the recording sessions in order to avoid a possible visual-cognitive effect of exhibiting the force level (Safri et al., 2006).

2.2.2. Experiment 2: Assessment of the after-effects of cTBS on MEP

Previous studies have demonstrated the long-term effect of cTBS on the cortico-spinal excitability (Huang et al., 2005; Ishikawa et al., 2007). And recently, it was shown that such an after-effect of cTBS could be modulated by a weak isometric muscle contraction performed immediately after cTBS (Huang et al., 2008). Therefore, we measured MEPs in an experimental condition matched to that of Experiment 1 mentioned above in order to (1) verify a long-term after-effect of cTBS on the cortico-spinal excitability even in the presence of an intermittent motor task after cTBS and (2) compare the cTBS after-effect on excitability with that on cortico-muscular coherence. As illustrated in Fig. 1 ("Experiment 2"), subjects were asked to perform the same contraction task, at nearly the same time points except 120 min, as in Experiment 1; i.e. in pre30 and pre0 sessions before cTBS, and at 2, 30, 60, and 90 min after cTBS. To obtain baseline MEP values (as the peak-to-peak amplitudes) for each subject, two recording sessions were made before cTBS application. In these sessions, MEP amplitude was measured 40 times each. After applying the same cTBS as in the Experiment 1, a post-hoc recording session was made 15 min later, and was subsequently repeated every 30 min (i.e. 15, 30, 60, 90, and 120 min after cTBS). Each of the post-hoc recording sessions consisted of 20 MEP recordings. There was a 6-min interval from the first post-hoc contraction task to the following recording session, in order to avoid possible post-exercise facilitation of MEPs.

2.2.3. EEG and EMG recordings

EEG signals were recorded from 19 scalp electrodes mounted on a cap (Electro-cap International, Inc., Eaton, OH) according to the conventional 10–20 electrode placement system. Electrode impedance was kept below 10 kΩ each for a high signal-to-noise ratio. Earlobe Ag–AgCl surface electrodes served as the reference. Surface EMG was recorded from the right FDI muscle with a reference surface electrode placed on the second metacarpal bone of the index finger. EEG and EMG signals were recorded by a bioamplifier (MME-3124; Nihon Kohden, Tokyo, Japan) with passbands of

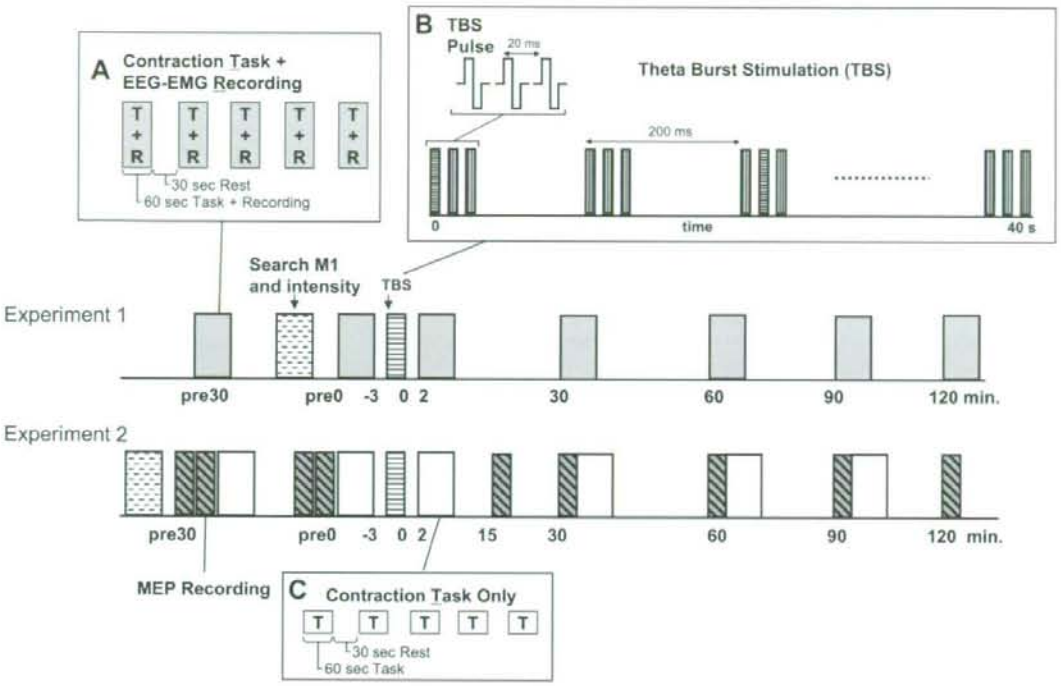

Fig. 1. Experimental design. Experiment (1): In each session (gray bars); EEG and EMG signals were recorded while subjects were performing 1-min-long contraction five times with 30-s-rests (inset A. T + R indicates Task and Recording). Motor hot spot and cTBS intensity were determined between two pre-sessions (horizontally dashed bar). cTBS was delivered at $t = 0$ (horizontally striped bar). cTBS consisted of three biphasic 50-Hz TBS pulses repeating themselves at every 200 ms (inset B). Experiment (2): In each session (white bars); subjects were asked to perform 1-min-long contraction five times with 30-s-long rests as in Experiment 1, but without EEG and EMG recording (inset C and T indicate Task without EEG–EMG recording). Each MEP recording session was consisted of 20 single-TMS pulses (diagonally striped bars). Motor hot spot and cTBS intensity were determined prior to two pre-MEP sessions (horizontally dashed bar). cTBS was delivered at $t = 0$ (horizontally striped bar).

0.5–200 and 5–300 Hz, respectively, and with 1-kHz sampling frequency. The EMG signals were rectified to be used for analyses (Halliday et al., 1998; Mima and Hallett, 1999a; Safri et al., 2006, 2007). As mentioned above, simultaneous recordings of EEG and EMG signals were made in Experiment 1.

2.2.4. MEP recording

MEP was recorded from the right FDI muscle using Ag–AgCl surface electrodes. The MEP signal was fed to a bioamplifier (Synax-1200; NEC, Japan) with a passband of 20–3000 Hz, and with 5-kHz sampling frequency. In order to ensure complete relaxation of the right FDI muscle during the MEP recording, the EMG was monitored online, and the data were stored to a personal computer for offline analysis (Signal Software, Cambridge Electronic Design, Cambridge, UK). A High Power Magstim 200 machine and a figure-of-eight coil with mean loop diameters of 70 mm (Magstim Co., Whitland, Dyfed, UK) were used to apply a monophasic single pulse of TMS (100 μs of rise time and 0.8 ms of pulse width) that evokes MEP. The current at the coil intersection flowed toward the handle during the rising phase of the magnetic field. The coil was held tangentially to the skull, the handle end pointed backwards and laterally at a 45° angle away from the midline. In this orientation, the coil was presumably placed perpendicular to the line of the central sulcus, and therefore it was the optimum positioning for inducing a posterior–anterior electric current in the brain thereby achieving the minimum motor threshold (Brasil-Neto et al., 1992; Mills et al., 1992). We determined the optimum scalp position for the activation of the right FDI muscle by moving the coil with 1-cm steps in the presumed M1 area. The position where the single TMS of slightly supra-threshold intensity could produce the largest MEP in the FDI muscle was marked as the "motor hot spot". For each subject, at the beginning of every experiment, the position of TMS was searched and then the single-pulse TMS intensity was set so that the evoked MEP amplitude was approximately 1 mV (Huang et al., 2005; Ishikawa et al., 2007; see also Section 3). MEPs were recorded only in Experiment 2.

2.2.5. Theta burst stimulation

Continuous TBS paradigm used in this study was designed as it was described previously (Huang et al., 2005). Three pulses of the TMS were given at 50 Hz and each of the pulse triplets was applied at 5 Hz for 40 s (600 pulses in total) to the M1 or S1 location (Fig. 1, inset). Each of the magnetic pulse had a 300-μs-long biphasic waveform. The stimulation was delivered using a Magstim Super Rapid stimulator (Magstim Co., Whitland, Dyfed, UK) and a figure-of-eight coil with mean loop diameters of 70 mm. The current at the coil intersection flowed toward the handle in the first phase, and then into reverse in the second phase. The intensity of each pulse for cTBS was set to 80% AMT for the FDI muscle as in the previous reports (Huang et al., 2005; Ishikawa et al., 2007).

2.2.6. Sham stimulation

A figure-of-eight coil connected to an uncharged magnetic stimulator was placed over the left M1 area so that the subject felt the presence of a coil as in the case of real stimulation. In addition, another coil connected to the charged stimulator was held 10 cm above the scalp, and was allowed to pass the same electric current as the real cTBS. This could produce the same sound as the real stimulation, and yet avoid induction of the electric current in the brain.

2.3. Data analysis

2.3.1. Coherence analysis

Coherence function was used to quantify the synchronization between signals of EEG and EMG. Coherence is the squared magnitude of the cross-power spectrum of a signal pair normalized by the product of their auto-power spectra, and is described by the expression

$$\kappa_{XY}^2(f) = \frac{|S_{XY}(f)|^2}{|S_{XX}(f)||S_{YY}(f)|}.$$

Here, $\kappa_{XY}^2(f)$ is the coherency; $S_{XY}(f)$ represents the cross-spectral density function between signals x and y; and $S_{XX}(f)$ and $S_{YY}(f)$ stand for the auto-spectral density function (spectral power) of the signals x and y, respectively. Since coherence is a normalized measure of the cross-correlation between the signal pair, $\kappa_{XY}^2(f) = 1$ represents a perfect linear dependence and $\kappa_{XY}^2(f) = 0$ indicates a lack of linear dependence within the signal pair. To calculate EEG–EMG coherence, the EEG and EMG signals recorded in Experiment 1 were segmented into non-overlapping epochs of 1024-ms duration. For the EEG, the current source density (CSD) method was utilized in order to achieve the spatially sharpened signals (Nunez et al., 1997; Mima and Hallett, 1999b; Safri et al., 2006, 2007). The fast Fourier transform with the epoch size of 1024, resulting in the frequency resolution of 0.98 Hz, was used to convert the signals in time domain into the frequency-domain signals. Cross-spectra between the EMG signal and each of the 19 CSD-transformed EEG signals, as well as auto-spectra of those signals, were calculated for the EEG–EMG coherence to be obtained by the abovementioned equation. For EEG–EEG coherence calculation, EEG signals were CSD-transformed, and the coherence between the signals from scalp electrode pairs was calculated by following the same procedure explained for the EEG–EMG coherence calculation.

2.3.2. Statistical analysis

Coherence magnitudes for $\kappa_{XY}^2(f) > 0$ were assumed to be statistically significant only if they were above the 95% confidence level, which was calculated as described in Rosenberg et al. (1989). Effects of cTBS on contraction force levels, peak coherence frequencies, peak coherence magnitudes and peak-to-peak MEP amplitudes were tested by the repeated-measures of ANOVA (analyses of variance) using within-subject factors. Arc hyperbolic tangent and logarithmic transformations were performed for the statistical evaluation of coherence and power values, respectively (Halliday et al., 1995). The interaction between the TBS site (Experiment 1: M1, S1, and sham; Experiment 2: M1 and sham) and time course of the effect (Experiment 1: pre30, pre0, 2, 30, 60, 90, 120 min; Experiment 2: pre30, pre0, 15, 30, 60, 90, 120 min) was evaluated using two-way repeated-measures of ANOVA as within-subject factors. In addition, the effects of cTBS on coherence or on MEPs were evaluated by employing separate one-way repeated-measures of ANOVA as within-subject factors. When necessary, the Greenhouse–Geisser correction was used to correct the non-sphericity. The post-hoc test with the Bonferroni correction for multiple comparisons was used to compare the coherence or MEP values between sessions. In order to examine correlation between the changes of coherence magnitude and MEP amplitude before and after cTBS, the linear correlation coefficients were calculated for the data from the five subjects who participated in both experiments (i.e. Experiment 1 and 2). Two-way mixed factorial ANOVA as between-subject factors was employed to compare MEP and coherence for unequal number of subjects in the two experiments.

In all analyses, the statistical significance was assumed for p values smaller than 0.05.

Table 1
Mean AMT and stimulus intensities in Experiment 1

cTBS position	AMT	cTBS intensity
M1	53.9 ± 3.5	43.3 ± 2.8
M1 + 2 cm	54.6 ± 3.1	43.8 ± 2.3
Sham	54.8 ± 2.8	43.8 ± 2.1

Values are means ± SEM. All values are given as percentage of maximum stimulator output.
AMT, active motor threshold; cTBS, continuous theta burst stimulation.

3. Results

3.1. Stimulation intensities

None of the subjects reported adverse effects during the cTBS application and the MEP assessment. Mean AMT and cTBS intensities in Experiment 1 are given in Table 1. Mean single-TMS intensities to evoke MEPs in Experiment 2 are presented in Table 2. ANOVA revealed that there was no statistically significant difference in the mean intensities of either AMT, cTBS, or single-TMS under all conditions.

3.2. Isometric contraction

Subjects successfully performed isometric contraction at 15% of MVC during Experiment 1 (Table 3). Two-way repeated measures of ANOVA on the mean contraction levels revealed that neither time (pre30, pre0, 2, 30, 60, 90, and 120 min) nor stimulation site (M1, S1, and sham) was a significant main factor. Also, the interaction of those factors did not show any significant effect on the contraction level. Separate one-way repeated measures of ANOVA revealed that the mean contraction levels did not change significantly with respect to time during either the cTBS-on-M1, cTBS-on-S1, or the sham condition. Mean (±SE) values of the force levels as percentage of MVC are presented in Table 3. These results showed that the subjects were able to perform the motor task in stable condition.

3.3. Cortico-muscular coherence

Fig. 2 shows examples of the EEG–EMG coherence spectra in a subject at 30 min before (pre30), 60 min after, and 120 min after either one of the cTBS (i.e. cTBS-on-M1, cTBS-on-S1, and as the sham). The coherence spectra were mapped according to the approximate locations of the EEG electrodes. Significant coherence values were observed only for the C3 scalp site and within the beta frequency band (13–30 Hz) for all conditions (Table 4). Respective 1-sec-long segments of the EMG signal and the CSD-transformed EEG signal at C3 are shown in the insets of Fig. 2 (upper-right of each scalp image). When cTBS was applied on M1, the peak coherence value decreased after 60 min, and recovered to the original level after 120 min. No significant change was observed in the EEG–EMG coherence spectra when cTBS was applied either on S1 or as

Table 2
Mean AMT, and stimulus intensities in Experiment 2

cTBS position	AMT	cTBS intensity	Stimulus intensity to evoke MEPs
M1	57.4 ± 1.9	46.1 ± 1.4	63.1 ± 4.2
Sham	55.7 ± 2.8	44.7 ± 2.2	63.6 ± 4.6

Values are means ± SEM. All values are given as percentage of maximum stimulator output.
AMT, active motor threshold; cTBS, continuous theta burst stimulation; MEP, motor-evoked potentials.

Table 3
Mean force levels of voluntary isometric contraction as percentage of MVC

cTBS position	Time (min)						
	Pre30	Pre0	2	30	60	90	120
M1	14.6 ± 0.9	14.5 ± 0.7	14.7 ± 0.8	14.8 ± 0.9	15.1 ± 0.9	16.2 ± 1.0	14.8 ± 0.8
M1 + 2 cm	12.8 ± 0.8	13.1 ± 0.8	13.7 ± 0.9	14.3 ± 0.9	13.7 ± 0.9	14.3 ± 0.8	14.0 ± 0.9
Sham	14.4 ± 0.8	14.8 ± 0.6	14.7 ± 0.8	14.1 ± 0.8	15.1 ± 0.7	14.3 ± 0.7	14.3 ± 0.8

Values are means ± SEM. All values are given as percentage of MVC.

the sham. For all subjects, significant magnitude of coherence was always observed for the C3 site, and the peak of coherence spectra was within the beta frequency band (13–30 Hz), before and after the cTBS. On the C3 site, peak coherence values were normalized with the control value, which was obtained by averaging the peak coherence values in the pre30 and pre0 sessions. One-way ANOVA did not reveal any significant difference between the coherence values of the pre30 and pre0 sessions under all conditions (i.e. cTBS-on-M1, cTBS-on-S1, and sham). The control values from the eight subjects (mean ± SE) were 0.067 ± 0.015, 0.068 ± 0.021, 0.054 ± 0.017 for cTBS-on-M1, cTBS-on-S1, and sham, respectively; and one-way ANOVA did not reveal any significant difference among those values. The normalized peak coherence before and after cTBS is shown in Fig. 3. Two-way repeated measures of ANOVA revealed a significant interaction between stimulation site (M1, S1, and sham) and time (pre30, pre0, 2, 30, 60, 90, and 120 min) ($F_{12,84} = 2.22$, $p < 0.05$). One-way repeated measures of ANOVA revealed a significant effect of time on the normalized peak coherence after TBS-on-M1 ($F_{6,42} = 4.61$, $p < 0.05$). And post-hoc Bonferroni multiple comparison test showed that the normalized peak coherence significantly decreased 30 and 60 min after TBS-on-M1 ($p < 0.05$; pre30 and pre0 vs. 30 and 60 min.), and recovered to almost the original level after 120 min ($p < 0.05$; 30 and 60 min vs. 120 min). The normalized peak coherence (mean ± SE) reached 53.9 ± 4.41% and 51.7 ± 4.39% of the control values at 30 and 60 min after TBS-on-M1, respectively. One-way ANOVA showed a significant effect of time for neither cTBS-on-S1 nor sham condition. These results demonstrate that cTBS affected the cortico-muscular coherence when it was applied on M1 but not on S1, and the after-effects of cTBS on the coherence persisted for 30 and 60 min, then faded away in 120 min. Statistical interpretations of the significance of the results on the arc hyperbolic tangent-transformed cortico-muscular coherence were still valid under untransformed condition of coherence (data not shown).

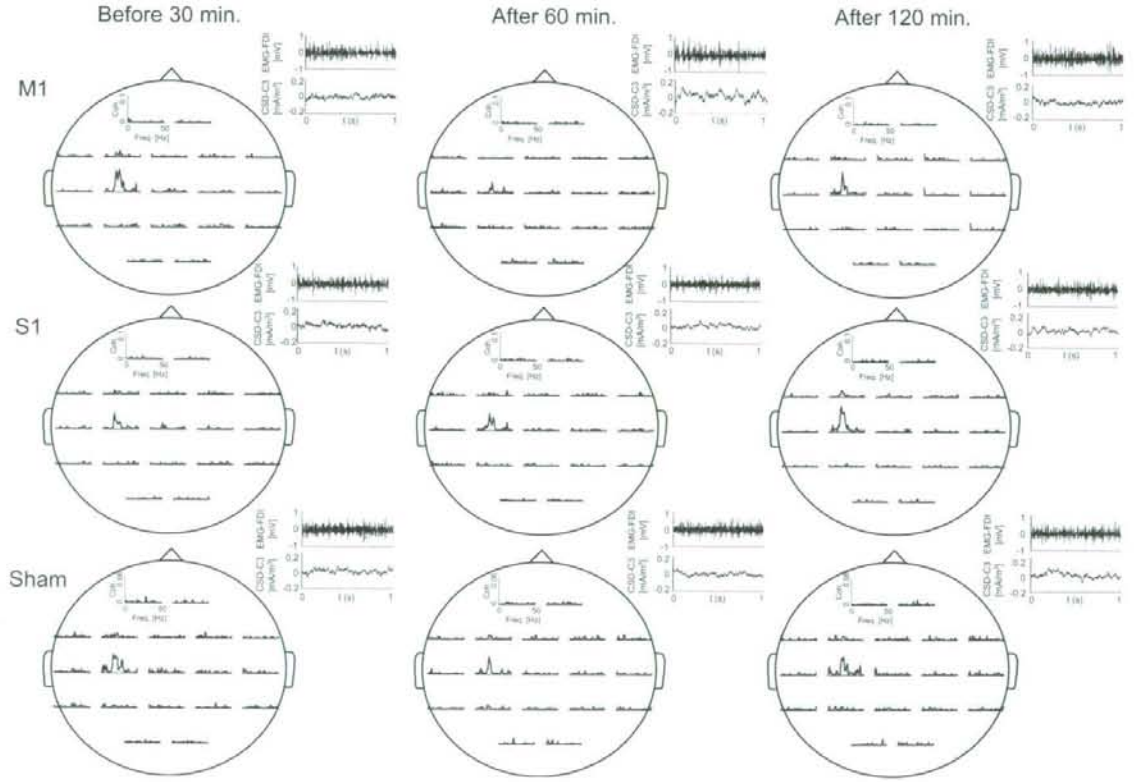

Fig. 2. Coherence spectra and raw traces from EEG and EMG (FDI) signals from one subject. Coherence responses 30 min before, 60 and 120 min after cTBS-on-M1, cTBS-on-S1, and sham are illustrated. Coherences between each of EEG electrodes and EMG are depicted on the approximate location of the corresponding EEG electrode. Horizontal dashed lines indicate the 95% confidence level. Insets right next to the skull images show 1-s segments of raw traces from typical EMG (FDI) and EEG (C3) signals.

Table 4
Frequencies of maximum cortico-muscular(C3-FDI) coherence

cTBS position	Time (min)						
	Pre30	Pre0	2	30	60	90	120
M1	19.5 ± 1.7	19.5 ± 1.2	20.3 ± 1.5	22.7 ± 0.9	19.3 ± 1.5	22.5 ± 1.7	20.3 ± 1.3
M1 + 2 cm	20.6 ± 1.7	20.9 ± 1.5	21.5 ± 1.7	18.7 ± 1.2	20.1 ± 1.6	20.4 ± 1.1	21.6 ± 1.3
Sham	18.2 ± 1.4	20.6 ± 1.6	21.6 ± 1.9	19.9 ± 1.6	18.3 ± 1.5	20.1 ± 1.4	21.7 ± 1.5

Values are means ± SEM. All values are given as Hertz.

3.4. Motor-evoked potentials

Next, we measured the MEPs before and after application of cTBS-on-M1. The peak-to-peak amplitudes of MEP were normalized with the control amplitude, which was obtained by averaging the peak-to-peak MEP amplitudes in the pre30 and pre0 sessions. One-way ANOVA did not reveal any significant difference between the MEP amplitudes of the pre30 and pre0 sessions under all conditions (i.e. cTBS-on-M1 and sham). The control values from the seven subjects (mean ± SE) were 1.25 ± 0.13 and 1.14 ± 0.12 mV for the cTBS-on-M1 and sham conditions, respectively; and paired-sample *t*-test did not reveal any significant difference between those amplitudes. Application of cTBS-on-M1 caused a decrease in the normalized MEP amplitude, as shown in Fig. 4. Two-way repeated measures of ANOVA revealed a significant interaction between stimulation site (M1 and sham) and time (pre30, pre0, 15, 30, 60, 90, and 120 min) ($F_{6,36} = 2.63$, $p < 0.03$). Separate one-way repeated measures of ANOVA revealed a significant effect of time on the normalized MEP amplitudes after cTBS-on-M1 ($F_{6,36} = 3.94$, $p < 0.01$), but not for the sham condition. And post-hoc Bonferroni multiple comparison test showed that the normalized MEPs were significantly suppressed 30 and 60 min after cTBS-on-M1 ($p < 0.05$; pre30 and pre0 vs. 30 and 60 min), and recovered to almost the original level after 120 min ($p < 0.05$; 30 and 60 min vs. 120 min). These results demonstrate that cTBS affected the cortico-spinal excitability when it was applied on M1, and the after-effects of cTBS on the excitability persisted for 30 and 60 min, then faded away in 120 min.

3.5. Comparison between cortico-muscular coherence and motor-evoked potentials

In order to test if the time courses of the changes in cortico-muscular synchronization and cortico-spinal excitability after the application of cTBS-on-M1 were similar, we made two-factor mixed-factorial ANOVA as between-subject factors. The analysis showed a significant time effect ($F_{6,78} = 6.70$, $p < 0.01$), but no significant interaction between time (pre30, pre0, 2, 30, 60, 90, and 120 min) and category (Coherence vs. MEP). The correlation coefficients between the normalized MEP amplitude and the normalized peak coherence value at all time points from the five subjects, who participated in the both experiments, were found to be $r = 0.344$ ($p < 0.05$) for TBS-on-M1 condition (Fig. 5), but not to be significant for the sham condition ($r = 0.015$, $p > 0.05$). These results suggest that the effects of cTBS-on-M1 on cortico-muscular coherence and cortico-spinal excitability followed the similar time course (Fig. 5, insets). Statistical interpretations of the significance of the results on the comparison between motor-evoked potentials and arc hyperbolic tangent-transformed cortico-muscular coherence were still valid for the comparison between motor-evoked potentials and untransformed coherence (data not shown).

3.6. EEG power and cortico-cortical coherence

Since the cortico-muscular coherence was observed specifically for the C3 site, in the beta range (13–30 Hz), and was altered by cTBS-on-M1 but not cTBS-on-S1, we examined whether beta-band oscillation at the C3 (closest electrode to M1) or at the P3 (closest electrode to S1) site, as well as beta-band synchronization between the C3 and P3 sites, was affected by the cTBS. Fig. 6 shows the beta-band power of the CSD-transformed EEG signals for C3 (A) and P3 (B), and the peak magnitude of the beta-band coherence between CSD-transformed EEG signals from C3 and P3 (C), before and after cTBS. In the figures, a vertical axis indicates the value normalized with the control value, which was the average of the power, or of the peak coherence, measured in the pre30 and pre0 sessions. The control values of the beta-band power for C3 from the eight subjects (mean ± SE) were 2.30 ± 0.06, 2.33 ± 0.01, and 2.23 ± 0.09

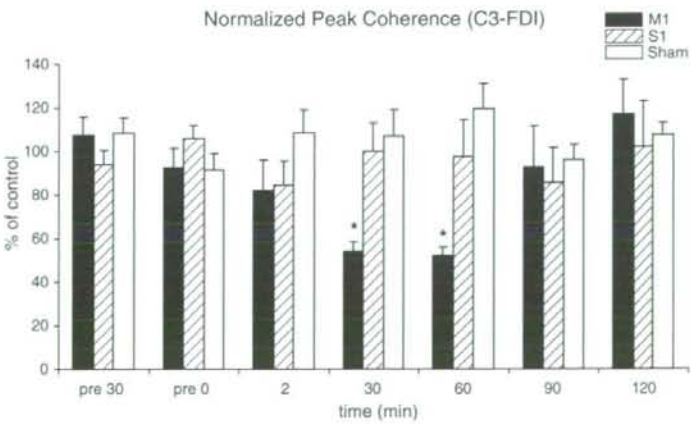

Fig. 3. Effects of cTBS on the arctanh-transformed and normalized peak cortico-muscular (C3-FDI) coherence ($n = 8$). Control value is obtained by averaging the peak coherence values in the pre30 and pre0 sessions. Cortico-muscular coherence is significantly suppressed under cTBS-on-M1 (black bars) but not for cTBS-on-S1(striped bars) and sham (white bars) conditions. Results are displayed as means ± SE (*$p < 0.05$, Bonferroni correction).

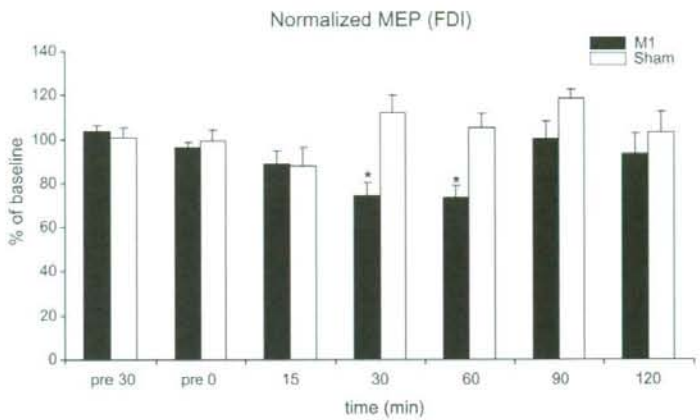

Fig. 4. Effects of cTBS on the normalized MEP amplitudes ($n = 7$). Control value is obtained by averaging the mean MEP amplitudes in the pre30 and pre0 sessions. MEP amplitudes are significantly suppressed under cTBS-on-M1 (black bars) but not for sham (white bars) condition. Results are displayed as means ± SE ($^*p < 0.05$, Bonferroni correction).

($\log_{10}(\mu A/m^3)^2$) for the cTBS-on-M1, cTBS-on-S1, and sham conditions, respectively. The control values of the beta-band power for P3 from the eight subjects (mean ± SE) were 2.24 ± 0.12, 2.23 ± 0.16, and 2.19 ± 0.12 ($\log_{10}(\mu A/m^3)^2$) for the cTBS-on-M1, cTBS-on-S1 and sham conditions, respectively. One-way ANOVA did not reveal any significant difference among the control values of the beta-band power under all conditions. The control values of the beta-band coherence between C3 and P3 (mean ± SE) were 0.43 ± 0.06, 0.42 ± 0.09, and 0.48 ± 0.12 for the cTBS-on-M1, cTBS-on-S1, and sham conditions, respectively. One-way ANOVA did not reveal any significant difference among these control values. We made two-way repeated measures of ANOVA to assess the after-effect of cTBS on the beta-band power and/or coherence. The analyses, however, revealed a significant effect of cTBS neither on the power nor on the coherence. Coherence analyses on the other electrode pairs also did not reveal any significant effect of cTBS on the inter-cortical coherence (data not shown). Statistical interpretations of the significance of the results on the log-transformed EEG power and the arc hyperbolic tangent-transformed cortico-cortical coherence were still valid under untransformed conditions of power and coherence (data not shown).

4. Discussion

Our results on cortico-muscular coherence are consistent with the "consensus" on the existence of beta-band cortico-muscular

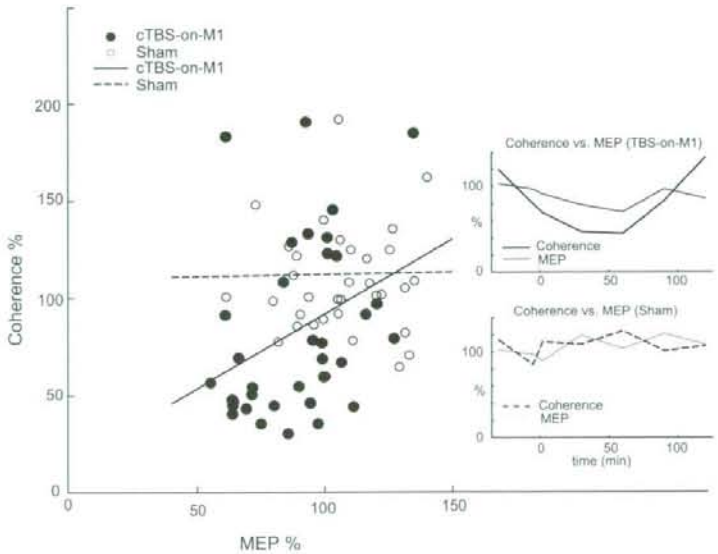

Fig. 5. Arctanh-transformed and normalized peak cortico-muscular (C3-FDI) coherence versus normalized MEP amplitudes ($n = 5$). Points regarding to cTBS-on-M1 and sham sessions are shown by black and white dots, respectively. In the main panel, best lines are depicted for cTBS-on-M1 (solid line) and sham experiments (dashed line). Top inset demonstrates the parallel change of MEP (solid line) and coherence (thick solid line) amplitudes. Bottom inset shows the lack of change in MEP (dashed line) and coherence (thick dashed line) in sham sessions.

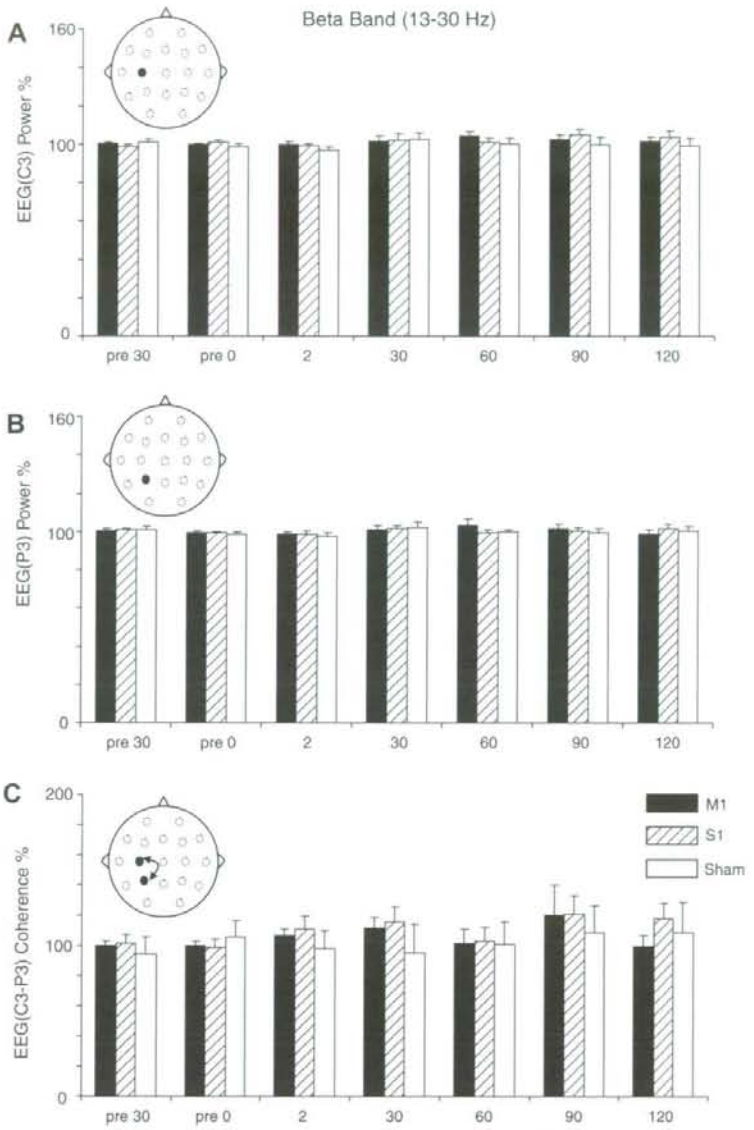

Fig. 6. Effects of cTBS on the normalized log-transformed EEG power and arctanh-transformed cortico-cortical (C3-P3) coherence ($n = 8$). Control values are obtained by averaging either the beta-band spectral power (A and B) or the peak beta-band coherence values (C) in the pre30 and pre0 sessions. Neither C3 (A) and P3 (B) power nor C3-P3 coherence(C) is suppressed under cTBS-on-M1 (black bars), cTBS-on-S1(striped bars) or sham (white bars) conditions. Results are displayed as means ± SE

synchronization, which was previously reported by a number of studies using different recording techniques (EEG: Halliday et al., 1998; Mima and Hallett, 1999b; Mima et al., 2001; Safri et al., 2006, 2007; MEG: Salenius et al., 1997; Murayama et al., 2001). Present results demonstrate that TBS given as a continuous paradigm (cTBS) can suppress cortico-muscular synchronization for more than 30 min. The effect of cTBS was found to be localized to M1, as there was no suppression in the coherence when cTBS was applied on S1 (2 cm posterior to M1). The suppression in coherence is likely to be due to cTBS because no changes were observed in response to sham stimulation. These results cannot be explained by any placebo effect because subjects were not informed of the difference between stimulations (M1, S1, or sham) and they did not notify any difference between those conditions. Previous studies showed that TMS or rTMS has access to cortico-muscular coherence. A single pulse of TMS-on-M1 can increase cortico-muscular coherence for up to 600–800 ms (Hansen and Nielsen, 2004) and 0.9 Hz rTMS on premotor cortex can suppress cortico-muscular coherence for less than 15 min (Chen et al., 2003). However, the number of reports on the rTMS-coherence interaction is very limited. Besides, the effects of cTBS on cortico-muscular coherence have not been investigated yet, therefore it is difficult to understand completely how the variety of rTMS paradigms modifies cortico-muscular coherence. The mechanism be-

hind the coherence suppression by cTBS-on-M1 could be explained by the synaptic inhibition in the primary motor area circuits rather than by the direct inhibition at the axons or cell bodies of the cortico-spinal neurons. Previous studies described the suppressive effects of rTMS patterns by the induction of LTD-like effects on synaptic connections (1 Hz rTMS Fitzgerald et al., 2002; Maeda et al., 2000 and cTBS: Huang et al., 2005, 2007). More specifically, Di Lazzaro et al. (2005) explained the MEP suppression phenomenon by showing that cTBS can suppress the cortico-spinal I1-wave in humans. It is previously shown that I-waves are evoked by trans-synaptic activation of cortico-spinal neurons (Ziemann and Rothwell, 2000). Thus, cTBS could probably inhibit those trans-synaptic activations. It is not easy to reconcile beta-band cortico-muscular oscillation with the I-wave frequencies; however, both I-waves and beta-band cortico-muscular coherence reflect motor-cortical drive from M1 to the spinal motoneuron pool (Salenius et al., 1997; Gerloff et al., 2006). Therefore, we can speculate that cTBS-on-M1 could alter beta-band trans-synaptic coupling of cortico-spinal neurons. In this study, neither strength nor frequency of coherence was affected by S1 stimulation. There could be two possible explanations: first, the conditioning effect of cTBS did not induce any effect on S1 area; however, previous studies showed that cTBS-on-S1 was significantly effective on SEP amplitudes (Ishikawa et al., 2007). Alternatively, S1 area was conditioned by cTBS but this change did not interfere with cortico-muscular coupling. It was previously shown that afferent sensory feedback from muscle to sensorimotor cortex highly interacts with the motor control system (Mima et al., 2001) and Pohja and Salenius (2003) demonstrated that the reduction of sensory feedback by ischemic sensory deafferentation does not alter the dominant frequency of coherence but affect the strength of the coherence indirectly. By contrast, there are other reports arguing that the sensory feedback may not be critical for coherence generation. In a previous report (Gerloff et al., 2006), patients with congenital hemiparesis which result in interhemispheric reorganization of the motor area were studied. In those patients, congenital lesions cause M1 to be relocated to ipsilateral hemisphere while corresponding S1 remained in the lesioned (contralateral) hemisphere. Remarkably, cortico-muscular coherence was only found in the ipsilateral hemisphere, identifying the origin of cortico-muscular coherence could be M1 rather than S1. On the other hand, such reorganizations due to neural lesions could also affect physiological properties of the sensory motor system as well. But another report (Mima et al., 2000) on healthy subjects supports the previous idea. In that study, vibratory stimulation of a muscle tendon, introducing modified somatosensory activity, had no effect on beta-band cortico-muscular coherence. Our results may further support that M1 is more important than S1 in cortico-muscular coherence generation.

Here, we showed that the cortico-muscular coherence and the MEP amplitude were suppressed 30 and 60 min after cTBS-on-M1 and recovered back to the original levels after 90 and 120 min. Conventional rTMS paradigms with more TMS pulses could produce similar but shorter effects on MEPs (Touge et al., 2001). Here, the parallel changes of cortico-muscular coherence and MEP amplitude confirm the efficiency of cTBS over conventional rTMS paradigms not only by means of cortico-muscular excitability but also cortico-muscular coherence.

Several reports have demonstrated that conventional rTMS patterns may also have some potential to temporarily improve the symptoms of neuropsychiatric disorders such as epilepsy (Tergau et al., 1999), Parkinson's disease (Siebner et al., 1999) and writer's cramp (Murase et al., 2005). Although these symptoms have diverse characteristics, they may show some rhythmic components (i.e. absence epilepsy: Guye et al., 2001), and some of these anomalous rhythmic components can be observed as a change in the cortico-muscular coherence. For example, previous studies reported abnormally enhanced cortico-muscular coherence in epilepsy patients (Unverricht–Lundborg type progressive myoclonus: Silén et al., 2002; cortical myoclonus: Grosse et al., 2003). Therefore, cortico-muscular coherence analysis could be regarded as a promising tool to quantify such an abnormality arising from the abnormal rhythmic brain activities. From this point of view, our results confirm that cTBS is capable of efficiently suppressing a rhythmic phenomenon (i.e. cortico-muscular coherence), and thus may have a potential in eliminating those abnormalities.

Previous studies showed that change in the cortico-muscular coherence was not necessarily accompanied by a change in motor performance (Safri et al., 2006, 2007). Consistently, the present results showed that motor performance was not affected by the suppression of cortico-muscular coherence, and the subjects were able to perform the assigned isometric contraction task with high precision (MSE < 1%, Table 3). However, cortico-muscular coherence was found to be related to specific parameters of hand motor function and it has been shown that the magnitude of the cortico-muscular coherence during a simple isometric contraction is smaller than that of the coherence during a relatively complex motor task (Kilner et al., 2000). Therefore, further studies on the relation between motor performance and cortico-muscular coherence (as well as cortico-spinal excitability) are necessary for characterizing the functional meaning of cortico-muscular coherence.

Our experimental design included 5-min-long sessions of intermittent isometric contraction of the target muscle in order to assess cortico-muscular coherence (see Section 2). Huang et al. (2008) demonstrated that contraction during or immediately after 20 s of cTBS delivery can alter the after-effect of stimulation. In that study, 1-min isometric contraction of FDI muscle during or immediately after cTBS delivery can abolish or reverse the inhibitory effect of cTBS, respectively, whereas contraction 10 min after the cTBS had only transient effect on the inhibition. In this study, the effect of cTBS on M1 remained inhibitory according to the MEP size, although the contraction of the target muscle was performed at 2, 30, 60, and 90 min after the end of cTBS. There are two possibilities may cause the discrepancy. First, as we have known that 40 s of cTBS produces much longer lasting after-effect than 20 s of cTBS does (60 min vs. 20 min) (Huang et al., 2005), it is possible that 40 s of cTBS and 20-s of cTBS behave differently and the inhibitory effect is consolidated within or very shortly after 40-s of cTBS. Second, although synaptic plasticity can be reduced or reversed by physiological activity after plasticity induction, previous animal studies have proved that the effect is greatest, the nearer it is to the end of the induction period (Chen et al., 2001). A 2-min gap may be long enough for the inhibitory effect of cTBS to be stabilized, and muscle contraction after the 2-min gap can no longer modify the inhibitory effect.

In conclusion, continuous paradigm of theta burst rTMS over M1 at an intensity of 80% AMT induced long-lasting inhibitions of cortico-muscular coherence and of cortico-spinal excitability with similar time courses. cTBS on 2 cm posterior to M1 did not exert any significant suppression on cortico-muscular coherence. These results further confirmed that cTBS is a prominent tool to induce temporal cortical plasticities which can be assessed not only by means of MEP amplitudes, but also by means of cortico-muscular coherence analyses during near-natural motor tasks.

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Comparison of monophasic versus biphasic stimulation in rTMS over premotor cortex: SEP and SPECT studies

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ABSTRACT

Objective: To optimize the clinical uses of repetitive transcranial magnetic stimulation (rTMS), we compared the effects of rTMS on somatosensory-evoked potentials (SEPs) and regional cerebral blood flow (rCBF) using different phases (monophasic *vs.* biphasic) or frequencies (0.2 Hz *vs.* 0.8 Hz) of stimulation.
Methods: In the first experiment, different phases were compared (0.2 Hz monophasic *vs.* 0.2 Hz biphasic). Biphasic 1 Hz or sham condition served as controls. The second experiment was to explore the effect of frequencies (0.2 Hz *vs.* 0.8 Hz) using the monophasic stimulation. Substhreshold TMS was applied 250 times over the left premotor cortex. Single photon emission computed tomography (SPECT) was performed before and after monophasic 0.2 Hz or biphasic 1 Hz rTMS.
Results: Monophasic rTMS of both 0.2 and 0.8 Hz significantly increased the ratio of N30 amplitudes as compared with sham rTMS, whereas biphasic stimulation showed no significant effects. SPECT showed increased rCBF in motor cortices after monophasic 0.2 Hz rTMS, but not after biphasic 1 Hz stimulation.
Conclusions: Monophasic rTMS exerted more profound effects on SEPs and rCBF than biphasic rTMS over the premotor cortex.
Significance: Monophasic rTMS over the premotor cortex could be clinically more useful than biphasic rTMS.

1. Introduction

Repetitive transcranial magnetic stimulation (rTMS) produces excitatory or inhibitory effects depending on the intensity and frequency of stimulation. High-frequency stimulation ($\geqslant$5 Hz) increases the cortical excitability, whereas low-frequency rTMS ($\leqslant$1 Hz) decreases it for an extended period of time (Chen et al., 1997; Chen, 2000). In recent years, these effects have been used as a tool for treating various disorders (Siebner et al., 1999; Hoffman and Cavus, 2002; Murase et al., 2005). In writer's cramp, monophasic 0.2 Hz rTMS over premotor cortex had therapeutic effects (Murase et al., 2005), but not biphasic 1 Hz rTMS over premotor cortex (Siebner et al., 2003). By contrast, biphasic 1 Hz rTMS over primary motor cortex (MC) was clinically effective (Siebner et al., 1999), but monophasic 0.2 Hz rTMS over MC was not (Murase et al., 2005). These varying effects of rTMS may result from different conditions including the phase or the frequency of stimulation.

Previous studies reported abnormal sensorimotor integration in dystonia (Tinazzi et al., 2000; Abbruzzese et al., 2001; Kaji et al., 2005), and sensory processing before movement is impaired in hand dystonia (Murase et al., 2000). Presumably rTMS for dystonia may influence the sensory modulation in the central nervous system and this modulation is, at least partly, responsible for the therapeutic effect.

We recently demonstrated significant changes of somatosensory-evoked potentials (SEPs) after monophasic 0.2 Hz rTMS, but not after biphasic 1 Hz rTMS over premotor cortex in normal subjects (Urushihara et al., 2006). In another study (Enomoto et al., 2001), biphasic 1 Hz rTMS over the MC, not over premotor cortex, affected the amplitude of SEPs. Although, in these studies, the authors used similar positions and numbers of magnetic stimulation, the pulse phases and frequencies of stimulation were different.

Previous studies on the effects of rTMS on the motor excitability have indicated that biphasic 1 Hz rTMS-induced inhibitory aftereffects on the MC (Chen et al., 1997; Wassermann et al., 1998a; Sommer et al., 2002), but few studies reported on the effect of very low-frequency ($\leqslant$1 Hz) monophasic rTMS (Sommer et al., 2002). Moreover, little is known about the difference between monophasic and biphasic rTMS. For example, monophasic stimulation increased motor-evoked potential (MEP) amplitude more than

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biphasic stimulation (Arai et al., 2005). Monophasic 1 Hz rTMS-induced larger inhibitory after-effects on the motor cortex than biphasic 1 Hz rTMS (Sommer et al., 2002). No studies compared the effects on SEPs between monophasic and biphasic rTMS over premotor cortex.

The aim of this study is to explore the optimum condition of rTMS (monophasic *versus* biphasic and 0.2 *vs.* 0.8 Hz) over premotor cortex for modulating the cortical sensory processing. In addition, we recorded single photon emission computed tomography (SPECT) immediately before and after monophasic 0.2 Hz or biphasic 1 Hz rTMS over premotor cortex to investigate changes in regional cerebral blood flow (rCBF) associated with those in SEPs.

2. Materials and methods

2.1. Subjects

Thirteen healthy right-handed volunteers [11 males, 2 females; mean age ± standard deviation (SD): 32.2 ± 9.8 years] participated in this study. The subjects were free from neurological and psychiatric diseases. All subjects gave their informed consent for this study, which was approved by the Ethics Committee of the University of Tokushima, School of Medicine.

2.2. Experimental design (Fig. 1)

2.2.1. Experiment 1 (SEP study using different phase-rTMS)

Eight subjects participated in this study (8 males; mean age ± SD: 32.3 ± 10.0 years). We compared the rTMS effect under the different condition of 0.2 Hz monophasic and biphasic stimulation. Biphasic 1 Hz (conventional stimulation) and sham rTMS served as controls.

In monophasic 0.2 Hz rTMS condition, we also recorded SEPs at 30 min after the end of rTMS in order to confirm the duration of after-effect in 7 of 8 subjects (mean age ± SD: 32.7 ± 10.7 years).

2.2.2. Experiment 2 (SEP study using different frequency-rTMS)

Nine subjects were included (7 males, 2 females; mean age ± SD: 33.7 ± 11.0 years). Four of 9 subjects participated in Experiment 1. We compared monophasic 0.2 Hz with monophasic 0.8 Hz rTMS to see the frequency effect in the SEPs. We could not use monophasic 1 Hz rTMS, because of technical limitations of the stimulator.

Subjects were blinded as to the phase of stimulation. SEP recording sessions were performed under five different rTMS conditions on five separate days (Fig. 1A). SEPs were recorded immediately before and after rTMS in each of the five conditions.

2.2.3. SPECT study

We evaluated the effect of monophasic 0.2 Hz and biphasic 1 Hz rTMS on cortical blood flow using SPECT. Seven of 8 subjects of the same group of experiment 1 participated in this study (mean age ± SD: 32.7 ± 10.7 years). All of them had SEP studies on separate days. SEPs and SPECT session were performed at least 1 week apart, according to the previous studies (Siebner et al., 2003; Urushihara et al., 2006), and the orders of the studies were randomly assigned.

2.3. rTMS

We used the same procedure for rTMS as the previous study on writer's cramp (Murase et al., 2005). Magnetic stimuli of 250 times were delivered to the left premotor cortex, 2 cm anterior and 1 cm medial to the hot spot (Schluter et al., 1998). We determined the optimal position for activation of the right first dorsal interosseous (FDI) muscle by moving the coil in 0.5-cm steps around the

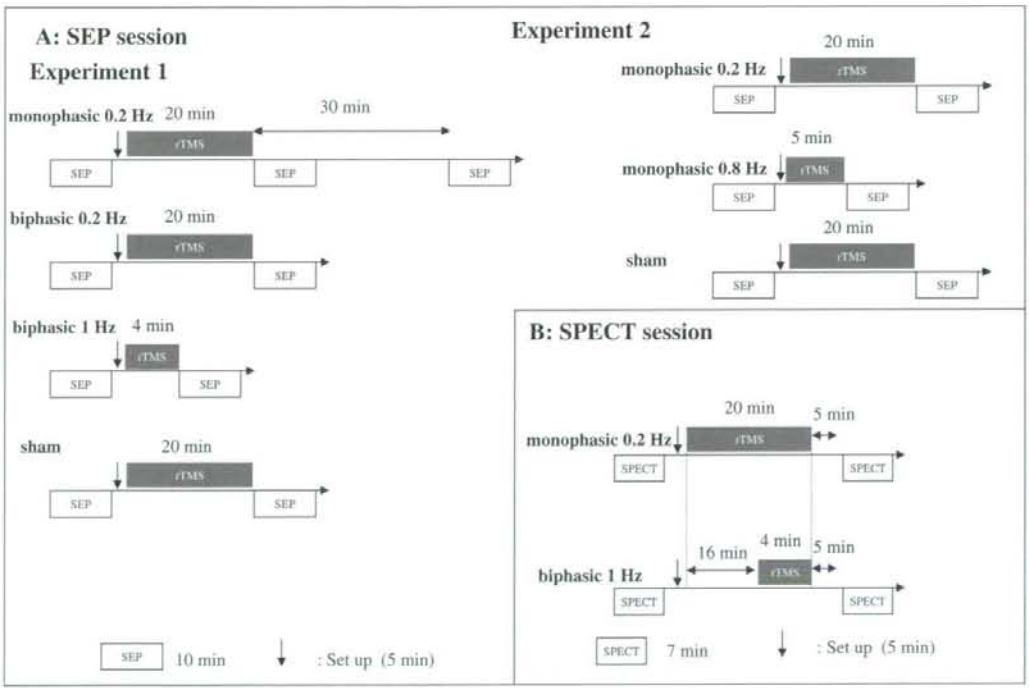

Fig. 1. Time course of each recording session. (A) shows the time course of SEP sessions, and (B) shows those of SPECT sessions. Setup: time to decide the position and intensity of magnetic stimulus.

presumed motor area. Motor response was recorded using electromyography. Threshold was defined as the minimum stimulation level necessary to evoke motor potentials of >50 μV peak-to-peak amplitude in 5 of 10 trials. The coil was positioned tangentially to the curvature of the head and handle of the coil formed a 45° angle with the subject's body midline. The current in the brain was in the postero-anterior direction for monophasic pulses and postero-anterior direction at the initial phase for biphasic pulses. Stimulation intensity was 85% of the resting motor threshold for the motor cortex.

In monophasic 0.2 Hz rTMS, figure-of-eight stimulation coil (outside diameter of one half-coil, 8.7 cm) connected to Magstim 200 stimulator (2.2 T at the coil surface when connected to Magstim 200; Magstim Co. Ltd., OHR Wales, UK) was placed over the premotor cortex. Stimulus frequency was set at 0.2 Hz. Stimuli were given for about 20 min.

In monophasic 0.8 Hz rTMS, a figure-of-eight stimulation coil (outside diameter of one half-coil, 8.7 cm) was placed over the premotor cortex. Two Magstim 200 stimulators (2.2 T at the coil surface when connected to Magstim 200; Magstim Co. Ltd., OHR Wales, UK) controlled by Bistim module (Magstim Co. Ltd., OHR Wales, UK) were connected to the TMS coil. Stimuli were given for about 5 min at 0.8 Hz.

In biphasic 1 Hz rTMS session, we used a figure-of-eight coil connected to Magstim rapid stimulator (Magstim Co. Ltd., OHR Wales, UK), placed over the premotor cortex. Stimuli of 250 times were given for 4 min.

For biphasic 0.2 Hz rTMS, we used a figure-of-eight coil connected to Magstim rapid stimulator (Magstim Co. Ltd., OHR Wales, UK), placed over the premotor cortex. Stimuli of 250 times were given for 20 min.

Sham coil stimulation (sham) was performed over the premotor cortex using a figure-of-eight sham coil in the same place as monophasic 0.2 Hz condition (a placebo system; Magstim Co. Ltd., OHR Wales, UK; outside diameter of one half-coil, 8.7 cm, the same shape as that of a true coil) connected to Magstim 200 stimulator (0.44 T at coil surface when connected to Magstim 200). It made sound similar to the real coil. Stimulus frequency was set at 0.2 Hz. These parameters of rTMS were in accordance with the International Safety Guidelines (Wassermann, 1998b).

2.4. SEPs

SEPs were evoked by median nerve stimulation at the right wrist. Electrical stimuli (0.2 ms duration) were delivered at 1 Hz by surface electrodes. The intensity was just above the motor threshold. SEPs were recorded with silver chloride disk surface electrodes at F3, F4, 2 cm posterior to C3 (C3′) and 2 cm posterior to C4 (C4′), according to the International 10–20 system. We examined topographical changes of SEPs after monophasic 0.2 Hz rTMS with 62-electrodes EEG recording system in the recent study (Urushihara et al., 2006). In this study, significant SEP changes were limited to the F3 region. Based on this, we used four recording positions in this study, because minimum numbers of electrodes were essential to examine the time course of SEP changes over a short period. The linked earlobe electrodes served as the reference. The impedance of these electrodes was kept below 3 kΩ. The electrooculogram (EOG) was also recorded with a pair of silver chloride disk electrodes at 2 cm above and 2 cm below the right outer canthus. Signals from scalp electrodes and EOG were amplified and acquired at a sampling rate of 10 kHz and filtered at 1–5000 and 0.5–1000 Hz, respectively (MEB2200 amplifier; Nihon Kohden, Tokyo, Japan).

We analyzed components which were detectable in all subjects: five components at C3′, an initial positive peak with a latency of 10–16 ms (P14), a following negative large peak (N20), a second positive peak (P26), a second negative peak (N34) and a third positive peak (P45). We identified only P14 (initial positive peak) at C4′. Recordings from F3, three components following P14 (initial positive peak) were analyzed: a second positive peak of 15–25 ms (P22) and two negative peaks (N30 and N60). We identified P14 (initial positive peak) and N30 (negative peak of 25–35 ms) at F4. We measured the baseline-to-peak amplitudes of these components. The baseline was defined as the segment between 2 and 6 ms after stimulation.

2.4.1. Statistical analysis (Experiment 1 and 2)

In Experiment 1 and 2, the ratios of amplitudes in each SEP component before and after rTMS were calculated, and were compared among conditions by one-way repeated-measures ANOVA (Experiment 1; monophasic 0.2 Hz, biphasic 1 Hz, biphasic 0.2 Hz, sham rTMS. Experiment 2; monophasic 0.2 Hz, monophasic 0.8 Hz, sham rTMS). When statistical significance was reached, for further post-hoc analysis, Dunnett's multiple comparison tests were used to compare the ratios of amplitude in real stimulating conditions with those in sham condition.

In monophasic 0.2 Hz, we also analyzed the time course of rTMS effects, N30 amplitudes at each time (before, immediately after and 30 min after rTMS) was tested with one-way repeated-measures ANOVA. For post-hoc test, Dunnett's multiple comparison test was used to compare N30 amplitudes immediately after or 30 min after rTMS with that before rTMS.

All data were analyzed with SPSS version 11.01 J for Windows (SPSS Japan Institute Inc., Tokyo). Results were considered significant at the level of $p < 0.05$.

2.5. SPECT

SPECT images were recorded immediately before rTMS for monophasic 0.2 Hz rTMS, and were obtained 16 min before rTMS for biphasic 1 Hz rTMS. The second SPECT images were taken 5 min after rTMS in both conditions (Fig. 1B).

We designed this SPECT protocol (single-day split-dose stress brain SPECT recording) to observe the qualitative differences in distribution of blood flow changed regions between after monophasic 0.2 Hz and biphasic 1 Hz rTMS. The previous study (Wong et al., 1996) confirmed that this protocol was suited for the qualitative evaluation of rCBF changes without any adjustment. Similar protocols as those of ours have been used in other imaging studies (Audenaert et al., 2000; Urushihara et al., 2006).

Each subject received an injection of 555 MBq 99mTc-ethyl Cysteinate dimmer (ECD). Data acquisition was started 5 min after the injection using double-head gamma camera (E.CAM Signature; Toshiba, USA) with a total acquisition time of 7 min. During this session, the subjects lay in the supine position on the bed and were instructed not to move. The head of each subject was immobilized using a head holder. The projection of data was obtained by a 128 × 128 format for 30 angles at 180° for each camera with 30 s per angle. A Butterworth filter was used before SPECT image reconstruction, and no attenuation correction was performed.

All images were reconverted into ANALYZE format for statistical parametric mapping analysis and underwent normalization onto the template and smoothing. The normalized data were then smoothed using a Gaussian kernel (full-width at half-maximum of system resolution: 12 mm). The resultant voxel size was 2 × 2 × 2 mm. All data were analyzed by SPM2 (Wellcome Department of Cognitive Neurology, University College London, UK). The difference in adjusted rCBF between before and after rTMS was determined by a voxel-by-voxel paired t-test setting at height threshold ($p = 0.001$), uncorrected for independent multiple comparison. These differences were considered significant if they survived a correction for multiple comparisons with cluster level at