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Effects of the motor cortical quadripulse transcranial magnetic stimulation (QPS) on the contralateral motor cortex and interhemispheric interactions

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Tsutsumi R, Hanajima R, Terao Y, Shirota Y, Ohminami S, Shimizu T, Tanaka N, Ugawa Y. Effects of the motor cortical quadripulse transcranial magnetic stimulation (QPS) on the contralateral motor cortex and interhemispheric interactions. *J Neurophysiol* 111: 26–35, 2014. First published October 9, 2013; doi:10.1152/jn.00515.2013.—Corpus callosum connects the bilateral primary motor cortices (M1s) and plays an important role in motor control. Using the paired-pulse transcranial magnetic stimulation (TMS) paradigm, we can measure interhemispheric inhibition (IHI) and interhemispheric facilitation (IHF) as indexes of the interhemispheric interactions in humans. We investigated how quadripulse transcranial magnetic stimulation (QPS), one form of repetitive TMS (rTMS), on M1 affects the contralateral M1 and the interhemispheric interactions. QPS is able to induce bidirectional plastic changes in M1 depending on the interstimulus intervals (ISIs) of TMS pulses: long-term potentiation (LTP)-like effect by QPS-5 protocol, and long-term depression-like effect by QPS-50, whose numbers indicate the ISI (ms). Twelve healthy subjects were enrolled. We applied QPS over the left M1 and recorded several parameters before and 30 min after QPS. QPS-5, which increased motor-evoked potentials (MEPs) induced by left M1 activation, also increased MEPs induced by right M1 activation. Meanwhile, QPS-50, which decreased MEPs elicited by left M1 activation, did not induce any significant changes in MEPs elicited by right M1 activation. None of the resting motor threshold, active motor threshold, short-interval intracortical inhibition, long-interval intracortical inhibition, intracortical facilitation, and short-interval intracortical inhibition in right M1 were affected by QPS. IHI and IHF from left to right M1 significantly increased after left M1 QPS-5. The degree of left first dorsal interosseous MEP amplitude change by QPS-5 significantly correlated with the degree of IHF change. We suppose that the LTP-like effect on the contralateral M1 may be produced by some interhemispheric interactions through the corpus callosum.

corpus callosum; interhemispheric inhibition; interhemispheric facilitation; plasticity; repetitive transcranial magnetic stimulation

CORPUS CALLOSUM connects the bilateral primary motor cortices (M1s) and plays an important role in bimanual motor control not only in primates (Donchin et al. 1998; Gribova et al. 2002) but also in humans (Liuzzi et al. 2011; Maki et al. 2008). With the use of transcranial magnetic simulation (TMS), transcallosal connection has been studied noninvasively in humans. Both the interhemispheric inhibition (IHI) and the interhemispheric facilitation (IHF) between the bilateral M1s have been detected by paired-pulse TMS protocols (Bäumer et al. 2006; Boroojerdi et al. 1996; Di Lazzaro et al. 1999; Ferbert et al. 1992; Hanajima et al. 2001; Salerno and Georgesco 1996; Ugawa et al. 1993). It has been reported that IHI dynamically changed during intermanual transfer tasks (Perez et al. 2007) and bimanual tasks (Bologna et al. 2012). These reports suggest that interhemispheric interactions could change, relating to plastic changes of the brain.

To study plasticity of the human motor cortex, various types of repetitive TMS (rTMS) have been used (Di Lazzaro et al. 2011; Ziemann et al. 2008). Usually, rTMS is applied over the M1 and used to estimate long-lasting changes of motor-evoked potentials (MEPs) to single-pulse TMS over stimulated M1 after rTMS. rTMS can be also used to evaluate plastic changes in nonstimulated sites through the corticocortical connection. The plastic change of the M1 contralateral to the stimulated M1 has been studied, but the results were variable. After 1-Hz M1 rTMS, MEP amplitude from the contralateral M1 varied from increase to decrease, which may be explained by the difference in the stimulation protocols (Gilio et al. 2003; Heide et al. 2006; Pal et al. 2005; Plewnia et al. 2003; Schambra et al. 2003; Wassermann et al. 1998). Another study showed the increase of contralateral MEP amplitude after 5-Hz M1 rTMS but no significant change after 0.5-Hz M1 rTMS (Gorsler et al. 2003). Studies using theta burst stimulation (TBS) are also variable in that continuous TBS increased (Stefan et al. 2008; Suppa et al. 2008) or decreased (Ishikawa et al. 2007) MEP amplitude from the nonstimulated M1, whereas intermittent TBS reduced MEP (Di Lazzaro et al. 2008; Suppa et al. 2008). A recent study using paired associative stimulation (PAS) showed increased MEP amplitude from the nonstimulated M1 (Shin and Sohn 2011). Moreover, the relationship between the MEP changes and IHI changes is also inconsistent. IHI is reported to be reduced by 1-Hz rTMS (Gilio et al. 2003; Pal et al. 2005), although the degrees of MEP change differ between the two reports. The IHI was not affected by TBS in one report (Suppa et al. 2008), whereas another study reported IHI reduction after PAS (Shin and Sohn 2012).

We recently reported one form of the patterned rTMS, quadripulse transcranial magnetic stimulation (QPS) (Hamada et al. 2007, 2008). QPS was able to induce bidirectional plasticity in the stimulated M1. We used the QPS for studying long-term effects on MEPs elicited by nonstimulated M1 in the present study for the following reasons. The intraindividual variability of the long-term effects may be due to associated effects on several intracortical circuits of M1 (Hamada et al. 2013). rTMS methods used in the previous reports, such as conventional rTMS, PAS, or TBS, induced changes in both

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intracortical inhibition and facilitation in the stimulated M1 in addition to the main effects on MEP. The balance between inhibition and facilitation in the stimulated M1 could produce complicating effects over the contralateral nonstimulated M1, because the associated induction of short-interval intracortical inhibition (SICI) or long-interval intracortical inhibition (LICI) would reduce IHI (Lee et al. 2007). This must make some intraindividual variability. In contrast, QPS induces no changes in SICI or LICI, although it induces some changes in short-interval intracortical facilitation (SICF) and MEP. This suggests that the effect induced by QPS on the contralateral M1 is much simpler than the other methods because of fewer associated effects. Moreover, the other stimulation methods, especially TBS, have much interindividual variability in the main effects (Hamada et al. 2013). In addition, according to the interindividual variability, effects of PAS or TBS are reported to deeply depend on the polymorphism of brain-derived neurotrophic factor (BDNF) gene (Cheeran et al. 2008). QPS, however, has no such interindividual variability (Nakamura et al. 2011). Therefore, the lower variability of QPS led us to study effects of QPS on the contralateral M1. This is the rationale for using QPS in the present work.

We hypothesize that the efficacy changes in the corpus callosum connection may induce some contralateral M1 excitability changes, even though the M1 plastic changes alone, without any changes in the corpus callosum function, may induce no effects on the contralateral M1. We studied whether QPS over M1 would induce plasticity on the contralateral M1 and also compared several parameters of the intracortical circuits and the interhemispheric interactions between before and after QPS.

METHODS

Subjects

We studied 12 healthy volunteers [2 women and 10 men; age 38.4 ± 7.1 yr (mean ± SD), range: 30–49 yr], who gave their written informed consent to participate in the experiments. No subjects had neurological, psychiatric, or other medical problems or had any contraindication to TMS (Rossi et al. 2009). All subjects were right-handed according to the Edinburgh inventory (Oldfield 1971). The protocol was approved by the Ethics Committee of the University of Tokyo in accordance with the ethical standards of the Declaration of Helsinki on the use of human subjects in experiments.

Recordings

MEPs were recorded from the bilateral first dorsal interosseous (FDI) muscle with 9-mm-diameter Ag-AgCl surface electrodes placed with a belly-tendon montage. Responses were input to an amplifier (Biotop; GE Marquette Medical Systems Japan, Tokyo, Japan) through filters set at 100 Hz and 3 kHz. They were then digitized with a sampling rate of 10 kHz and stored in a computer for later off-line analyses (TMS Bistim Tester; Medical Try System, Tokyo, Japan).

Motor cortical excitability was assessed by measuring the peak-to-peak amplitude of MEPs from both FDI muscles. During the experiments, subjects were seated on a comfortable chair and both FDI muscles were relaxed as confirmed by an oscilloscope monitor (except *experiment 5*, described below). Trials contaminated with unexpected voluntary electromyogram (EMG) activities were discarded from analyses.

Transcranial Magnetic Stimulation

Focal TMS was given using a hand-held figure-of-eight coil (9-cm external diameter at each wing; Magstim, Whitland, UK). Single monophasic TMS pulses were delivered by a magnetic stimulator (Magstim 200; Magstim). The optimal site for eliciting MEPs from both FDI muscles (i.e., hot spot for FDI) was determined before each experiment. A figure-of-eight coil was placed tangentially over the scalp with the handle pointing backwards at about 45° laterally. We stimulated several positions in 1-cm increments in the anteroposterior and mediolateral direction from each other using the same intensity and determined the optimal site at which the largest responses were elicited. This position was marked with a pen on the scalp for repositioning the coil.

Before each experiment, resting (RMT) and active motor thresholds (AMT) for both FDIs were measured. RMT was defined as the lowest stimulator output intensity capable of eliciting MEPs of 50-μV peak-to-peak amplitude in the relaxed FDI muscle in more than 5 of 10 consecutive trials (Rossini et al. 1994). AMT was determined as the lowest stimulator output intensity to evoke MEPs of 100-μV peak-to-peak amplitude when the participant maintained a slight contraction of FDI muscle (5–10% of the maximum voluntary contraction) in more than 5 of 10 consecutive trials. The stimulus intensity was changed in steps of 1% of the maximum stimulator output (MSO).

Quadripulse Transcranial Magnetic Stimulation

For QPS, monophasic TMS pulses were delivered by four magnetic stimulators (Magstim 200^2; Magstim) connected with a specially designed combining module (Magstim). The device combines the outputs from four stimulators to allow a train of four monophasic magnetic pulses to be delivered through a single coil. The QPS protocol consists of trains of four monophasic TMS pulses separated by an interstimulus interval (ISI) of 5 ms (QPS-5) or 50 ms (QPS-50) with an interburst interval of 5 s (i.e., 0.2 Hz) for 30 min (Fig. 1*A*). This delivers a total of 360 trains, or 1,440 pulses. We chose these two parameters because QPS-5 is the most effective parameter for a long-term potentiation (LTP)-like effect over the stimulated M1, and QPS-50 is most effective for a long-term depression (LTD)-like effect over the stimulated M1 (Hamada et al. 2008).

We applied QPS over the left M1. A figure-of-eight coil was positioned over the optimum point for FDI, tangentially over the scalp and angled 45° to the parasagittal plane so that currents flowed in an anteromedial-to-posterolateral direction at the center of the coil. The stimulus intensity for QPS was fixed at 90% of the AMT of the right FDI. We used this stimulus intensity because it is sufficient to induce plastic changes (Hamada et al. 2007). Both FDI muscles were kept relaxed during QPS.

Study Design

To study the effects of QPS on the contralateral M1, we recorded various parameters before (pre) and 30 min after (post) the end of the 30-min QPS protocol over the left M1 (Fig. 1*B*). Measurement of each parameter takes about 10–15 min. Therefore, we separated measurements of parameters into five experiments. The experimental sessions were separated by 1 wk or longer in the same subject. The order of the experiments and the parameters were randomized and balanced among subjects.

Experiment 1: effects of QPS on MEPs to activation of bilateral M1s, and AMT and RMT for both FDI. In *experiment 1*, we recorded MEPs from both FDIs as the main experiment of this study. All 12 subjects were enrolled. We recorded MEPs to single-pulse TMS over the right or left M1, where the hot spot for left and right FDI was determined, respectively. During the pre-QPS evaluation period, we fixed the stimulus intensity to elicit ~0.5-mV MEPs, and we used the

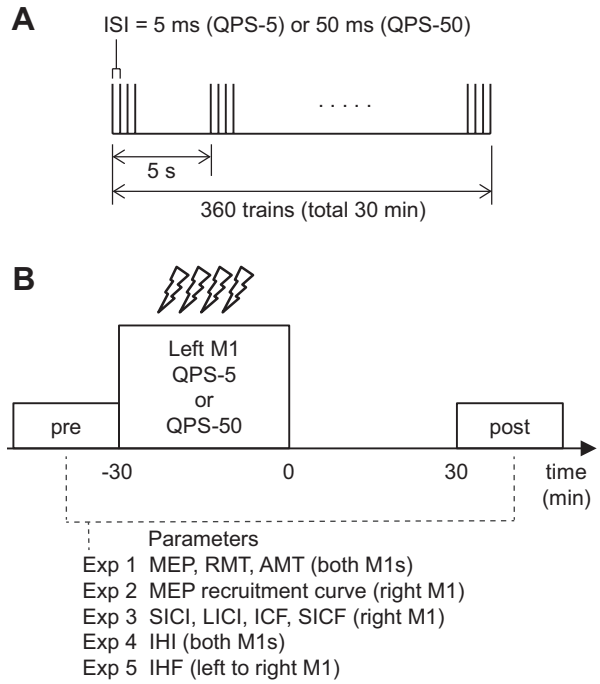

Fig. 1. Stimulus protocol and experimental design. *A*: stimulus protocol of the quadripulse transcranial magnetic stimulation (QPS). A train of 4 monophasic transcranial magnetic stimulation (TMS) pulses is delivered with an interburst interval of 5 s for 30 min. Interstimulus interval (ISI) in each train was 5 (QPS-5) or 50 ms (QPS-50). *B*: QPS-5 or QPS-50 was delivered over the left primary motor cortex (M1) for 30 min. There were evaluation periods before (pre) and 30 min after the end of QPS (post). We evaluated motor-evoked potential (MEP), resting motor threshold (RMT), active motor threshold (AMT), MEP recruitment curve, short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI), intracortical facilitation (ICF), short-interval intracortical facilitation (SICF), interhemispheric inhibition (IHI), and interhemispheric facilitation (IHF) in 5 separate experiments (Exp).

same stimulus intensity in post-QPS evaluation periods. We performed 20 single-pulse TMS for each hemisphere with the intertrial intervals at 6 ± 0.5 s. RMT and AMT for both FDIs were also measured in each evaluation period.

Experiment 2: effects of QPS on MEP recruitment curves of the nonstimulated motor cortex. Eight of the 12 subjects were enrolled in the MEP recruitment curve experiment for left FDI. We applied single-pulse TMS over the hot spot for the left FDI. Ten stimuli for each stimulus intensities at 100, 110, 120, 130, 140, 150, and 160% of the RMT were applied every 6 ± 0.5 s.

Experiment 3: effects of QPS on SICI, LICI, ICF, and SICF of the nonstimulated motor cortex. The excitatory and inhibitory circuits of the contralateral M1 were studied using a paired-pulse TMS paradigm. We studied SICI and intracortical facilitation (ICF) (Kujirai et al. 1993), LICI (Valls-Solé et al. 1992; Wassermann et al. 1996), and SICF (Tokimura et al. 1996; Ziemann et al. 1998) in 9 of the 12 subjects. In this experiment, TMS was applied to the right M1, the contralateral hemisphere of the QPS, and MEPs were recorded from the left M1.

SICI was examined at an ISI of 3 ms using a conditioning stimulus (CS) intensity of 70, 80, or 90% AMT, and ICF was examined at an ISI of 10 and 15 ms with a CS intensity of 90% AMT. LICI was examined at an ISI of 100 ms with a CS intensity of 110% RMT. The intensity of the test stimulus (TS) was adjusted to elicit MEPs of ~0.5 mV in relaxed left FDI.

SICF was examined at an ISI of 1.3, 1.5, or 1.7 ms (Hanajima et al. 2002; Tokimura et al. 1996; Ziemann et al. 1998). The intensity of the first stimulus (TS) was adjusted to elicit MEPs of ~0.5 mV in the relaxed left FDI, and the second stimulus (CS) intensity was set at 90% AMT.

Since AMT did not change significantly after QPS, the CS intensity was kept constant over the whole experiment. The TS intensity after QPS, however, was adjusted to elicit MEPs as large as ~0.5 mV.

Experiment 4: effects of QPS on IHI. All 12 subjects were enrolled. The experiment was conducted using a paired-pulse paradigm as reported previously (Ferbert et al. 1992; Gerloff et al. 1998). TS was given over the left or right M1, and CS over the opposite M1, preceding TS by 10 or 40 ms. We used two figure-of-eight coils (outer diameter of each wing was 9 cm) positioned at the hot spot for FDI. Both coils were placed tangentially over the scalp and angled 45° to the parasagittal plane so that currents flowed in an anteromedial-to-posterolateral direction at the center of the coil. The intensity of TS and CS was adjusted to elicit MEPs of ~0.5 mV at pre- and post-QPS.

Experiment 5: effects of QPS on IHF. Eight of the 12 subjects were enrolled. Hanajima et al. (2001) reported that there is an early interhemispheric facilitation between the human hand motor areas that is mediated through the corpus callosum. This early facilitation was only observed in responses to I3 waves, so we performed this experiment under slight contraction of the target muscle to see changes in I3 waves purely. TS was given over the right M1, and CS over the left M1, preceding TS by 4 ms. We used two figure-of-eight coils (outer diameter of each wing was 9 cm) positioned at the hot spots for bilateral FDIs. The TS coil for the right M1 was placed tangentially over the scalp and angled backwards so that currents flowed in a posterior-to-anterior direction at the center of the coil. The CS coil for the left M1 was set toward the sagittal plane so that currents flowed in a medial-to-lateral direction at the center of the coil. The intensity of CS was set at 120% AMT for the right FDI. The intensity of TS was adjusted to elicit MEPs of ~0.3 mV under slight contraction of the left FDI (~10% of the maximum voluntary contraction). The amount of contraction was confirmed by an oscilloscope monitor during the experiment.

Data Analysis

Experiment 1. We analyzed the absolute MEP amplitudes by two-way repeated-measures analysis of variance (ANOVA) using QPS (QPS-5 and QPS-50) and time (pre and post) as within-subject factors in each muscle. For motor thresholds, we analyzed the %MSO by three-way repeated-measures ANOVA using QPS (QPS-5 and QPS-50), contraction (RMT and AMT), and time (pre and post) as within-subject factors in each muscle.

Experiment 2. We performed three-way repeated-measures ANOVA using QPS (QPS-5 and QPS-50), time (pre and post), and intensity (7 conditions; 100, 110, 120, 130, 140, 150, and 160% RMT) as within-subject factors.

Experiments 3–5. Ten trials for each condition were randomly intermixed with 10 unconditioned trials in which TS was delivered alone. The ratio of the mean peak-to-peak amplitude of conditioned MEPs to that of unconditioned MEPs (MEP size ratio) was calculated for each condition in each subject.

We applied three-way repeated-measures ANOVA as follows: for SICI, using QPS (QPS-5 and QPS-50), intensity (70, 80, and 90% AMT), and time (pre and post) as within-subject factors in each muscle; for ICF, QPS (QPS-5 and QPS-50), ISI (10 and 15 ms), and time (pre and post); for SICF, QPS (QPS-5 and QPS-50), ISI (1.3, 1.5, and 1.7 ms), and time (pre and post); and for IHI, QPS (QPS-5 and QPS-50), ISI (10 and 40 ms), and time (pre and post). For LICI and IHF, we used two-way repeated-measures ANOVA using QPS (QPS-5 and QPS-50) and time (pre and post) as within-subject factors. To ensure that the experiments were not affected by the test MEP size difference between the conditions, we used paired *t*-tests to compare the test MEP (unconditioned MEP) amplitudes in each experiment between pre-QPS-5 and post-QPS-5, and also between pre-QPS-50 and post-QPS-50.

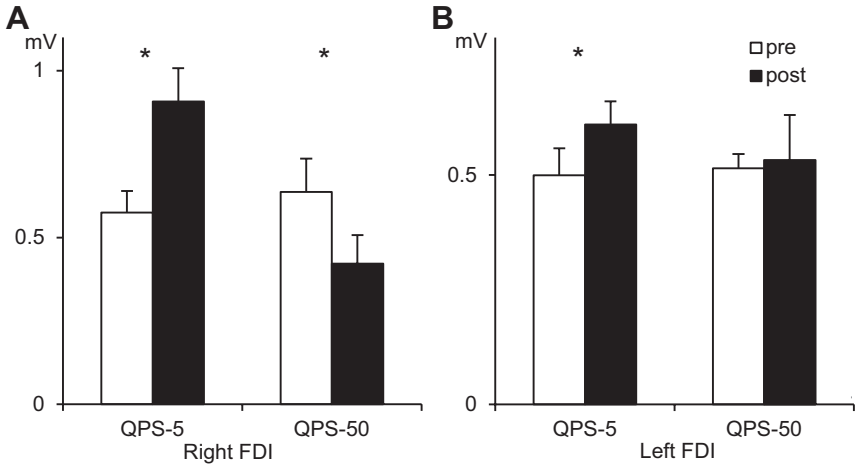

Fig. 2. Changes of MEP amplitude. *A*: MEP amplitude to single-pulse TMS over left M1, recorded from right first dorsal interosseous (FDI), increased after QPS-5 and decreased after QPS-50. *B*: MEP amplitude to single-pulse TMS over right M1, recorded from left FDI, increased after QPS-5. Vertical axis shows the absolute MEP amplitude (mV). Error bars indicate SE. $*P < 0.05$.

Correlation study. We conducted a correlation analysis to evaluate if any correlation exists between the change of MEP amplitude and that of the interhemispheric interactions measured by TMS. We performed the multiple regression analysis using ΔMEP_{LtFDI} (post-MEP amplitude − pre-MEP amplitude of the left FDI) as a dependent variable and ΔMEP_{RtFDI} (post-MEP amplitude − pre-MEP amplitude of the right FDI), ΔIHI (post-MEP ratio − pre-MEP ratio of the left-to-right M1 IHI of 10 ms), and ΔIHF (post-MEP ratio − pre-MEP ratio of the left-to-right M1 IHF) as independent variables at the QPS-5 condition. We did not perform the analysis of QPS-50 condition because the MEP change was not significant.

When necessary, Greenhouse-Geisser correction was used to correct for nonsphericity in ANOVA. Statistical analyses were performed using PASW Statistics 18.0.0 (IBM, New York, NY). *P* values <0.05 were judged to be significant.

RESULTS

Experiment 1: Effects of QPS on MEPs to Activation of Bilateral M1s, and AMT and RMT for Both FDI

After QPS was applied over left M1, MEP amplitude to single-pulse TMS over left M1, recorded from right FDI, increased after QPS-5 and decreased after QPS-50 (Fig. 2*A*). There was a tendency toward a main effect of QPS [$F(1,11) = 4.7$, $P = 0.053$] but no significant main effect of time [$F(1,11) = 0.7$, $P = 0.43$]. There was a significant interaction of QPS × time [$F(1,11) = 21.6$, $P < 0.001$]. Simple main effect tests revealed a significant change of QPS-5 by time [$F(1,11) = 8.4$, $P = 0.015$] and QPS-50 by time [$F(1,11) = 11.7$, $P = 0.006$].

After QPS was applied over left M1, MEP amplitude to single-pulse TMS over right M1, recorded from left FDI, increased after QPS-5 but did not change significantly after QPS-50 (Fig. 2*B*). There was no significant main effect of QPS [$F(1,11) = 0.3$, $P = 0.62$] or time [$F(1,11) = 1.9$, $P = 0.19$] and no interaction of QPS × time [$F(1,11) = 1.0$, $P = 0.34$]. Simple main effect tests revealed a significant change in QPS-5 effect by time [$F(1,11) = 8.9$, $P = 0.012$] but no change in QPS-50 effect by time [$F(1,11) = 0.04$, $P = 0.84$].

Neither RMT nor AMT of either hemisphere changed after QPS-5 or QPS-50 (Table 1). In left M1, recorded from right FDI, there was a significant main effect of contraction [$F(1,11) = 63.6$, $P < 0.001$] but no significant main effect of QPS [$F(1,11) = 0.001$, $P = 0.97$] or time [$F(1,11) = 0.001$, $P = 0.97$] and no interaction of QPS × contraction × time [$F(1,11) = 1.04$, $P = 0.33$]. Simple-simple main effect tests revealed no significant change by time at any conditions. In right M1, recorded from left FDI, there was a significant main effect of contraction [$F(1,11) = 102.5$, $P < 0.001$] but no significant main effect of QPS [$F(1,11) = 1.54$, $P = 0.24$] or time [$F(1,11) = 3.3$, $P = 0.094$] and no interaction of QPS × contraction × time [$F(1,11) = 0.70$, $P = 0.42$]. Simple-simple main effect tests revealed no significant changes by time at any conditions.

Experiment 2: Effects of QPS on MEP Recruitment Curves of the Nonstimulated Motor Cortex

Recruitment curve showed significant increase of MEP amplitude after QPS-5 (Fig. 3*A*) but no change after QPS-50 (Fig. 3*B*). There was a significant main effect of intensity [$F(1,42) = 24.7$, $P < 0.001$] and a tendency toward a main effect of time [$F(1,7) = 5.5$, $P = 0.052$] but no significant main effect of QPS [$F(1,7) = 1.0$, $P = 0.35$]. There was no significant interaction of QPS × time × intensity [$F(6,42) = 1.2$, $P = 0.32$]. Simple-simple main effect tests revealed a significant change of QPS-5 by time at 130% RMT [$F(1,7) = 9.5$, $P = 0.018$], 150% RMT [$F(1,7) = 7.6$, $P = 0.028$], and 160% RMT [$F(1,7) = 6.3$, $P = 0.040$] and also a tendency at 120% RMT [$F(1,7) = 4.5$, $P = 0.071$] and 140% RMT [$F(1,7) = 4.3$, $P = 0.077$]. There were no significant changes in QPS-50 effect by time at any intensity.

Table 1. *Cortical motor threshold before and after QPS*

	QPS-5		QPS-50	
	Pre	Post	Pre	Post
Right FDI				
RMT	47.2 ± 2.8	46.1 ± 3.0	46.7 ± 2.9	47.5 ± 3.1
AMT	29.2 ± 1.6	28.8 ± 1.8	28.3 ± 1.9	28.8 ± 1.9
Left FDI				
RMT	49.9 ± 3.3	51.8 ± 3.3	48.9 ± 2.7	49.8 ± 3.5
AMT	33.2 ± 1.8	33.7 ± 1.7	30.9 ± 1.5	32 ± 2.1

Values are means ± SE of cortical resting (RMT) and active motor threshold (AMT) measured as %maximum stimulator output before (Pre) and after (Post) quadripulse transcranial magnetic stimulation (QPS) with an interstimuls interval of 5 (QPS-5) or 50 ms (QPS-50) in right and left first dorsal interosseous (FDI) muscle.

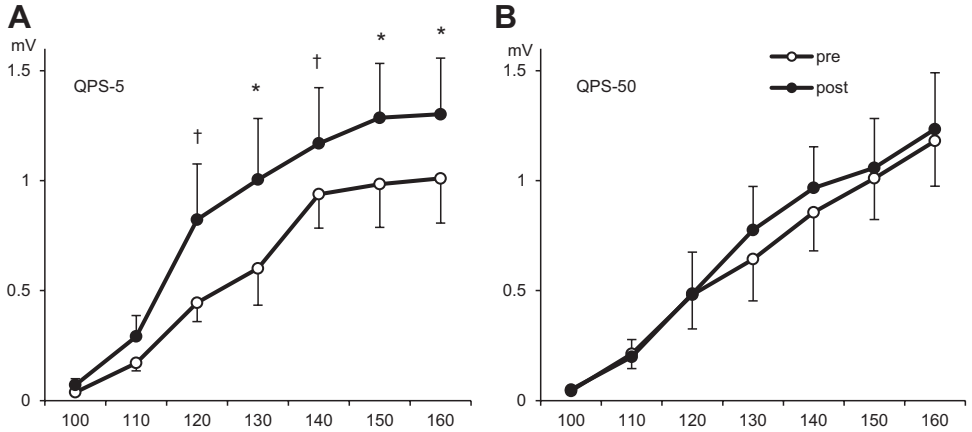

Fig. 3. MEP recruitment curves. *A*: MEP recruitment to single-pulse TMS over right M1, recorded from left FDI, increased after QPS-5. *B*: MEP recruitment to single-pulse TMS over right M1, recorded from left FDI, did not change after QPS-50. Horizontal axis shows %RMT for each subject. Vertical axis shows the absolute MEP amplitude (mV). Error bars indicate SE. *$P < 0.05$; †$P < 0.1$.

Experiment 3: Effects of QPS on SICI, LICI, ICF, and SICF of the Nonstimulated Motor Cortex

Test MEP size did not differ significantly by time at any conditions (Table 2). No significant change was observed after QPS-5 or QPS-50 for SICI, LICI, ICF, and SICF (Fig. 4).

For SICI, there was a significant main effect of intensity [$F(2,16) = 10.7$, $P = 0.001$], which showed a difference between 70 and 90% AMT ($P = 0.018$) and between 80 and 90% AMT ($P = 0.012$). There was no significant main effect of QPS [$F(1,8) = 0.008$, $P = 0.93$] or time [$F(1,8) = 0.02$, $P = 0.89$] and no interaction of QPS × intensity × time [$F(2,16) = 0.04$, $P = 0.96$]. Simple-simple main effect tests revealed no significant change by time at any conditions.

For LICI, there was no significant main effect of QPS [$F(1,8) = 0.007$, $P = 0.93$] or time [$F(1,8) = 0.004$, $P = 0.95$] and no interaction of QPS × time [$F(1,8) = 0.001$, $P = 0.97$]. Simple main effect tests revealed no significant change by time at any conditions.

For ICF, there was no significant main effect of QPS [$F(1,8) = 0.06$, $P = 0.82$], ISI [$F(1,8) = 1.8$, $P = 0.21$], or time [$F(1,8) = 0.06$, $P = 0.82$] and no interaction of QPS × ISI × time [$F(1,8) = 0.62$, $P = 0.45$]. Simple-simple main effect tests revealed no significant change by time at any condition.

For SICF, there was no significant main effect of QPS [$F(1,8) = 0.05$, $P = 0.83$], ISI [$F(2,16) = 0.39$, $P = 0.68$], or time [$F(1,8) = 0.07$, $P = 0.80$] and no interaction of QPS × ISI × time [$F(2,16) = 2.5$, $P = 0.11$]. Simple-simple main effect tests revealed no significant change by time at any condition.

Experiment 4: Effects of QPS on IHI

After QPS over left M1, IHI (ISI 10 ms) from left to right M1 increased after QPS-5, although there were no changes after QPS-50 in IHI from right to left M1 (Fig. 5). For IHI from left to right M1, recorded from left FDI, there was no significant main effect of QPS [$F(1,11) = 1.6$, $P = 0.23$], ISI [$F(1,11) = 0.02$, $P = 0.89$], or time [$F(1,11) = 2.8$, $P = 0.13$] and no interaction of QPS × ISI × time [$F(1,11) = 4.0$, $P = 0.07$]. Simple-simple main effect tests revealed a significant change by time only at the condition of QPS-5 and ISI of 10 ms [$F(1,11) = 11.7$, $P = 0.006$]. For IHI from right to left M1, recorded from right FDI, there was no significant main effect of QPS [$F(1,11) = 0.07$, $P = 0.79$], ISI [$F(1,11) = 1.38$, $P = 0.26$], or time [$F(1,11) = 0.03$, $P = 0.87$] and no interaction of QPS × ISI × time [$F(1,11) = 0.03$, $P = 0.86$]. Simple-simple main effect tests revealed no significant changes by time at any conditions.

Experiment 5: Effects of QPS on IHF

After QPS over left M1, IHF from left to right M1 increased after QPS-5 but not after QPS-50 (Fig. 6). There was no significant main effect of QPS [$F(1,7) = 0.63$, $p = 0.45$] or time [$F(1,7) = 1.5$, $P = 0.26$] and no interaction of QPS × time [$F(1,7) = 1.1$, $P = 0.33$]. Simple main effect tests revealed a significant change in IHF after QPS-5 [$F(1,7) = 7.7$, $P = 0.027$] but no change after QPS-50 [$F(1,7) < 0.001$, $P = 0.99$].

Correlation Study

Multiple regression analysis revealed a significant effect of the model [$F(3,8) = 5.79$, $P = 0.044$]. Standardized partial regression coefficient values were −0.27 for ΔMEP_{RtFDI} ($t = -0.87$, $P = 0.43$), −0.36 for ΔIHI ($t = -1.47$, $P = 0.20$), and 0.89 for ΔIHF ($t = 3.15$, $P = 0.025$). ΔIHF only had significant correlation with ΔMEP_{LtFDI}. The multiple correlation

Table 2. *Test motor-evoked potential size in each experiment*

	QPS-5			QPS-50		
	Pre	Post	*P*	Pre	Post	*P*
SICI/ICF/LICI	0.46 ± 0.07	0.46 ± 0.11	0.97	0.49 ± 0.07	0.48 ± 0.08	0.83
SICF	0.45 ± 0.10	0.45 ± 0.09	0.99	0.51 ± 0.08	0.48 ± 0.08	0.81
IHI right FDI	0.57 ± 0.06	0.70 ± 0.10	0.34	0.63 ± 0.10	0.68 ± 0.22	0.56
IHI left FDI	0.50 ± 0.06	0.54 ± 0.06	0.60	0.51 ± 0.03	0.49 ± 0.07	0.72
IHF	0.34 ± 0.03	0.33 ± 0.01	0.69	0.36 ± 0.08	0.33 ± 0.05	0.64

Values are means ± SE of test motor-evoked potential size (mV) in each experiment. SICI, short-interval intracortical inhibition; ICF, intracortical facilitation; LICI, long-interval intracortical inhibition; SICF; short-interval intracortical facilitation; IHI, interhemispheric inhibition; IHF, interhemispheric facilitation.

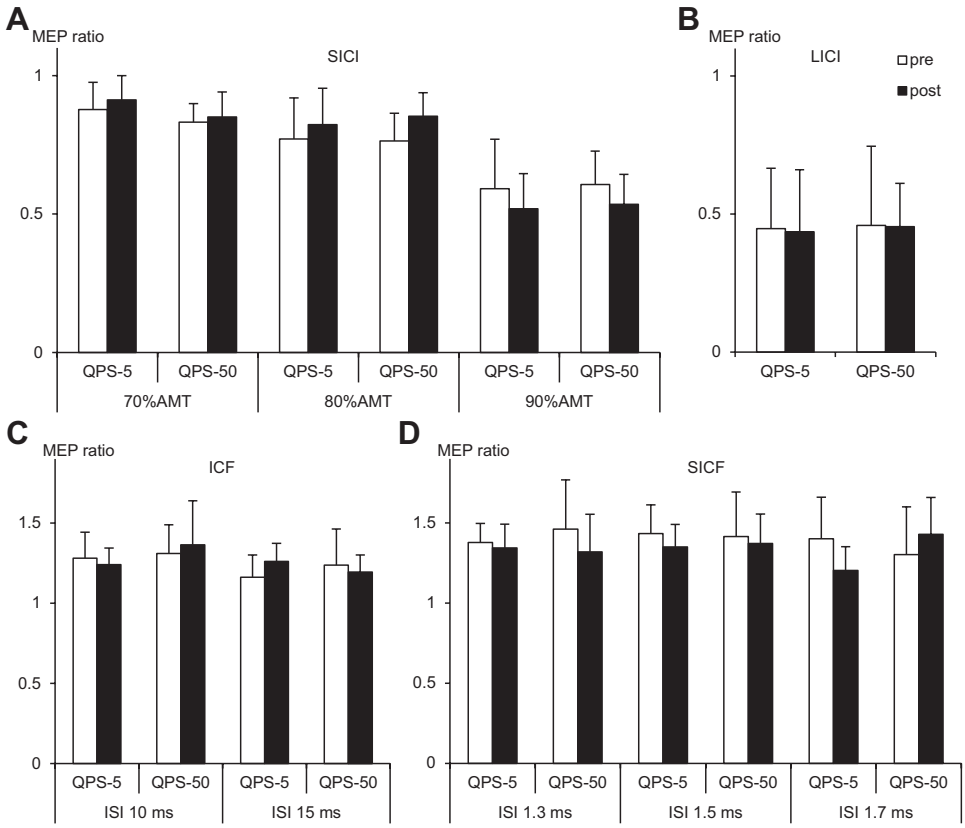

Fig. 4. Changes of intracortical inhibition and facilitation. *A*: SICI at the conditioning stimulus intensity of 70, 80, and 90% AMT of the right M1, recorded from left FDI, did not change after QPS-5 or QPS-50. *B*: LICI did not change after QPS-5 or QPS-50. *C*: ICF at the ISI of 10 and 15 ms did not change after QPS-5 or QPS-50. *D*: SICF at the ISI of 1.3, 1.5, and 1.7 ms did not change after QPS-5 or QPS-50. Vertical axis shows the MEP ratio. Error bars indicate SE.

coefficient was 0.88, and the coefficient of determination was 0.78. The scatter chart between ΔMEP_{LtFDI} and ΔIHF is shown in Fig. 7.

DISCUSSION

In the present study, we have shown that the plastic change of M1 could be induced by QPS over the contralateral M1 associated with changes of the interhemispheric interactions. QPS-5 over the left M1, inducing LTP in the stimulated left M1, also induced LTP in the nonstimulated right M1. Meanwhile, QPS-50 over the left M1, inducing LTD in the stimulated left M1, did not induce any significant changes in the nonstimulated right M1. None of RMT, AMT, intracortical inhibition (SICI and LICI), and intracortical facilitation (ICF and SICF) in the nonstimulated right M1 changed significantly after either QPS-5 or QPS-50 over the left M1. The degrees of inhibition of IHI and facilitation of IHF from left to right M1 significantly increased after left M1 QPS-5, and the MEP amplitude change of the left FDI significantly correlated with the change of IHF. This is the first report that IHF as well as IHI had changed following M1 rTMS, and it was IHF that significantly correlated with changes of the contralateral MEP amplitude. Moreover, the result that MEP changes did not correlate significantly between both hands indicates that the plastic changes in the two hemispheres may occur independently.

Contralateral M1 Changes by rTMS

In previous studies, rTMS inducing LTP in the stimulated M1 has been reported to affect the contralateral M1 in incon-

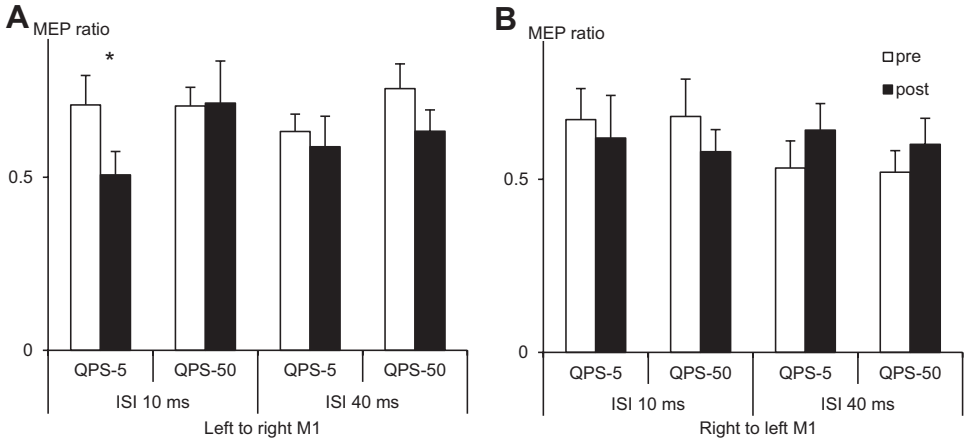

Fig. 5. Changes of IHI. *A*: IHI at the ISI of 10 and 40 ms, from left to right M1, were studied. IHI from left to right M1 with ISI of 10 ms increased after QPS-5 but did not change after QPS-50 or at ISI of 40 ms. *B*: IHI from right to left M1 did not change after QPS-5 or QPS-50. Vertical axis shows the MEP ratio. Error bars indicate SE. $*P < 0.05$.

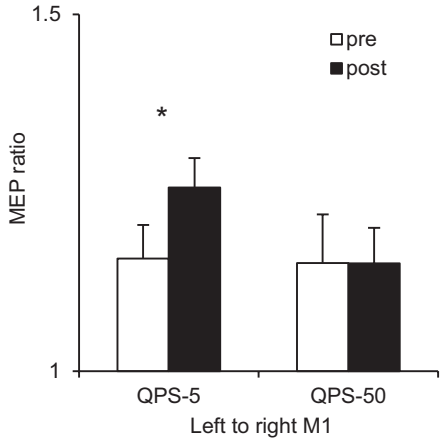

Fig. 6. Changes of IHF. IHF from left to right M1 increased after QPS-5 but not after QPS-50. Vertical axis shows the MEP ratio. Error bars indicate SE. $*P < 0.05$.

sistent manners. Five-Hz rTMS and PAS increased MEPs evoked by the nonstimulated M1 activation (Gorsler et al. 2003; Shin and Sohn 2011). This same directional change in both M1s is consistent with our results. However, intermittent TBS inducing an LTP-like effect on the stimulated M1 reduced excitability of contralateral M1 (Di Lazzaro et al. 2008; Suppa et al. 2008). According to results of rTMS inducing LTD in the stimulated M1, effects on the contralateral M1 were again variable. After 1-Hz M1 rTMS, both contralateral M1 excitability increments and decrements were reported (Gilio et al. 2003; Heide et al. 2006; Pal et al. 2005; Plewnia et al. 2003; Schambra et al. 2003; Wassermann et al. 1998). Continuous TBS, inducing LTD in stimulated M1, increased (Stefan et al. 2008; Suppa et al. 2008) or decreased (Ishikawa et al. 2007) the excitability of nonstimulated M1. The variability of the rTMS remote effects could be caused by the difference in rTMS protocol. However, in a recent article comparing the effects of different rTMS protocols, with the exception of QPS, no significant effect was observed in the contralateral M1 after any rTMS protocols (Di Lazzaro et al. 2011). We have shown that QPS-5 induced a significant, same-directional effect on the contralateral M1.

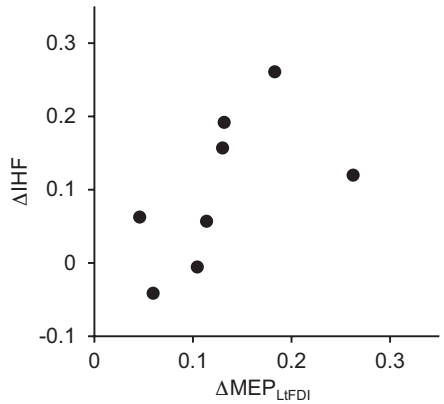

Fig. 7. Correlation between the changes of MEP amplitude and IHF. After QPS-5, change of the IHF from left to right M1 (ΔIHF) had significant correlation between the MEP amplitude changes to single-pulse TMS over right M1, recorded from left FDI (ΔMEP_{LtFDI}). Horizontal axis shows the change of the absolute MEP amplitude (mV). Vertical axis shows the change of MEP ratio of IHF.

Contralateral M1 changes and IHIs

The relation between plastic change in the contralateral M1 and changes in the interhemispheric interactions has not been elucidated so far. The degree of IHI has been studied when some LTP-like plastic changes occurred at the nonstimulated M1 after various types of rTMS (Gilio et al. 2003; Pal et al. 2005; Suppa et al. 2008; Shin and Sohn 2011). TBS did not affect IHI (Suppa et al. 2008), and 1-Hz rTMS and PAS (25 ms) induced MEP enhancement and IHI reduction (Gilio et al. 2003; Shin and Sohn 2011). The common interpretation for this is that the reduction of IHI leads to the disinhibition of the nonstimulated M1. We showed that QPS-5 enhanced both MEPs to the nonstimulated M1 activation and IHI from stimulated to nonstimulated M1. This means that the LTP-like effect on the contralateral M1 by QPS-5 could be produced by some mechanism different from those by the other protocols. In this work, we first studied changes in IHF after QPS. IHF from stimulated to nonstimulated M1 also increased after QPS-5.

We have shown that the LTP-like effects on M1 are associated with both the inhibitory and facilitatory interhemispheric interactions after QPS-5. Moreover, IHF increment significantly related to the degree of LTP at the nonstimulated M1, whereas IHI decrement had no significant correlation with the degree of LTP. We consider these three findings to be explained as follows. In the case of LTP of the stimulated M1, both facilitatory and inhibitory transcallosal connections would be potentiated, as well (Fig. 8). Synaptic potentiation may occur between transcallosal fibers and either facilitatory or inhibitory interneurons, as well as axonal plastic changes of the interneurons at the nonstimulated M1. Neurons for the transcallosal facilitation would interact with the contralateral corticospinal tract (CST) neurons in shorter intervals, whereas those for the transcallosal inhibition would interact with the contralateral CST neurons via inhibitory interneurons, taking longer intervals. Because MEPs to TMS are mediated by the potentiated facilitatory interneurons but not through the potentiated inhibitory interneurons, MEPs to the contralateral M1 activation are potentiated after LTP-like effects on M1. There may be several other possibilities to explain these three induced events. However, the causal correlation between these three events is not a main target of this study. Whichever the

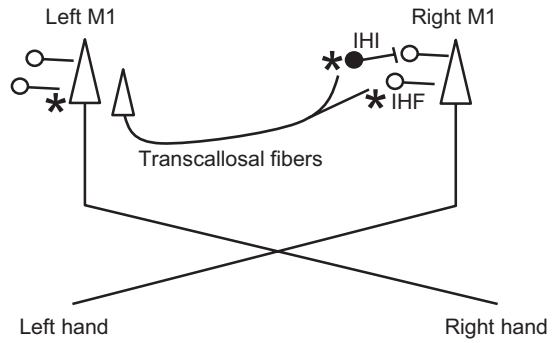

Fig. 8. Schema of the effects of motor cortical QPS on the contralateral motor cortex and interhemispheric interactions. QPS over the left M1 would induce long-term potentiation (LTP) not only to the left M1 pyramidal neurons but also to the transcallosal fibers from left M1 to right M1. Both facilitatory interneurons and inhibitory interneurons of the right M1 would be potentiated, as well. Asterisks show the sites of potentiated synapses.

underlying mechanisms are, it is conspicuous that QPS-5 definitely induced these three events differently from other rTMS methods. In contrast, however, because QPS-50 induces no changes in the callosal function for some reason, no MEP changes were provoked in the right M1. This point is discussed later.

Another issue to stress is the difference in IHI effects between ISIs of 10 and 40 ms. The short-latency IHI (SIHI; ISI = 10 ms) was increased, whereas the long-latency IHI (LIHI; ISI = 40 ms) was unaffected. This dissociation is the same as that reported by Gilio et al. (2003) and supports the idea that the SIHI and LIHI are produced by different mechanisms (Chen et al. 2003). Moreover, SIHI and LIHI are reported to be modulated by different neurotransmitter systems, such as $GABA_A$ and $GABA_B$ (Irlbacher et al. 2007). The difference in transmitter system may be one of the factors to explain the difference between SIHI and LIHI.

Difference between LTP and LTD

In our study, the effect on the contralateral M1 was only observed by QPS-5 inducing LTP in the stimulated M1 but not by QPS-50 inducing LTD in the stimulated M1. Why did QPS-50 not induce LTD in the contralateral M1? One simple possibility is that the effect of QPS-50 was weaker than that of QPS-5 and was not powerful enough to induce the contralateral M1 plasticity. Another possibility is that QPS-5 and QPS-50 may affect transcallosal fibers and CST fibers differently. Since these two fibers have different physiological properties (Hallman et al. 1988; Kasper et al. 1994; Molnár and Cheung 2006), their reactivity to rTMS may differ. Some results may depend on the stimulus protocols. In previous reports of 1-Hz rTMS, the different results are reported and the stimulus intensity, coil orientation, or other conditioning stimulation parameters are thought to affect the results (Gilio et al. 2003; Heide et al. 2006; Pal et al. 2005; Plewnia et al. 2003; Schambra et al. 2003; Wassermann et al. 1998). Interestingly, regardless of the rTMS protocols, in the case of LTP-like effects, most results show the facilitatory effects on the contralateral M1, whereas only few show the inhibitory effects.

Clinical application

In application to clinical use, attention has been paid to IHI in stroke patients (Hummel and Cohen 2006). There are several reports of the effect of rTMS in stroke patients: low-frequency stimulation ($\leq$1 Hz) over the unaffected M1 to reduce the interhemispheric inhibition of the contralesional hemisphere (Avenanti et al. 2012; Kirton et al. 2008; Mansur et al. 2005) or high-frequency stimulation ($>$ 1 Hz) over the affected M1 to increase the excitability of the ipsilesional hemisphere (Corti et al. 2012). These methods are based on the fact that IHI is abnormal in stoke patients (Murase et al. 2004) and on the reduction of IHI after rTMS (Avenanti et al. 2012; Kirton et al. 2010). We have shown in this study that not only IHI but also IHF changed after rTMS. Although the causal correlations are unknown, these results imply that some new rTMS approach may be used in the restoration of motor functions in patients with unilateral motor dysfunction.

One limitation of this study is the time window for evaluation. We only performed the poststimulus evaluation at 30 min after the end of QPS. We chose this time point because the strong effect on the stimulated M1 was reported at this time window (Hamada et al. 2008). We could not completely exclude the possibility that some changes may be induced in other parameters at different time points. Another limitation may be the experiment of IHF done during voluntary contraction. There are some concerns about the plasticity disappearance by muscle contraction in TBS or PAS. The method we used in measuring IHF needs slight muscle contraction because of the recruitment of I3 waves (Hanajima et al. 2001). Since the muscle contraction effects on the plasticity are minimal in the case of QPS (Kadowaki 2012), we suppose that the experiment during voluntary contraction did not seriously affect our results.

Additionally, when interhemispheric interactions are studied in humans, the age of the subjects (Fling and Seidler 2012) and the latent possibility of the difference between dominant and nondominant hemispheres (Bäumer et al. 2007; De Gennaro et al. 2004; Nelson et al. 2009; Netz et al. 1995; Salerno and Georgesco 1996) should be considered as confounding factors. In this study, all of the participants were right-handed so that we observed the effects from the dominant to nondominant hemisphere. The age and dominant side effects on the present interactions will be one of the future projects. Moreover, the gender difference could be another confounding factor (De Gennaro et al. 2004; Weis and Hausmann 2010). This will be also studied in the near future. The low number of subjects would be another limitation. Our positive conclusion should not be affected by a small number of subjects because we showed statistically significant differences even with small numbers; however, some negative results may be due to small numbers. Also, even though IHI and IHF are known to be mediated through the corpus callosum, we could not completely exclude the possibility that the contralateral effect of the QPS might be mediated through some subcortical route and not through the corpus callosum. Further study of a patient with corpus callosum agenesis might be expected. Beyond these difficulties, further studies are desired to investigate the complicated mechanism of the corpus callosum and its interactions in humans.

In summary, we have shown in this study that left M1 QPS-5 induced facilitatory MEP change to the contralateral right M1. SIHI and IHF from left to right M1 increased, and IHF showed significant correlation, with the right M1 MEP change. These results showed that the LTP-like effect on the contralateral M1 is related to the interhemispheric interactions through the corpus callosum.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.T., R.H., Y.T., Y.S., S.O., T.S., N.T., and Y.U. conception and design of research; R.T., R.H., Y.T., Y.S., S.O., T.S., and N.T. performed experiments; R.T. analyzed data; R.T. interpreted results of experiments; R.T. prepared figures; R.T. drafted manuscript; R.T., R.H., Y.T., Y.S., S.O., T.S., N.T., and Y.U. edited and revised manuscript; R.T., R.H., Y.T., Y.S., S.O., T.S., N.T., and Y.U. approved final version of manuscript.

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