Development/Plasticity/Repair

Movement-Related Cortical Stimulation Can Induce Human Motor Plasticity

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Repeated paired associative stimulation combining peripheral nerve stimulation and transcranial magnetic stimulation (TMS) of the primary motor cortex (M1) can produce human motor plasticity. However, previous studies used paired artificial stimuli, so that it is not known whether repetitive natural M1 activity associated with TMS can induce plasticity or not. To test this hypothesis, we developed a movement-related cortical stimulation (MRCS) protocol, in which the left M1 was stimulated by TMS at specific timing with respect to the mean expected reaction time (RT) of voluntary movement during a simple reaction time task using the right abductor pollicis brevis (APB) muscle. Seventeen normal volunteers were subjected to repeated MRCS intervention (0.2 Hz, 240 pairs). Motor function was assessed before and after MRCS. When TMS was given 50 ms before the RT of movement [MRCS(−50)], motor-evoked potential (MEP) amplitude of the right APB, but not other muscles, increased for up to 15 min post-MRCS. The RT of the right APB was also shortened. However, spinal excitability measured by F-wave did not change. When TMS was given 100 ms after the RT [MRCS(+100)], MEP amplitude was decreased. These findings show that this new MRCS protocol can produce timing-dependent motor associative plasticity, which may be clinically useful.

Introduction

Synaptic plasticity refers to an activity-dependent modification of synaptic strength (Citri and Malenka, 2008). Hebb's postulate of synaptic modification by correlated activity has become a cornerstone in our understanding of activity-dependent neural plasticity (Hebb, 1949), and was linked to the processes of long-term potentiation (LTP) and/or inhibition (LTD) (Bliss and Lomo, 1973). Long-term potentiation can be induced by use of pairing protocols (associative or Hebbian LTP), which imply synchronous activation of two inputs to a neuron, or synchronous presynaptic and postsynaptic depolarization. Together, LTP and LTD allow activity-dependent modification of synaptic strength, thus serving as promising candidates for the synaptic basis of learning and memory (Martin et al., 2000).

Stefan and others succeeded to induce plasticity in the human primary motor cortex (M1) by pairing low-frequency peripheral nerve stimulation with transcranial magnetic stimulation (TMS) over the contralateral M1. This paired associative stimulation (PAS), rapidly induces a long lasting, reversible, and somatotopically specific increase in the cortical excitability, which may be related to the associative LTP (Stefan et al., 2000).

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The associative LTP in animal models can be induced when an action potential of the postsynaptic neuron was preceded by the EPSP induced by another input. If the order of stimulation was reversed, LTD of that input is induced (Allen and Tsukahara, 1974; Markram et al., 1997; Bi and Poo, 1998; Debanne et al., 1998; Feldman, 2000). This form of associative plasticity is governed by a strict temporally asymmetric rule. Similarly in human, following PAS, when the median nerve stimulation preceded TMS pulse by 25 ms, M1 excitability increased, whereas PAS with ISI of 10 ms led to its depression (Wolters et al., 2003).

In previous forms of PAS, the peripheral electrical stimulation induces activity in M1 through thalamocortical "vertical" and/or corticocortical fibers from the somatosensory cortex (Wolters et al., 2005). However, it has been rarely investigated whether other types of afferent input to M1 combined with TMS can produce similar associative LTP-like effects (Koganemaru et al., 2009; Rizzo et al., 2009) or not. One recent animal study showed that the repetitive activation of the artificial connection between M1 neurons via implantable electronic circuits can produce long-term plasticity (Jackson et al., 2006). If associative stimulation is a general principle for human neural plasticity, it is possible that natural physiological activation of M1 during the reaction time task synchronized with TMS can also produce associative LTP/LTD-like plasticity.

Instead of pairing a peripheral nerve stimulation (Stefan et al., 2000, 2002; Wolters et al., 2003) or contralateral M1 stimulation (Koganemaru et al., 2009; Rizzo et al., 2009) with TMS, we consistently paired voluntary thumb abduction movement with TMS over M1; movement-related cortical stimulation (MRCS). As a paired stimulation which was repeated in MRCS, we applied

TMS over M1, before or after the mean RT of thumb movements during the overlearned reaction time task. We hypothesized that MRCS combining TMS with endogenous movement-related activity in M1 can induce timing-dependent plasticity in motor function.

Materials and Methods

Subjects. Experiments were performed on 17 healthy volunteers (9 males, and 8 females) aged 19–43 years (29.5 ± 6 years). All subjects were right handed as determined by Oldfield handedness inventory (Oldfield, 1971). None of the subjects had a history of neurological or psychiatric disorders or was under drug treatment during experiments. All subjects gave written informed consent before experiments. The protocol was approved by the Ethics Committee of Kyoto University Graduate School of Medicine.

Recordings. Each subject was seated comfortably on an armchair with his or her arms placed on the armrest with the hands facing upward. Surface electromyogram (EMG) was recorded from the abductor pollicis brevis (APB) and abductor digiti minimi (ADM) muscles on both sides (experiment 1) or on the right side (experiments 2, and 3), using pairs of silver electrodes. The EMG signals were amplified, bandpass filtered (5–2000 Hz), digitized at a rate of 10 kHz and stored for offline analysis. The subjects were asked to keep relaxation throughout the experiments with the aid of visual feedback from the EMG monitor.

TMS. TMS was given using a figure-of-eight coil (9 cm for the outer diameter) connected to a Magstim 200 stimulator (Magstim Company). The optimal motor point for eliciting the best MEP (hot spots) for APB muscle was established by a suprathreshold stimulus over the M1 contralateral to the target muscle with the coil held ~45° to the midsagittal line (approximately perpendicular to the central sulcus). The optimal position was marked on the scalp by a soft tip pen to ensure identical placement of the coil throughout the experiment. The direction of the induced current was from posterior to anterior.

TMS measurements. The resting motor thresholds (rMT) for relaxed APB muscle(s) were determined to the nearest 1% of the stimulator output and defined as the lowest stimulus intensity required for eliciting MEP with peak to peak amplitude >50 μV in at least 5 of 10 trials (Rossini et al., 1994). The active motor threshold (aMT) was recorded as the minimum intensity at which MEPs with an amplitude of ~200 μV can be distinguished from the background activity in 50% of trials (Rothwell et al., 1999).

For the evaluation of the corticospinal excitability, we measured the peak-to-peak MEP amplitudes of the right and left APB and ADM muscles for 20 trials and the averages were taken. The intensity of the test stimulus was adjusted to produce MEP of ~0.5–1 mV from the relaxed APB muscle before MRCS (SI_{1mV}).

For silent period (SP) recording, the stimulation intensity was adjusted to be 120% of the rMT of the right APB before MRCS. The SP was assessed during the isometric contraction of the right APB at ~15% of the maximum force. Its duration was taken from the onset of TMS to the return of voluntary EMG activity.

For the measurement of short interval intracortical inhibition (SICI), paired pulse magnetic stimuli were applied over the hot spot of the right APB muscle (Kujirai et al., 1993). The intensity of the conditioning stimulus was adjusted to 95% of aMT measured before MRCS, and that of the test stimulus was adjusted to SI_{1mV} with an interstimulus interval (ISI) of 3 ms (Ziemann et al., 1996b). For further analysis, the ratio of the conditioned MEP divided by the test MEP was used.

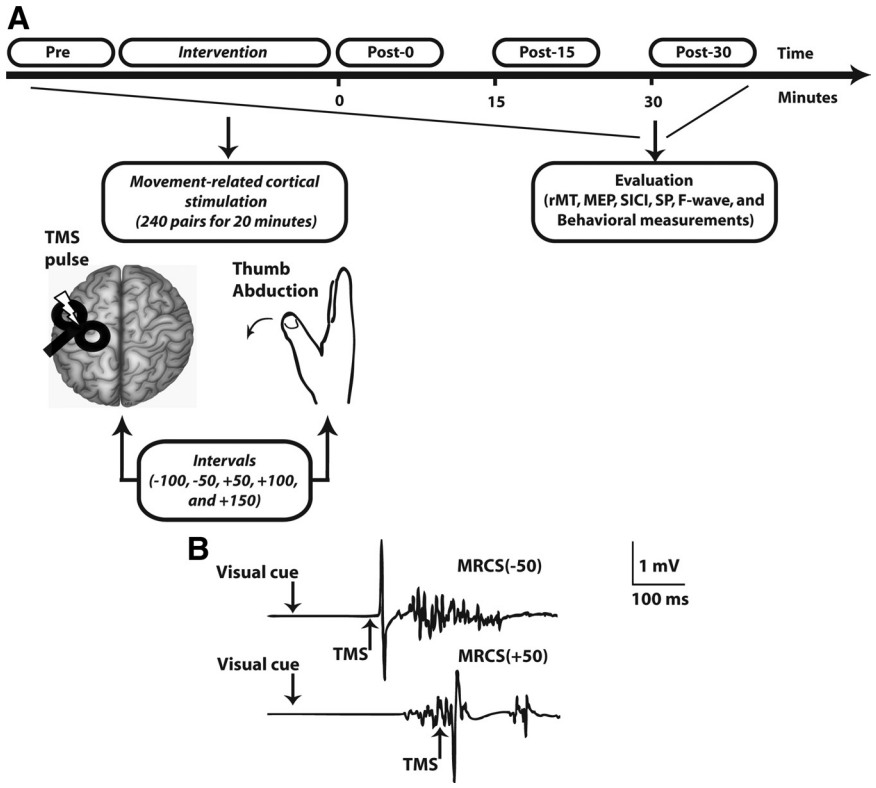

Figure 1. Schematic illustration of experimental design. ***A***, The movement-related cortical stimulation (MRCS) intervention was applied as repetitive TMS pulses preceding/following the mean reaction time of thumb abduction movement of the SRT. The interval between TMS pulses and movements was −50 and +50 (experiment 1), −50 (experiment 2), and −100, −50, +50, +100, and +150 (experiment 3). The intervention consists of 240 pairs at 0.2 Hz for 20 min. Evaluation (rMT, MEP, SICI, SP, F-wave, and behavioral measurements) was done just before, immediately, 15 min, and 30 min after intervention. EMG traces during MRCS ***B***, Single traces of EMG recorded from the right APB muscle were shown during MRCS(−50) (top), and MRCS(+50) (bottom).

F-wave measurements. Excitability of spinal motor neurons was examined by F-wave (Mercuri et al., 1996). The F-wave at right APB muscle was evoked by supramaximal electrical stimulation of the median nerve at wrist before and after the intervention. Twenty F-waves were recorded and averaged.

Pinch force. Pinch forces of both hands were determined using Jamar pinch gauge (Sammons Preston Inc.). Subjects were instructed to press the handle of the pinch gauge with the maximum power by using the thumb and index fingers.

Simple reaction time task. Subjects were comfortably sitting on an armchair with padded armrests, facing a computer monitor situated at ~100 cm before the eyes of subjects. Their forearms were relaxed on top of a small table of adjustable height. They were requested to position their thumb on keyboard buttons in a comfortable position, and instructed to react to a visual cue (the green circle, visual angle: 1.7°) that appeared on the computer monitor (ISI = 5 s) by pressing the button as rapid as possible after stimulus presentation, by brisk thumb abduction movement. Twenty five trials were measured for each hand separately, and the mean RTs were computed.

MRCS. During the intervention protocol, subjects continuously performed the simple reaction time task (SRT) for 20 min (ISI = 5 s) (Fig. 1). The only difference was that subjects performed the thumb abduction without holding the button. After subjects overlearned the SRT, the mean reaction time of movement EMGs (RT_{emg}) for each subject was determined by measuring the time between the onset of the cue and that of the EMG recorded from the right APB (20 trials).

For MRCS, TMS was given at the left M1 in association with the mean estimated RT_{emg}. The pairs of TMS and movement were repeated at a rate of 0.2 Hz for 240 pairs along 20 min. The TMS intensity was 120% of the rMT of the right APB before the intervention. During the main ex-

Table 1. Behavioral and electrophysiological changes after MRCS(+50) and MRCS(−50) in experiment 1

Measures	Types of MRCS	Site	Time			
			Pre	Post-0	Post-15	Post-30
Pinch force (kg)	MRCS(−50)	Right hand	3.5 ± 0.3	3.6 ± 0.4	3.4 ± 0.4	3.6 ± 0.4
		Left hand	3.2 ± 0.3	3.3 ± 0.4	3.4 ± 0.4	3.4 ± 0.4
	MRCS(+50)	Right hand	3.3 ± 0.5	3.3 ± 0.4	3.3 ± 0.4	3.4 ± 0.5
		Left hand	3.3 ± 0.4	3.3 ± 0.4	3.2 ± 0.4	3.4 ± 0.4
Motor evoked potential amplitude (μV)	MRCS(−50)	Right APB	611 ± 59	1323 ± 248*	1113 ± 149*	714 ± 66
		Right ADM	633 ± 170	714 ± 173	904 ± 224	842 ± 227
	MRCS(+50)	Right APB	937 ± 237	848 ± 159	901 ± 170	1077 ± 325
		Right ADM	627 ± 135	630 ± 123	562 ± 142	560 ± 119
Resting motor threshold (%)	MRCS(−50)	Right APB	54.2 ± 3.7	53 ± 3.9	53 ± 3.6	52.6 ± 3.7
		Left APB	60.3 ± 4.1	59.1 ± 3.9	60.7 ± 4.0	60 ± 4.2
	MRCS(+50)	Right APB	55.8 ± 2.5	55.5 ± 2.7	53.8 ± 2.8	54.9 ± 2.7
		Left APB	54.6 ± 2.8	56.3 ± 2.9	55.3 ± 2.9	54.7 ± 2.7

Mean ± SEM, *$p < 0.05$.

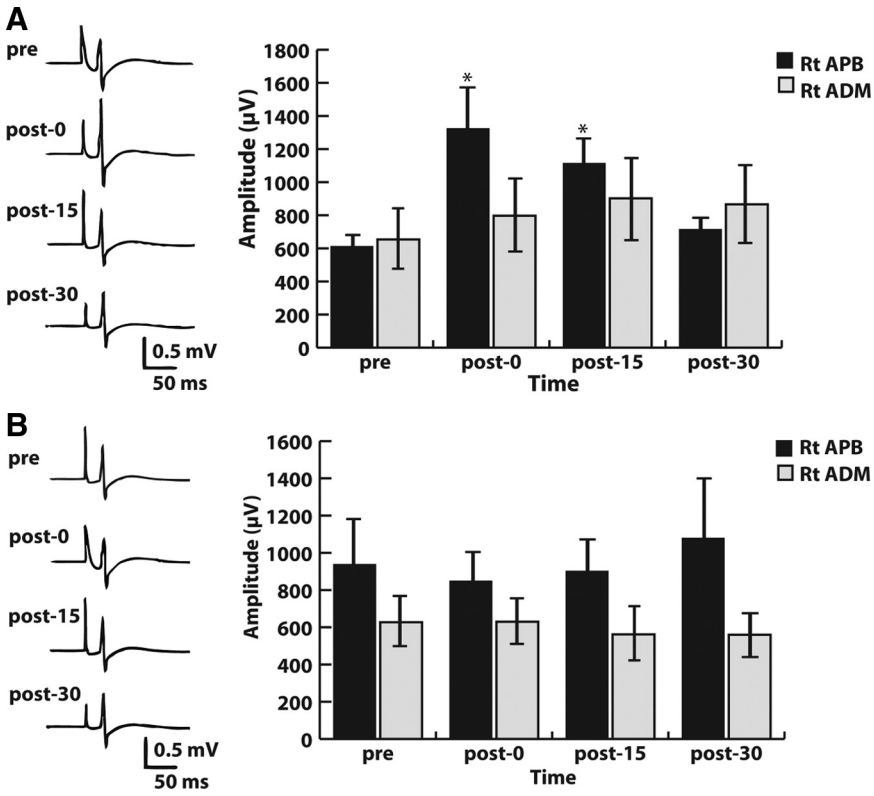

Figure 2. Effect of MRCS(−50) on MEP amplitudes. ***A***, EMG traces in one representative subject (left side) and the MEP amplitudes (mean ± SEM) of the right APB and ADM for pre, post-0, post-15, and post-30 min of the intervention (right side) were shown. Significant increase was observed only for the right APB at post-0 and post-15 conditions. In this and other figures, asterisks represent the significant change (*t* test, *$p < 0.05$, **$p < 0.01$). Effect of MRCS(+50) on MEP amplitudes. ***B***, The mean EMG traces in one representative subject (left side) and the MEP amplitudes (mean ± SEM) of the right APB and ADM for pre, post-0, post-15, and post-30 min of the intervention (right side) were shown. There was no significant change for this intervention.

periment (experiment 1), TMS was given 50 ms before and after the RT_{emg} [MRCS(−50), and MRCS(+50), respectively]. In preliminary experiments, the SDs of RT_{emg} were 20–30 ms. We used −50/+50 ms of time delay to ensure that TMS would be delivered just before/after the estimated onset of APB contraction. MRCS with other timings (−100, +100, +150 ms) were also tested (experiment 3). Visual monitoring of the voluntary EMG activity was continued to monitor the EMG silence during the experiment, except for the thumb abduction task triggered by the cue.

Experiment 1: MRCS(−50) versus MRCS(+50) and somatotopy. Thirteen subjects participated in MRCS(−50), and 12 participated in MRCS(+50). Ten subjects participated in both experiments on different days separated by at least 1 week to counteract any crossover effect. We measured the MEP amplitudes of the right and left APB and ADM, rMT for the right and left APB, and pinch powers for both hands, before (pre), immediately (post-0), 15 min (post-15), and 30 min (post-30) after the end of MRCS.

Experiment 2: alterations of detailed motor parameters after MRCS(−50). Eight subjects participated in this experiment. In addition to the MEP amplitude, we evaluated the F-wave amplitude, SICI and SP duration of the right APB muscle. The mean RT of the right and left thumb were measured before and after MRCS(−50) (pre, post-0, post-15, post-30).

Experiment 3: effects of ISI on MRCS-induced changes. In six subjects, we studied the effect of different ISI (−100, −50, +50, +100, and +150 ms) on the MRCS-induced changes of MEP amplitudes. We measured the MEP amplitudes of the right APB and ADM before and after MRCS (pre, post-0, post-15, and post-30). The order of experiments for different ISI was randomized across subjects. At least a period of 1 week has elapsed between experiments in each subject to counteract any crossover effect.

Data analysis. For statistical analysis, repeated-measures ANOVA was used. The factors tested in each experiment are given in more details in the results. The Greenhouse-Geisser method was used for adjustment of sphericity if needed. Two-tailed paired *t* test with Bonferroni correction was used for *post hoc* analysis. Effects were considered significant if $p < 0.05$. Data are presented as mean ± SEM.

Results

The mean RT_{emg} ± SD was 162.9 ± 16.4 ms ($n = 17$). The mean ± SD of rMT before MRCS was 54.2 ± 10.9 and 58.5 ± 11.6% for the right and left APB, respectively ($n = 17$ and 13). The mean ± SD of SI_{1mV} was 68.3 ± 14.9 and 71.5 ± 13.6% for the right and left APB, respectively ($n = 17$ and 13).

Experiment 1

The means ± SEM of this experiment's data are presented in Table 1, which showed the significant increase of MEP amplitudes only after MRCS(−50). For both MRCS(−50) and MRCS(+50), repeated-measures ANOVA for pinch force with time (pre, post-0, post-15, post-30) and side (right, left) showed no significant effects of time or time × side. For MRCS(−50),

Table 2. Behavioral and electrophysiological changes of the right APB after MRCS(−50) in experiment 2

	Time			
Measures	Pre	Post-0	Post-15	Post-30
RT (ms)	245 ± 10	218 ± 10*	220 ± 9*	234 ± 11
Motor evoked potential amplitude (μV)	551 ± 69	944 ± 152*	944 ± 159	614 ± 103
Short-interval intracortical inhibition (ratio)	0.35 ± 0.04	0.45 ± 0.04	0.48 ± 0.07	0.4 ± 0.04
Silent period duration (ms)	180 ± 9	206 ± 7*	201 ± 8*	193 ± 10
F-wave amplitude (μV)	347 ± 62	343 ± 58	348 ± 32	396 ± 58

Mean ± SEM, *$p < 0.05$.

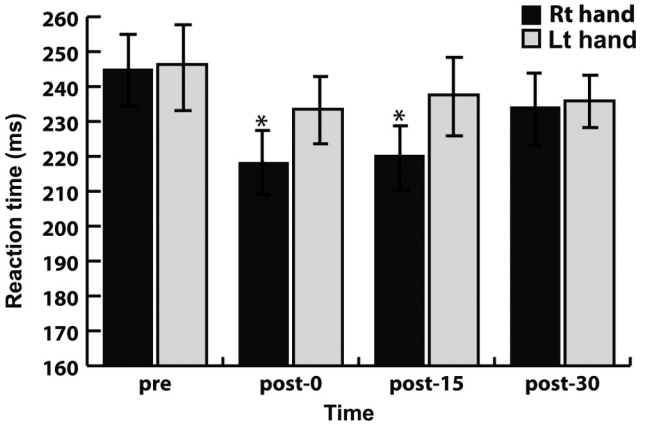

Figure 3. Effect of MRCS(−50) on simple reaction time. The mean reaction times in both right and left hands for pre, post-0, post-15, and post-30 conditions were shown. The significant shortening of the reaction time was observed for post-0 and post-15 conditions.

two-way repeated-measures ANOVA for MEP amplitude of the right APB and ADM with time and muscle as the within-subject variables revealed significant main effect of time ($F = 7.602$, $p = 0.005$), and time × muscle interaction ($F = 6.438$, $p = 0.009$). *Post hoc* analysis for each time point revealed significant effects for pre versus post-0 ($p = 0.048$), and pre versus post-15 ($p = 0.012$) in the right APB (Fig. 2*A*). For the right ADM, there was no significant effect. For the left hand, two-way repeated-measures ANOVA showed no significant effect for time and time × muscle. However, for MRCS(+50), two-way repeated-measures ANOVA with time and muscle as the within-subject variables revealed no significant main effect of time and time × muscle interaction for both right and left hands. (Fig. 2*B*). For rMT, repeated-measures ANOVA showed insignificant effect of time for the right and left APB muscles for MRCS(−50) and MRCS(+50).

Experiment 2

Since the pinch force measurement in experiment 1 showed insignificant change, we measured the RT during SRT in addition to detailed electrophysiological measures and found the increase of MEP amplitude and SP duration as well as the shortening of RT. The means ± SEM of the data of this experiment are presented in Table 2.

The mean ± SD of TMS intensity used for recording the SP was 73.5 ± 16.5% ($n = 8$). The mean ± SD of aMT was 42.8 ± 9.7% and for SI_{1mV} was 68.8 ± 15.7% of maximum stimulator output.

Repeated-measures ANOVA with time as the within-subject variable for RT of the right hand was significant ($F = 8.816$, $p = 0.001$). *Post hoc t* test revealed significant decrease of RT during post-0 and post-15 conditions ($p = 0.033$ and 0.03, respectively, Fig. 3). However, for the left hand, it was insignificant (the mean RT was 246 ± 13, 234 ± 11, 238 ± 12, and 236 ± 9 ms for pre, post-0, post-15 and post-30 conditions).

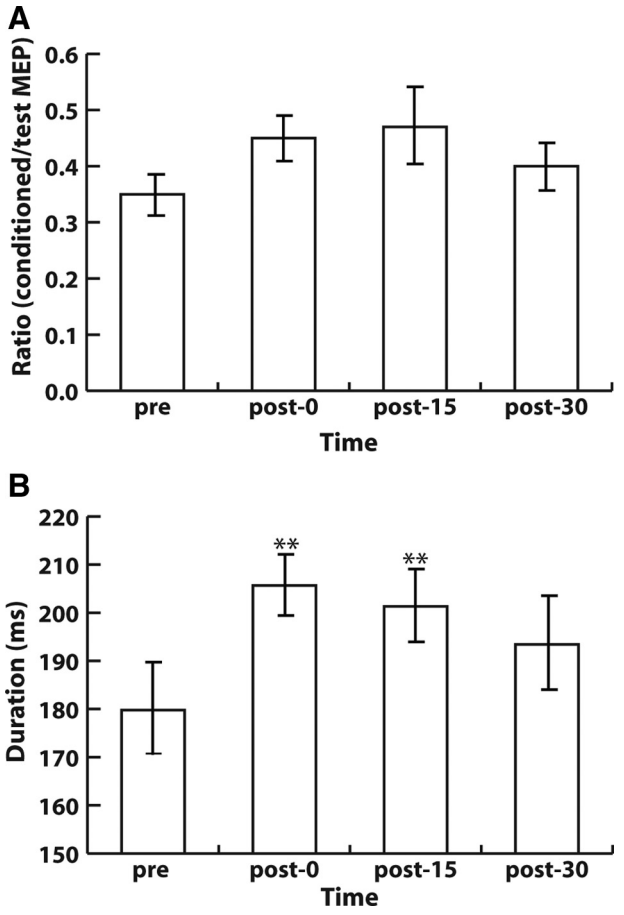

Figure 4. ***A***, ***B***, Effect of MRCS(−50) on the cortical inhibitory system measured by short interval intracortical inhibition (***A***) and silent period (***B***). The mean intracortical inhibition (conditioned/test MEP ratio) in the right APB measured for pre, post-0, post-15, and post-30 conditions showed no significant difference. The mean duration of SP in the right APB for post-0, and post-15 conditions showed the significant increase after intervention.

Regarding the mean MEP amplitude for the right APB, repeated-measures ANOVA showed the significant effect of time ($F = 6.087$; $p = 0.004$). *Post hoc t* test showed the increase of MEP amplitudes for post-0 compared with pre condition ($p = 0.027$).

The change in SICI was insignificant after intervention (Fig. 4*A*). However repeated-measures ANOVA for the durations of SP of the right APB showed significant effect of time ($F = 15.968$, $p < 0.001$, Fig. 4*B*). *Post hoc t* test revealed significantly longer SP duration for post-0 and post-15 compared with pre condition ($p = 0.003$ and 0.003, respectively). The mean F-wave amplitudes recorded from the right APB were insignificant (Fig. 5).

Experiment 3

By changing the interval between the movement onset and TMS, we found the timing-dependent biphasic pattern of changes in-

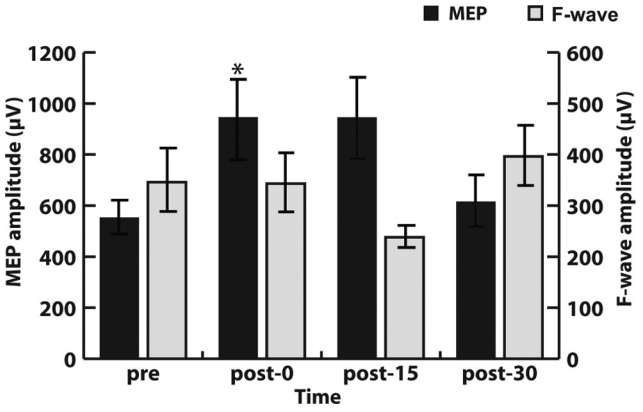

Figure 5. Effect of MRCS(−50) on the corticospinal and spinal excitability. The mean MEP and F-wave amplitudes measured from the right APB for pre, post-0, post-15, and post-30 conditions are shown. Only the MEP for post-0 condition, but not F-wave, showed significant increase.

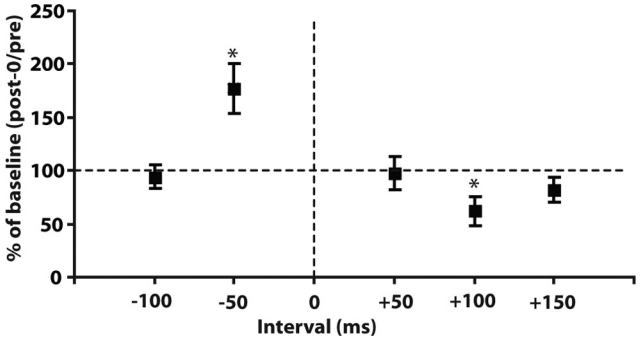

Figure 6. The mean MEP amplitude ratios (post-0/pre) recorded from the right APB as a function of intervals between TMS and the mean reaction time. Significant increase of MEP for post-0 compared with pre was observed for MRCS(−50), while significant decrease was demonstrated for MRCS(+100).

duced by MRCS. Since the largest change of MEP amplitudes was observed at the post-0 condition, we performed two-way repeated-measures ANOVA for interaction between time (pre, post0), and ISI (−100, −50, +50, +100, +150) as the within-subjects factors, which was found to be significant ($F = 6.722, p = 0.028$, Fig. 6). *Post hoc t* test (pre vs post-0) revealed the significant increase for MRCS(−50) and decrease for MRCS(+100) ($p = 0.023$ and 0.036, respectively, Fig. 6) but not for other ISIs.

Discussion

We found that rTMS of M1 paired with voluntary movement (MRCS) can induce change in the corticospinal excitability and motor behavior that outlasted the stimulation period. The characteristics of this change are similar to associative LTP/LTD in animal models; as it rapidly developed (within 20 min), sustained after intervention, showed associativity (TMS associated with voluntary movement), and was input-specific (as M1 excitability changed only in right APB "the moving" rather than right ADM "the resting" muscles). Additionally, this form of induced plasticity was timing-dependent, as its direction was governed by the order of TMS and the onset of voluntary movement. Absence of F-wave change following MRCS suggests cortical origin of induced plasticity.

Many earlier studies induced human associative plasticity in the motor (Stefan et al., 2000, 2002, 2004; Ridding and Taylor, 2001; Wolters et al., 2003; Müller-Dahlhaus et al., 2008; Di Lazzaro et al., 2009) and somatosensory (Wolters et al., 2005) cortices by pairing peripheral electrical nerve stimulation with TMS. Also, it was found that associative stimulation of motor cortices bilaterally can induce lasting excitability changes in the target M1 (Koganemaru et al., 2009; Rizzo et al., 2009).

Recent animal studies have stressed the importance of the temporal order of presynaptic and postsynaptic spiking of associative stimulation, and have defined temporal windows of tens of milliseconds for the direction of induced plasticity (Markram et al., 1997; Debanne et al., 1998; Zhang et al., 1998; Egger et al., 1999; Feldman, 2000; Sjöström et al., 2001; Froemke and Dan, 2002; Tzounopoulos et al., 2004). This form of synaptic plasticity is known as spike timing-dependent plasticity (STDP). If postsynaptic spiking occurs within a specific time window after synaptic activation, LTP is induced, whereas spiking before synaptic activation leads to LTD (Bi and Poo, 1998). This time window was found to be variable from one site to another within the brain and can be 50 ms or more (Bell et al., 1997; Markram et al., 1997; Debanne et al., 1998; Zhang et al., 1998; Egger et al., 1999; Feldman, 2000; Froemke and Dan, 2002; Tzounopoulos et al., 2004).

There are two forms of synaptic plasticity, which are homo- and heterosynaptic plasticity. The homosynaptic plasticity refers to changes in the strength of a synapse due to its own activity, however the heterosynaptic plasticity, is a change in the strength of a synapse due to activity in another pathway (Kelso and Brown, 1986; Bradler and Barrioneuvo, 1989). In previous PAS studies (Stefan et al., 2000; Wolters et al., 2003, 2005) and in our MRCS study, the induced M1 plasticity may be related to homosynaptic form of LTP/LTD as the change in MEP amplitude occurred in muscles innervated by the stimulated peripheral nerve in PAS, and in the moving rather than resting muscle in MRCS. The direction of change in the M1 excitability induced by PAS and MRCS is determined by the timing rule similar to STDP (Wolters et al., 2003).

The increase of MEP amplitude in the present study was not associated with change in F-wave amplitude recorded in the same muscle, suggesting that M1 excitability change induced by MRCS occurred at the cortical rather than spinal level (Mercuri et al., 1996; Stefan et al., 2000).

Since the change of MEP was not associated with any change of rMT, the effect of MRCS may not be related to the resting membrane potential properties of the corticospinal neurons (Ziemann et al., 1996a). This finding is in agreement with other PAS and bihemispheric stimulation protocols (Stefan et al., 2000; Wolters et al., 2003; Fratello et al., 2006; Koganemaru et al., 2009; Rizzo et al., 2009).

Intracortical inhibitory system plays an important role in changing M1 excitability (Peinemann et al., 2000; Sanes and Donoghue, 2000; Di Lazzaro et al., 2002; Plewnia et al., 2003) and motor performance (Ridding et al., 1995; Garry et al., 2004; Sale and Semmler, 2005; Rosenkranz et al., 2007). Thus, we studied SICI which partly reflects GABA-A, and SP which is related to GABA-B inhibitory cortical neurons activities (Chen, 2004; Ziemann, 2004). The SICI was not changed by MRCS, suggesting that the-induced M1 plasticity is not directly related to GABA-A neuronal activity. Other PAS protocols showed similar results (Ridding and Taylor, 2001; Stefan et al., 2002; Sale et al., 2007). However, MRCS(−50) significantly increased SP duration similar to other PAS protocols (Stefan et al., 2000; Sale et al., 2007; Cirillo et al., 2009).

Long-term potentiation of inhibitory synapses was observed in many brain areas (Morishita and Sastry, 1991; Kano et al., 1992;

Komatsu and Iwakiri, 1993; Aizenman et al., 1998). The change in SP duration might reflect the MRCS-induced LTP-like effect on inhibitory synapses within M1 (Nicoll et al., 1996; Buonomano and Merzenich, 1998). Since SP duration is thought to reflect the movement-related activity of GABA-B inhibitory circuits during voluntary contraction (Ziemann, 2004), MRCS(−50) might induce increase in synaptic strength within these circuits similar to the excitatory circuits responsible for MEP, leading to simultaneous increase in the MEP amplitude and SP duration.

MRCS(−50) significantly shortened RT in addition to MEP amplitude increase of the right APB. Previous studies showed improvement in motor behavior due to LTP-like effects in M1 induced by high-frequency rTMS (Khedr et al., 2003, 2005; Bütefisch, 2004), associative stimulations (Koganemaru et al., 2009; Rizzo et al., 2009), and anodal transcranial direct current stimulation (DCS) (Hummel and Cohen, 2005, 2006). Therefore, it is likely that shortened RT might reflect increased M1 excitability.

We found significant increase and decrease in MEP amplitude of the right APB muscle for MRCS(−50) and MRCS(+100). However this bidirectional change in excitability was not generalized to the right ADM muscle, which was not involved in the voluntary movement task. This finding suggests that the M1 plasticity in MRCS is governed by strict somatotopy based on muscle activity in movement task. This somatotopy is consistent with other associative plasticity protocols (Stefan et al., 2000; Wolters et al., 2003, 2005).

In human studies, the motor potential (MP) component of movement related cortical potential (MRCP), synonymously called initial slope motor potential (isMP) (Tarkka and Hallett, 1991) and N-10 potential (Shibasaki et al., 1980), occurs partly before and after the EMG onset. This potential probably represents activation of pyramidal tract neurons in M1, and persists for 30–50 ms after the onset of EMG activity (Hallett, 1994; Shibasaki and Hallett, 2006). If we applied the STDP rule induced in animals to our protocol, we may find an explanation to our results as; TMS pulse will precede/follow the activation of M1 neurons which are somatotopically related (as discussed before) to movement execution in MRCS(−50) and MRCS(+100) leading to increase/decrease of synaptic strength within those neuronal circuits.

Animal studies have shown that M1 directly and indirectly receives multiple inputs from other cortical and subcortical regions that may play important roles in motor processing, including premotor, supplementary motor, cingulated motor, somatosensory and prefrontal cortex, and anterior thalamic nuclei which indirectly connect cerebellum and basal ganglia outputs to M1(Strick, 1985; Orioli and Strick, 1989; Hoover and Strick, 1993; Stepniewska et al., 1993; Kaneko et al., 1994; Lu et al., 1994; Farkas et al., 1999). During movement execution, top-down synchronous firing of pyramidal neurons (Weiler et al., 2008) occurs in all layers (including layers 2/3 and 5) of M1 (Isomura et al., 2009). The activity of those neurons stops at the end of movement execution and replaced by activation of other neuronal subsets in layers 4 and 6 during movement-off and post-movement phases of motor action (Maiorov, 2003; Isomura et al., 2009).

In conclusion, this study provides a new associative stimulation TMS protocol that can be used for induction of M1 plasticity. In previous protocols, peripheral electrical stimulation and contralateral M1 conditioning TMS were used (Stefan et al., 2000, 2002; Wolters et al., 2003; Koganemaru et al., 2009; Rizzo et al., 2009) to induce M1 plasticity. However in our MRCS, intrinsic M1 activation was used. Our study may provide the evidence that associative stimulation-induced plasticity is a rather general principle independent of the nature of used stimuli. The finding that MRCS may induce M1 plasticity supports the possibility of its use for rehabilitation of neurological disability after vascular (Hummel and Cohen, 2005; Khedr et al., 2005; Fregni et al., 2006; Kim et al., 2006), inflammatory (Centonze et al., 2007a,b; Koch et al., 2008) or degenerative brain diseases (Khedr et al., 2003, 2006; Lomarev et al., 2006). Since topographical specificity is an important characteristic of MRCS-induced plasticity, it can be used to induce movement-specific M1 plasticity, rather than generalized increase/decrease of M1 plasticity induced by rTMS (Chen et al., 1997; Wu et al., 2000; Gilio et al., 2003; Quartarone et al., 2005) or DCS (Nitsche and Paulus, 2000, 2001; Nitsche et al., 2003), which can be tailored to match different rehabilitation situations.

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