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Bidirectional modulation of sensory cortical excitability by quadripulse transcranial magnetic stimulation (QPS) in humans

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HIGHLIGHTS

- The newly designed quadripulse transcranial magnetic stimulation (QPS) was applied to the study of long-lasting sensory cortical excitability changes in humans.
- Sensory cortical excitability was modulated by QPS over the primary motor cortex or dorsal premotor cortex, but not by QPS over the primary sensory cortex itself.
- QPS might be a useful method in studies of heterotopic long-term potentiation/depression (LTP/LTD)-like effects in humans.

ABSTRACT

Objective: Quadripulse transcranial magnetic stimulation (QPS) is a newly designed patterned repetitive transcranial magnetic stimulation (TMS). Previous studies of QPS showed bidirectional effects on the primary motor cortex (M1), which depended on its inter-stimulus interval (ISI): motor evoked potentials (MEPs) were potentiated at short ISIs and depressed at long ISIs (homotopic effects). These physiological characters were compatible with synaptic plasticity. In this research, we studied effects of QPS on the primary sensory cortex (S1).
Methods: One burst consisted of four monophasic TMS pulses at an intensity of 90% active motor threshold. The ISI of four pulses was set at 5 ms (QPS-5) or at 50 ms (QPS-50). Same bursts were given every 5 s for 30 min. QPS-5 and QPS-50 were performed over three areas (M1, S1 and dorsal premotor cortex (dPMC)). One sham stimulation session was also performed. Excitability changes of S1 were evaluated by timeline of somatosensory evoked potentials (SEPs).
Results: QPS-5 over M1 or dPMC enhanced the P25–N33 component of SEP, and QPS-50 over M1 depressed it. By contrast, QPSs over S1 had no effects on SEPs.
Conclusions: QPSs over motor cortices modulated the S1 cortical excitability (heterotopic effects). Mutual connections between dPMC or M1 and S1 might be responsible for these modulations.
Significance: QPSs induced heterotopic LTP or LTD-like cortical excitability changes.

1. Introduction

Most long-term effects induced by non-invasive brain stimulation techniques in humans have been focussed on the motor cortical homotopic effect, that is, aftereffects on the primary motor cortex (M1) by stimulation over M1 itself (Chen et al., 1997; Berardelli et al., 1998; Huang et al., 2005, 2011; Gilio et al., 2009). A newly designed quadripulse transcranial magnetic stimulation (QPS) has also been applied to study homotopic effects after stimulation over M1 (Hamada et al., 2007a,b, 2008a,b). They showed bidirectional cortical plastic and metaplastic changes as predicted by the Bienenstock–Cooper–Munro (BCM) rule (Bienenstock et al., 1982).

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Several investigators have also studied long-term effects on the primary sensory cortex (S1) by repetitive transcranial magnetic stimulation (rTMS), theta burst stimulation (TBS) or direct current stimulation (tDCS) over M1 (heterotopic effect). They showed long-lasting effects on somatosensory evoked potential (SEP) (Enomoto et al., 2001; Ishikawa et al., 2007; Kodama et al., 2009), perception or pain (Satow et al., 2003; Matsunaga et al., 2004; Tamura et al., 2004; Saitoh et al., 2006; Hirayama et al., 2006; Kodama et al., 2009). The effects on S1 by S1 stimulation (homotopic effect) were controversial. Some articles reported considerable effects (Dieckhöfer et al., 2006; Ishikawa et al., 2007; Katayama and Rothwell, 2007; Katayama et al., 2010) and others reported no or only limited homotopic effects (Enomoto et al., 2001; Satow et al., 2003; Hirayama et al., 2006). Several previous investigations also studied effects of premotor cortex (PMC) stimulation on S1. The SEP was not affected by rTMS over PMC (Enomoto et al., 2001; Urushihara et al., 2006; Hosono et al., 2008). In summary, according to the long-lasting effects on S1, the heterotopic effects by stimulation over M1 and PMC are consistent in previous studies, but the homotopic effects by sensory cortical stimulation are inconsistent.

Based on these previous studies, we hypothesised that the heterotopic effects on S1 should be induced by QPS over M1, but not over dorsal PMC (dPMC). We also would like to know whether or not QPS has homotopic effects on S1. To solve these issues, in this article, we studied the sensory cortical effects by QPSs over several cortical areas using SEPs.

2. Methods

2.1. Subjects

Eleven right-handed healthy volunteers aged 32–55 years (mean, 39.6 ± 7.2 years) participated in this study. None of them had neurological or psychiatric disorders, head injuries and alcohol or drug abuse. Ten subjects participated in all the experiments and the other subject only in QPSs over M1. In each subject, two successive experiments were separated by at least 1 week. Written informed consent was obtained from all the subjects before the experiments. The protocol was approved by the Ethics Committees of Fukushima Medical University and the University of Tokyo. No adverse effects were noted in any individuals.

2.2. QPS stimulation

Prior to QPS, the active motor threshold (AMT) was measured by single-pulse transcranial magnetic stimulation (TMS) during a slight voluntary contraction of the right first dorsal interosseous (FDI) muscle. The coil was placed over the hot spot for the FDI. We used a specially designed figure-of-eight coil of 9-cm external diameter whose handle was attached vertically to the connecting point of the wings. The direction of induced current in the brain was adjusted as posterolateral to anteromedial at a 45° angle. Surface EMG was recorded from the right FDI muscle with an active electrode placed over the muscle belly and a reference electrode over the metacarpophalangeal joint of the index finger. Responses were amplified (Neuropack μ, Nihon-Kohden, Japan) through filters set at 100 Hz and 3 kHz and stored at a sampling rate of 10 kHz, and analysed offline (TMS bistim tester; Medical Try System, Japan). The mean (±standard error) AMT was 38.0% (±0.6) of the maximum stimulator output.

In QPS, the coil was connected with four magnetic stimulators through a special connecting device (MagStim 200^2; The MagStim Co. Ltd., UK), as previously reported (Hamada et al., 2007a,b, 2008a,b). During QPS, subjects lay on a comfortable reclining chair, with the target muscle relaxed. We set a large pillow under the subject's head on a reclining chair to fix the coil stably. One burst consisted of four monophasic TMS pulses at the same intensity (90% AMT) and the same bursts were given every 5 s (inter-burst interval). Interstimulus intervals (ISIs) of four pulses were set at 5 ms (QPS-5) or at 50 ms (QPS-50), which induced the most powerful LTP-like and LTD-like effects on M1, respectively (Hamada et al., 2007a,b, 2008a,b). QPS was given over three sites: the hot spot for the FDI muscle (M1), 3 cm anterior to M1 (dPMC) (Siebner et al., 2003) and C3′ (2 cm behind C3 of the International 10–20 system) which is usually used for recording median nerve SEPs. Each session consisted of 360 bursts (1440 pulses) for 30 min.

For the sham stimulation, we used the 'realistic sham' technique. This method was used in previous studies (Okabe et al., 2003; Nagel et al., 2008) and had considerable placebo effects (Hamada et al., 2008a,b). We placed a coil over the scalp connecting to an uncharged stimulator to give a pressure sensation similar to real stimulation. To mimic real QPS, stimulation noises of QPS-50 were given by firing another stimulator through a non-stimulating coil beside the subject's head, and skin sensation was given with an electric stimulus through the electrodes placed directly over the scalp at the same time. Any sham method has pros and cons (Borckardt et al., 2008) and it is difficult to mimic correctly the muscle twitch of real stimulation in a sham procedure. The sham technique with electrodes placed directly on the scalp, however, was useful even though the stimulation was not identical to rTMS (Mennemeier et al., 2009).

In every subject, the order of seven QPS sessions (sham stimulation, QPS-5 and QPS-50 over three cortical areas) was randomised to avoid the order effect.

2.3. SEP

For SEP recording, we stimulated the right median nerve at the wrist at 2 Hz. The recording electrode was placed over the hand

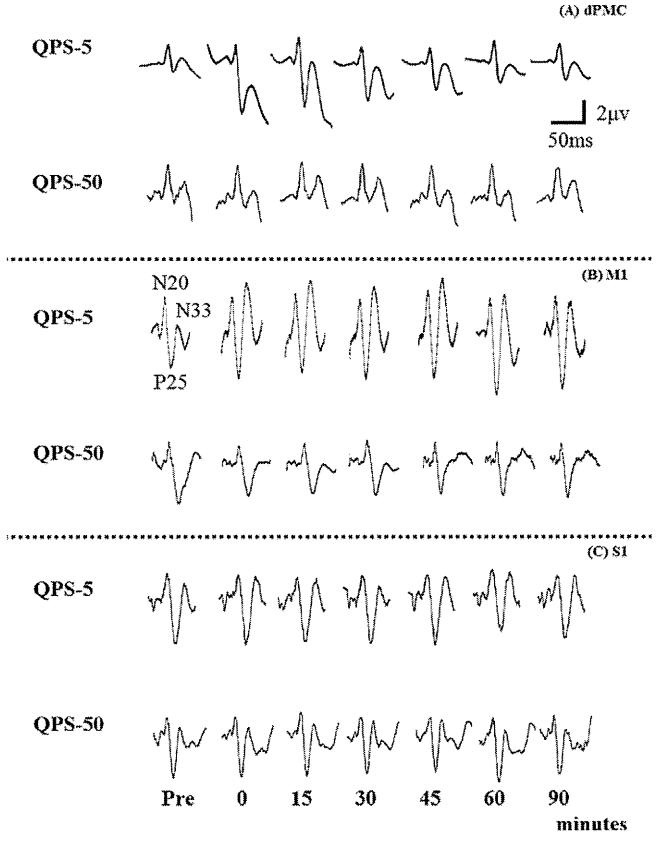

Fig. 1. Typical SEP waveforms before and after QPSs. (A) shows SEPs for the dPMC stimulation experiment, (B) for M1 stimulation and (C) for S1 stimulation. The upper rows are those for QPS-5 and the lower for QPS-50 in each site of stimulation.

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Table 1
Amplitudes of SEP components, Erb's potential and sensory nerve action potential (SNAP).

		Pre	0	5	10	15	30	45	60	75	90
N20o–N20p	Sham	1.8 ± 0.3	1.7 ± 0.3	1.8 ± 0.3	1.8 ± 0.4	1.8 ± 0.3	1.7 ± 0.3	1.8 ± 0.3	1.8 ± 0.3	1.9 ± 0.3	1.8 ± 0.3
	dPMC-5	1.7 ± 0.4	1.4 ± 0.2	1.6 ± 0.3	1.6 ± 0.3	1.5 ± 0.3	1.6 ± 0.3	1.7 ± 0.4	1.8 ± 0.4	1.8 ± 0.4	1.6 ± 0.3
	dPMC-50	2.0 ± 0.3	2.1 ± 0.4	2.0 ± 0.4	2.2 ± 0.3	2.2 ± 0.4	2.0 ± 0.3	2.0 ± 0.3	2.0 ± 0.3	2.0 ± 0.4	2.1 ± 0.4
	M1-5	1.8 ± 0.3	1.8 ± 0.3	1.9 ± 0.3	1.9 ± 0.3	1.8 ± 0.3	2.0 ± 0.4	1.9 ± 0.3	1.8 ± 0.3	1.9 ± 0.3	1.8 ± 0.3
	M1-50	1.6 ± 0.3	1.4 ± 0.2	1.5 ± 0.3	1.5 ± 0.3	1.5 ± 0.3	1.5 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	1.4 ± 0.3	1.4 ± 0.2
	S1-5	1.7 ± 0.2	1.4 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.7 ± 0.2	1.5 ± 0.2	1.7 ± 0.2	1.5 ± 0.2
	S1-50	1.8 ± 0.3	1.9 ± 0.2	1.8 ± 0.3	1.8 ± 0.3	1.9 ± 0.3	1.9 ± 0.2	2.0 ± 0.3	1.8 ± 0.3	1.8 ± 0.3	1.9 ± 0.3
N20p–P25	Sham	3.8 ± 0.6	3.7 ± 0.6	3.7 ± 0.6	3.9 ± 0.6	3.7 ± 0.5	3.5 ± 0.6	3.5 ± 0.6	3.6 ± 0.6	3.6 ± 0.6	3.6 ± 0.5
	dPMC-5	3.7 ± 0.4	4.5 ± 0.7	4.1 ± 0.5	4.5 ± 0.9	4.1 ± 0.7	4.0 ± 0.5	4.1 ± 0.6	4.3 ± 0.6	4.3 ± 0.5	4.0 ± 0.5
	dPMC-50	4.3 ± 0.9	4.3 ± 0.9	4.5 ± 1.0	4.3 ± 1.0	4.3 ± 0.9	4.5 ± 1.0	4.2 ± 1.0	4.1 ± 1.0	4.2 ± 1.1	4.6 ± 1.0
	M1-5	3.8 ± 0.9	3.3 ± 0.6	3.3 ± 0.7	3.4 ± 0.7	3.4 ± 0.7	3.6 ± 0.7	3.3 ± 0.7	3.4 ± 0.7	3.6 ± 0.8	3.3 ± 0.5
	M1-50	4.1 ± 1.1	3.7 ± 0.7	3.7 ± 0.8	3.8 ± 0.8	3.8 ± 0.9	3.6 ± 0.9	3.6 ± 0.9	3.8 ± 0.9	3.7 ± 0.9	3.4 ± 0.7
	S1-5	3.1 ± 0.7	3.1 ± 0.7	3.1 ± 0.7	3.0 ± 0.7	3.1 ± 0.7	3.1 ± 0.7	3.0 ± 0.6	3.1 ± 0.7	3.0 ± 0.7	3.1 ± 0.7
	S1-50	3.3 ± 0.4	3.4 ± 0.5	3.4 ± 0.5	3.4 ± 0.5	3.2 ± 0.5	3.7 ± 0.6	3.4 ± 0.5	3.5 ± 0.6	3.6 ± 0.7	3.3 ± 0.5
P25–N33	Sham	1.9 ± 0.4	1.8 ± 0.4	1.8 ± 0.4	1.8 ± 0.4	1.7 ± 0.3	1.7 ± 0.3	1.7 ± 0.4	1.8 ± 0.3	1.8 ± 0.3	1.8 ± 0.4
	dPMC-5	1.9 ± 0.2	2.6 ± 0.3	2.5 ± 0.5	3.0 ± 0.6	2.6 ± 0.5	2.4 ± 0.2	2.3 ± 0.3	2.4 ± 0.4	2.5 ± 0.3	2.4 ± 0.3
	dPMC-50	1.3 ± 0.5	1.3 ± 0.5	0.9 ± 0.4	1.2 ± 0.5	1.2 ± 0.5	1.1 ± 0.4	1.2 ± 0.4	1.3 ± 0.5	1.3 ± 0.5	1.3 ± 0.5
	M1-5	1.7 ± 0.2	2.0 ± 0.4	2.0 ± 0.4	2.0 ± 0.4	2.0 ± 0.3	2.2 ± 0.4	2.1 ± 0.4	2.1 ± 0.4	2.1 ± 0.4	2.1 ± 0.4
	M1-50	2.2 ± 0.5	1.9 ± 0.3	1.7 ± 0.4	1.8 ± 0.3	1.8 ± 0.4	1.7 ± 0.4	1.7 ± 0.4	1.9 ± 0.4	2.0 ± 0.5	1.8 ± 0.4
	S1-5	1.6 ± 0.4	1.5 ± 0.4	1.6 ± 0.4	1.6 ± 0.4	1.7 ± 0.5	1.8 ± 0.4	1.5 ± 0.4	1.6 ± 0.4	1.6 ± 0.5	1.5 ± 0.5
	S1-50	2.3 ± 0.7	2.2 ± 0.6	2.2 ± 0.6	2.1 ± 0.5	2.4 ± 0.8	2.0 ± 0.5	2.4 ± 0.7	2.1 ± 0.5	1.7 ± 0.4	2.2 ± 0.6
Erb	Sham	10.6 ± 2.1	10.5 ± 2.2	10.6 ± 2.3	10.5 ± 2.1	10.5 ± 2.3	11.5 ± 2.6	11.1 ± 2.5	10.9 ± 2.4	11.4 ± 2.4	10.7 ± 2.3
	dPMC-5	7.7 ± 1.6	8.3 ± 1.6	8.5 ± 1.7	8.3 ± 1.6	8.3 ± 1.4	8.4 ± 1.6	8.5 ± 1.8	8.3 ± 1.7	8.4 ± 1.5	8.6 ± 1.7
	dPMC-50	8.4 ± 1.6	7.8 ± 1.6	8.4 ± 1.6	7.5 ± 1.6	7.9 ± 1.5	8.2 ± 1.4	7.7 ± 1.8	8.1 ± 1.7	8.3 ± 1.5	8.6 ± 1.7
	M1-5	8.6 ± 2.1	8.8 ± 1.9	8.7 ± 2.3	8.6 ± 2.2	9.1 ± 2.3	9.1 ± 2.3	8.8 ± 2.2	9.0 ± 2.3	9.1 ± 2.1	9.1 ± 2.2
	M1-50	8.4 ± 2.7	6.8 ± 1.9	7.6 ± 2.6	7.7 ± 2.6	7.7 ± 2.8	7.6 ± 2.5	7.8 ± 2.7	7.9 ± 2.4	7.2 ± 2.3	8.1 ± 2.4
	S1-5	8.6 ± 1.7	8.9 ± 1.5	9.2 ± 1.8	9.0 ± 1.5	9.1 ± 1.6	9.4 ± 1.6	8.9 ± 1.7	9.5 ± 1.7	9.5 ± 1.9	9.5 ± 1.9
	S1-50	7.3 ± 1.2	6.9 ± 0.7	7.0 ± 1.0	7.1 ± 1.1	7.1 ± 1.2	7.0 ± 1.0	7.0 ± 1.3	6.9 ± 1.0	6.9 ± 0.9	6.8 ± 1.0
SNAP	Sham	46.2 ± 8.3	44.0 ± 8.1	50.2 ± 7.9	47.5 ± 7.3	47.4 ± 7.5	43.8 ± 7.7	46.6 ± 7.1	47.0 ± 7.2	48.0 ± 7.7	51.4 ± 8.1
	dPMC-5	39.1 ± 7.2	40.7 ± 7.2	42.0 ± 7.2	40.4 ± 7.4	39.0 ± 6.8	40.8 ± 7.8	40.8 ± 6.8	39.2 ± 7.7	39.5 ± 7.8	37.5 ± 7.8
	dPMC-50	47.1 ± 3.9	49.3 ± 4.4	52.9 ± 3.7	51.0 ± 4.3	48.9 ± 4.3	44.9 ± 6.1	47.9 ± 3.5	50.9 ± 3.8	49.3 ± 3.1	51.6 ± 5.0
	M1-5	47.8 ± 9.8	47.1 ± 10.3	45.8 ± 10.2	46.8 ± 9.7	47.5 ± 9.7	47.2 ± 9.4	48.7 ± 8.6	46.8 ± 10.2	47.5 ± 9.5	46.4 ± 9.3
	M1-50	49.7 ± 15.6	50.8 ± 15.3	49.3 ± 16.4	50.5 ± 16.9	51.3 ± 17.5	50.5 ± 18.6	51.8 ± 18.5	52.6 ± 19.0	52.9 ± 18.9	50.5 ± 18.3
	S1-5	40.6 ± 9.3	42.4 ± 10.5	42.1 ± 11.4	43.4 ± 11.7	43.6 ± 11.7	43.7 ± 11.7	42.6 ± 10.7	43.1 ± 11.7	42.2 ± 11.5	41.1 ± 11.1
	S1-50	23.8 ± 5.8	24.9 ± 5.8	25.2 ± 5.8	25.3 ± 6.0	25.9 ± 5.9	26.4 ± 6.0	27.6 ± 6.5	27.4 ± 6.1	26.9 ± 5.9	26.3 ± 5.6

Mean amplitudes (± standard error) of each component (μv) before and after QPSs are shown. (Pre) prior to QPSs, (0–90) 0 to 90 min after QPSs; (dPMC-5/dPMC-50) QPS-5/QPS-50 over dPMC, (M1-5/M1-50) QPS-5/QPS-50 over M1, (S1-5/S1-50) QPS-5/QPS-50 over S1.

sensory area (C3′) and the reference on Fz, according to the International 10–20 system. The impedance of these electrodes was maintained below 3 kΩ. The band-pass filter was set between 2 Hz and 2 kHz (Neuropack 8 or Neuropack μ; Nihon-Kohden, Japan). A brief electrical rectangular pulse of 0.2 ms duration was set at an intensity of 2.5–3 times the sensory threshold, which evoked a small muscular twitch in the thenar muscle. The antidromic sensory nerve action potential (SNAP) was recorded at the index finger tip, and the afferent volley at the ipsilateral Erb's point to confirm that the median nerve was stably activated during the experiments. The time window was 80 ms after the onset of median nerve stimulation. We collected and averaged 250 sweeps without artefacts for one SEP waveform.

SEPs were recorded three times before QPS (T_{pre}) and nine time points at 0, 5, 10, 15, 30, 45, 60, 75 and 90 min after QPS (T_0, T_5, T_{10}, T_{15}, T_{30}, T_{45}, T_{60}, T_{75} and T_{90}). After QPS, we recorded SEPs once, and did not record twice to confirm the reproducibility of findings. We did not have enough time for multiple recordings at one time point after QPS because we followed SEPs in time. SEPs were well measured, and they were stably recorded. We, therefore, considered that our SEP time courses were good enough for later analyses even without double traces.

We identified three SEP components of an initial negative peak as N20, following a positive peak as P25 and a second negative peak as N33. We determined an initial rise from the baseline of the N20 component as N20o (N20 onset). They were automatically determined by the computer software. Peak-to-peak amplitudes of N20o–N20p, N20p–P25 and P25–N33 were measured. Aftereffects of QPSs on these parameters were evaluated using the size ratios of SEP after QPS to the baseline before QPS.

2.4. Statistical analyses

The ratio of SEP amplitude against pre-QPS baseline was subjected to the following analyses as a dependent variable. We examined whether or not the SEP size changed depending on the target area (AREA: dPMC, M1, S1 and sham), ISIs (ISI: 5 and 50 ms) of the QPS and time after QPS (TIME: T_0, T_5, T_{10}, T_{15}, T_{30}, T_{45}, T_{60}, T_{75} and T_{90}) by using three-way repeated measures analysis of variance (ANOVA). For the factors that yielded significant main effect and interaction, we performed post hoc pair-wise comparisons with Bonferroni correction ($p < 0.05$). We used Matlab for Windows with Statistics Toolbox (MathWorks) for statistical analyses.

3. Results

No subjects complained of any adverse effects after QPSs. Fig. 1 shows exemplary SEP waveforms before and after QPS. Table 1 summarises the measured amplitudes of SEP components, Erb's potential and SNAPs. Neither SNAPs nor Erb's potentials changed significantly throughout experiments, indicating stable activation of the median nerve.

Fig. 2 shows the time courses of the SEP P25–N33 component after QPS over the three cortical areas ((A) dPMC; (B) M1; (C) S1 and sham stimulation (D)). Three-way ANOVA (AREA × ISI × TIME) revealed significant main effects of AREA ($F_{3,666} = 54.75$, $p < 0.001$)

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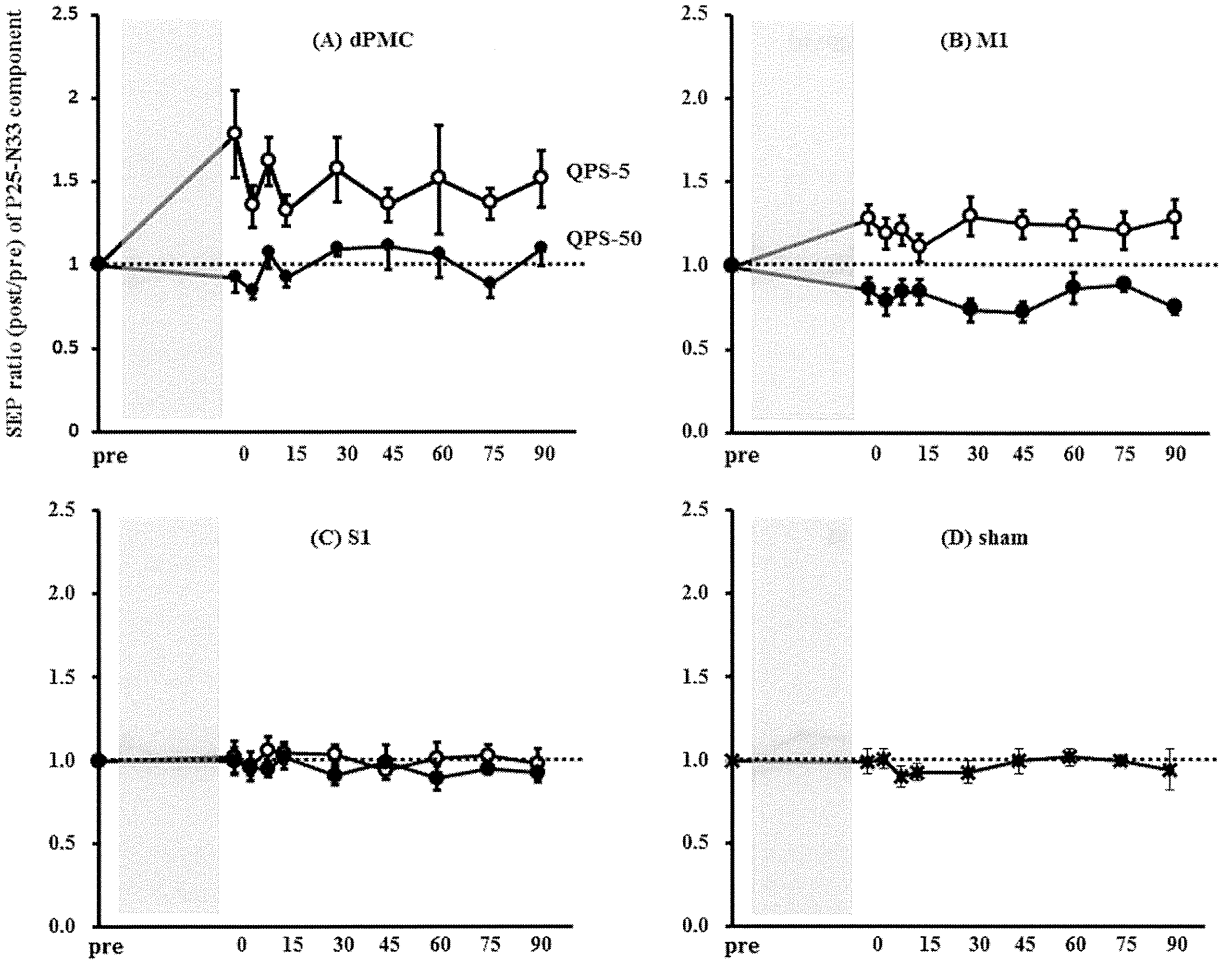

Fig. 2. Time courses of P25–N33 size ratio when QPSs were applied over dPMC (A), M1 (B), S1 (C) and in sham stimulation (D). SEP was recorded 10 times: before (T_{pre}) QPS, immediately after (T_0) and 5, 10, 15, 30, 45, 60, 75, 90 min (T_5, T_{10}, T_{15}, T_{30}, T_{45}, T_{60}, T_{75}, T_{90}) after QPS. The ordinate indicates the size ratio (post/pre). The abscissa is the time line. QPSs were applied in the grey time zone. Three-way ANOVA (AREA × ISI × TIME) revealed significant main effects of AREA ($F_{3,666} = 54.75$, $p < 0.001$) and ISI ($F_{1,666} = 183.84$, $p < 0.001$). AREA and ISI showed significant interaction ($F_{3,666} = 49.87$, $p < 0.001$), and post hoc pairwise comparisons against the sham condition showed significant SEP potentiation when QPS-5 was given over dPMC or M1 ($p < 0.001$). The potentiation effect over dPMC was larger than over M1 ($p < 0.001$). The P25–N33 component was significantly depressed by QPS-50 over M1 ($p < 0.001$). The main effect of TIME was not significant ($F_{8,666} = 1.21$, $p = 0.289$). Interactions between ISI × TIME ($F_{8,666} = 0.45$, $p = 0.890$), AREA × TIME ($F_{24,666} = 0.79$, $p = 0.747$), and AREA × ISI × TIME ($F_{24,666} = 0.73$, $p = 0.819$) were not significant.

and ISI ($F_{1,666} = 183.84$, $p < 0.001$). Post-hoc tests for AREA revealed that SEP amplitude in the dPMC condition was significantly larger than the other three conditions (M1, S1, sham; $p < 0.001$). As for ISI, the SEP amplitude was larger after QPS-5 ms than QPS-50. AREA and ISI showed significant interaction ($F_{3,666} = 49.87$, $p < 0.001$), and post hoc pair-wise comparisons against the sham condition showed significant SEP potentiation when QPS-5 was given over dPMC or M1 ($p < 0.001$). The potentiation effect over dPMC was larger than over M1 ($p < 0.001$). The P25–N33 component was significantly depressed by QPS-50 over M1 ($p < 0.001$). The main effect of TIME was not significant ($F_{8,666} = 1.21$, $p = 0.289$), indicating that SEP after QPS was stable during the observed period. Interactions between ISI × TIME ($F_{8,666} = 0.45$, $p = 0.890$), AREA × TIME ($F_{24,666} = 0.79$, $p = 0.747$) and AREA × ISI × TIME ($F_{24,666} = 0.73$, $p = 0.819$) were not significant.

The earlier SEP components were unaffected by QPSs significantly (N20o–N20p, Fig. 3; N20p–P25, Fig. 4). Three-way ANOVA of the N20o–N20p component showed a significant main effect of AREA ($F_{3,666} = 2.95$, $p = 0.032$) and a significant interaction between AREA × ISI ($F_{3,666} = 13.52$, $p < 0.001$). However, post hoc tests showed that QPS caused no significant SEP changes in any of the tested conditions with reference to the sham condition ($p > 0.1$). The N20p–P25 component showed significant main effects of AREA ($F_{3,666} = 3.75$, $p = 0.011$) and ISI ($F_{1,666} = 17.35$, $p < 0.001$). The interaction between AREA × ISI was also significant ($F_{3,666} = 5.9$, $p < 0.001$). However, post hoc pair-wise comparisons against the sham condition revealed no significant SEP changes in any of the QPS conditions ($p > 0.1$).

4. Discussion

Previous reports showed aftereffects of QPSs on M1 when it was applied over M1 itself (homotopic effects) (Hamada et al., 2007a,b, 2008a,b). In this study, QPS induced heterotopic effects on S1 when it was applied over M1, which was consistent with previous reports (Enomoto et al., 2001; Ishikawa et al., 2007; Kodama et al., 2009). QPS applied over dPMC also had some long-lasting effects on S1. However, it was not affected by QPSs over S1 itself.

4.1. QPS over M1

In this research, SEP components were modulated bidirectionally by QPSs over M1, that is, SEP was potentiated by QPS-5 and depressed by QPS-50. Only the P25–N33 component was influenced by QPSs, but N20o–N20p and N20p–P25 were not affected. Lack of significant interaction between AREA × TIME and ISI × TIME of P25–N33 indicate that aftereffects of QPS began immediately after

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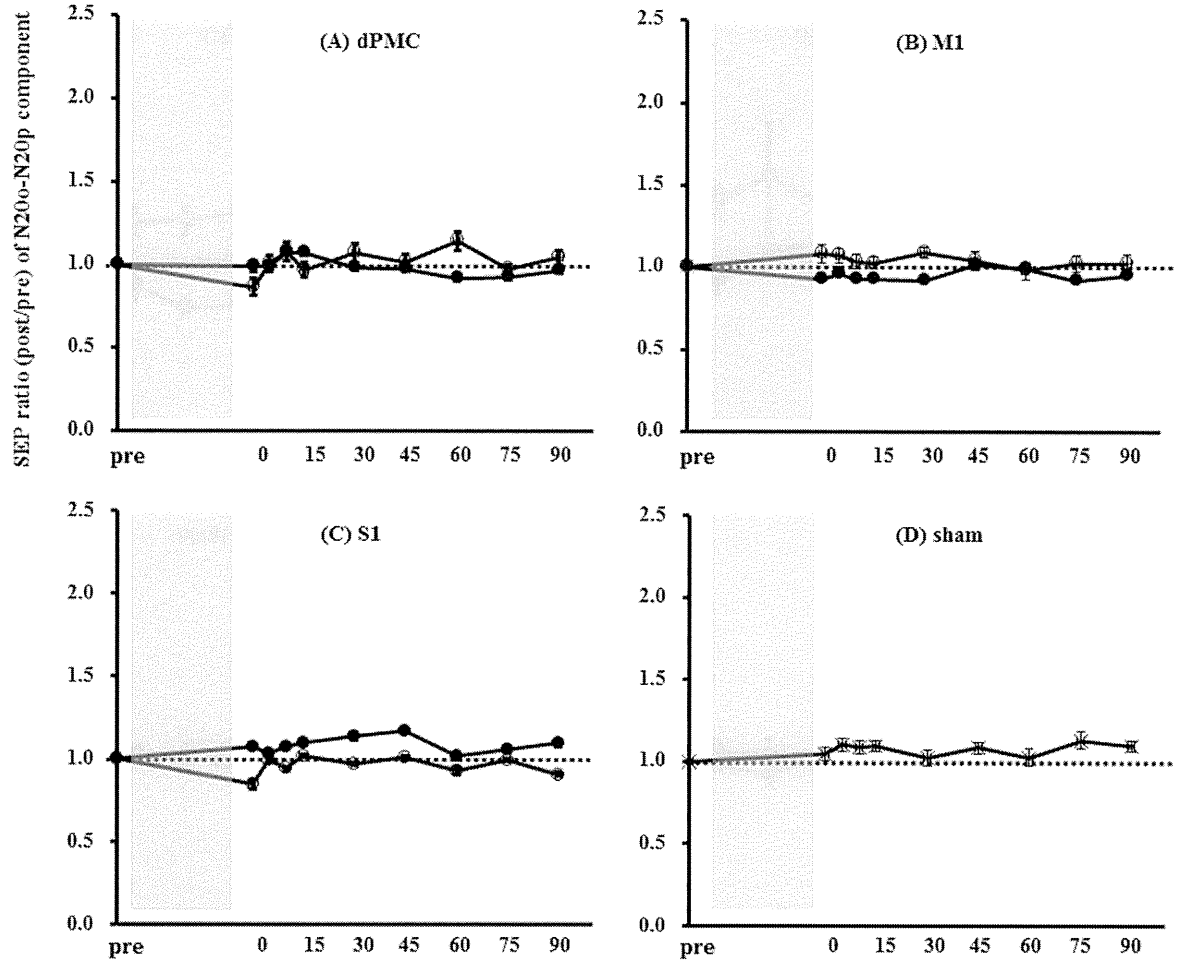

Fig. 3. Time courses of N20o–N20p size ratio when QPSs were applied over dPMC (A), M1 (B), S1 (C) and in sham stimulation (D). Three-way ANOVA of N20o–N20p component showed a significant main effect of AREA ($F_{3,666} = 2.95$, $p = 0.032$) and a significant interaction between AREA × ISI ($F_{3,666} = 13.52$, $p < 0.001$). However, post hoc tests showed that QPS caused no significant SEP changes in any of the tested conditions with reference to the sham condition ($p > 0.1$).

the end of QPS and lasted for 90 min stably. The effect of QPS was likely to fade out if we continued to observe for a much longer period.

The P25–N33 change without altered N20p–P25 suggested that potentiation and depression occurred at the sensory cortex. However, we cannot completely exclude a possibility of subcortical component changes with our present results because we monitored no subcortical components. Considering these factors, we suppose that QPSs affected the sensory cortical excitability even though some subcortical changes might contribute to our findings partly. The modulation by QPSs could be generated by LTP- and LTD-like mechanisms. In previous reports, low-frequency (1-Hz) rTMS over M1 reduced the amplitude of the P25–N33 component (Enomoto et al., 2001) and depressed motor cortical excitability (Chen et al., 1997). These physiological characteristics are compatible with LTP and LTD. This heterotopic effect on S1 from M1 may be produced by a tight cortico-cortical connection between M1 and S1 (Enomoto et al., 2001). SEP size changes in amyotrophic lateral sclerosis patients also support the idea of tight connection between these two cortical areas. SEP amplitudes were increased when weakness was moderate, but decreased when it was severe (Hamada et al., 2007b). M1 might bidirectionally modify the sensory cortical function through cortico-cortical connection when needed.

4.2. QPS over dPMC

Previous reports showed no significant effect of conventional rTMS applied over PMC on SEPs (Enomoto et al., 2001; Urushihara et al., 2006; Hosono et al., 2008). In this research, QPS-5 over dPMC potentiated SEP more powerfully than that over M1. However, QPS-50 over dPMC had no effects on SEP. What mechanism can explain these results? Current spread to M1 may not explain the results because aftereffects were unidirectional, that is, only a facilitatory effect was observed in QPS over dPMC, but bidirectional in QPS over M1. Furthermore, QPS-5 over dPMC potentiated SEP stronger than that over M1. A previous combination study of TMS and positron emission tomography indicated that low-frequency rTMS over dPMC affected larger areas including parietal and prefrontal areas compared with rTMS over M1 (Philippe et al., 2003). Another study showed that rTMS over dPMC and M1 induced illusory movement sensation (Christensen et al., 2010). These influences on the sensory function by dPMC stimulation may be produced by the same mechanisms for our present results.

Which pathways mediate this influence from dPMC to S1? One candidate is the association between these cortices through the cortico–thalamo–cortical pathway (Cappe et al., 2009), and the other is a controlling S1 by dPMC via M1 (Philippe et al., 2003).

4.3. QPS over S1

Interestingly, SEP did not change after QPS over S1 in this study. Previous studies of rTMS over S1 had no or limited effects on conventional SEP (Enomoto et al., 2001; Satow et al., 2003), which was consistent with the present results. In another study, however, the paired-pulse SEP technique showed homotopic S1 modulation after rTMS over S1 (Ragert et al., 2004). The paired-pulse SEP might

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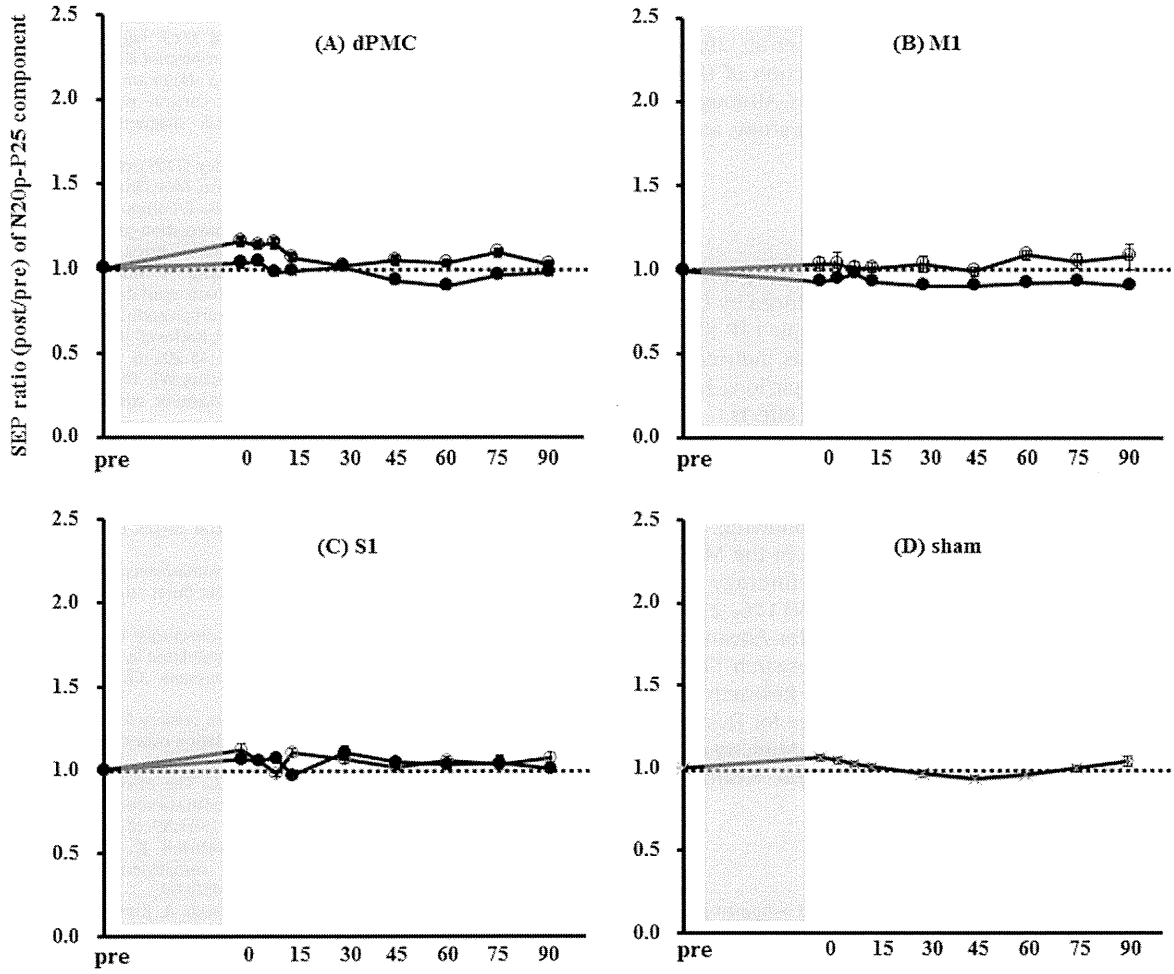

Fig. 4. Time courses of N20p–P25 size ratio when QPSs were applied over dPMC (A), M1 (B), S1 (C) and in sham stimulation (D). Three-way ANOVA of N20p–P25 component showed significant main effects of AREA ($F_{3,666} = 3.75$, $p = 0.011$) and ISI ($F_{1,666} = 17.35$, $p < 0.001$). The interaction between AREA × ISI was also significant ($F_{3,666} = 5.9$, $p < 0.001$). However, post hoc pairwise comparisons against the sham condition revealed no significant SEP changes in any of the QPS conditions ($p > 0.1$).

show small changes after QPS. Unfortunately, however, we did not perform paired-pulse SEPs. Direct current stimulation altered SEP components when it was applied over the sensorimotor cortex (Matsunaga et al., 2004; Dieckhöfer et al., 2006). These inconsistent results may be explained by the difference in size of activated area between rTMS and tDCS. tDCS may affect more broad areas than rTMS or TBS. tDCS over S1 may activate M1 or premotor cortex in addition to S1. Activation of M1 or premotor cortex may contaminate the sensory cortical homotopic effects in tDCS over S1. This contamination of motor-related cortical area activation must explain some homotopic effects by tDCS over S1. TBS over S1 also affected SEP components (Ragert et al., 2004; Ishikawa et al., 2007; Katayama and Rothwell, 2007; Katayama et al., 2010). We have no good explanation for the difference between QPS and TBS. The homotopic effects on S1 may be stimulation method dependent, namely it is induced by TBS but not by QPS, for some reason.

In any case, the present study shows the conspicuous finding that SEP is heterotopically modulated by QPS over M1 or dPMC but not homotopically by QPS over S1. The lack of homotopic effects is partly expected. The giant SEP was produced by motor cortical stimulation, but not by sensory cortical stimulation (Kujirai et al., 1993). Motor cortical stimulation reduced pain in patients with intractable pain, but that over S1 had no effect (Tamura et al., 2004; Saitoh et al., 2006; Hirayama et al., 2006; Kodama et al., 2009). Another explanation for the lack of SEP changes by S1 QPS may be as follows. Some sensory cortical plastic change is actually induced by QPS over S1, and it is undetectable by SEPs but detectable by other methods of sensory functional analyses, such as tactile perception, two-point discrimination test or sensory modality detection test.

4.4. Affected SEP components by QPS

QPSs over M1 and dPMC affected only P25–N33 in this study, but had no effect on N20o–N20p and N20p–P25 components. As mentioned above, SEP size changes must be explained by sensory cortical potentiation or depression. The straightforward interpretation of our results is that LTP/LTD-like effects may occur at the levels of origins of P25 or N33 components. The N20 and N20–P25 components are generated by area 3b. The P25–N33 component is generated by area 3b and area 1, but area 1 is the most probable candidate (Allison et al., 1991). Projections from motor to sensory cortex are mainly to areas 1 and 2 (Jones et al., 1978; Ishikawa et al., 2007). Mutual connection between motor and sensory cortices through these connections can explain the phenomenon that the P25–N33 component was affected by QPS over M1 or dPMC.

4.5. Age dependency

Subjects' age was considerably restricted between 32–55 years in this research. Paired-pulse suppression was reduced in aged rat neurons (David-Jürgens and Dinse, 2010). The age dependence

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of neuroplasticity has been shown by some investigators using various stimulation methods in humans (Tecchio et al., 2008; Fathi et al., 2010; Todd et al., 2010). The age restriction of this study has some limitation to reach definite conclusions. Although this issue is interesting, it is out of scope of the present study, and will be solved by future studies.

5. Conclusion

This is the first report of QPS effects on the sensory cortex in humans. A heterotopic LTP/LTD-like effect was induced by QPSs over M1. QPS over dPMC also induced the heterotopic LTP-like effect. However, no homotopic LTP/LTD aftereffect was induced by QPS over S1. QPS is applicable for studying heterotopic long-lasting effects in the human brain, as well as homotopic effects.

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LETTER TO THE EDITOR

Some evidence supporting the safety of quadripulse stimulation (QPS)

To the Editor: Quadripulse transcranial magnetic stimulation (QPS) is a newly designed patterned repetitive transcranial magnetic stimulation (rTMS), which induces bidirectional, long-lasting after effects on the human motor cortex. Although QPS is a powerful tool for neurophysiologic research, there are concerns about its possible adverse effects, including induction of seizure and EEG abnormalities. In the original reports, the occurrence rates of after discharges were not significantly different between QPS and sham stimulation, and no spread of excitation was observed.[1,2] Although these studies tentatively showed the safety of QPS, further investigation is needed. In this communication, we provide further evidence of the safety of QPS comparing the vital signs, neurologic status, serum prolactin (PRL) levels, and EEGs before and after QPS.

We used eight healthy subjects aged 31 to 54 years (mean, 40.5 years) without any neurologic disorders. Before QPS, we determined the active motor threshold (AMT) of the right first dorsal interosseus (FDI) muscle by recording motor evoked potentials (MEPs; sampling rate of 10 kHz, high pass 100 Hz, low pass 3 kHz; Neuropack μ, Nihon-Kohden, Japan). TMS was given over the hot spot of FDI by a figure-of-eight coil connected to MagStim 200^2 (The MagStim Co Ltd, UK). We used QPS protocols of successive four monophasic pulses delivered with interstimulus intervals of 5 milliseconds (QPS-5) or 50 milliseconds (QPS-50). One experimental session consisted of 360 trains of four pulses (1440 pulses in total) at the intensity of 90% AMT.[1,2] Intertrain intervals were fixed at 5 seconds. QPS-5 and QPS-50 were used because previous studies reported that these protocols induced powerful potentiation and depression on the motor cortex, respectively.[1,2]

We measured vital signs in supine position for three times: before (T_{pre}), immediately after (T_0), and 180 seconds after (T_{180}) QPS. We also checked emotional state, headache, visual abnormality, dizziness, subjective hearing loss, tinnitus, aural fullness, weakness, paresthesia, and gait instability. We collected venous blood samples three times: before (T_{pre}), immediately after (T_0), and 10 minutes after (T_{10}) QPS. Because all subjects were awake from 2 hours before and throughout the experiment, the serum PRL was not affected by the sleep.[3] Serum PRL was measured with a chemiluminescence immunoassay method. Conventional EEG was recorded using a digital polygraph recording system. Ag-AgCl electrodes were attached to the scalp with conductive paste at 16 positions according to the international 10-20 system (sampling rate, 1 kHz; time constant, 0.3; low pass filter 100 Hz). EEG data were preprocessed to exclude segments with artefacts. Residual parts were divided into epochs of approximately 4 seconds (2048 points). These epochs were filtered digitally into five frequency bands from δ to γ. Approximately 100 epochs were analyzed per record.

To statistically test the effects of QPS on the blood pressure (BP), heart rate (HR), and PRL, we used two-way repeated measures analysis of variance (ANOVA) with factors of QPS-TYPE (QPS-5, QPS-50) and TIME (pre, T_0, T_{180} for BP and HR; pre, T_0, T_{10} for PRL). To examine the effects of QPS on EEG power-spectral densities, we conducted three-way ANOVA with factors of QPS-TYPE (QPS-5, QPS-50), TIMING (pre-QPS, post-QPS), and SIDE (C3, C4).

No subjects showed any emotional or neurologic symptoms during or after QPS. Hearing side effects as well as subjective symptoms were not observed. Figure 1A–C shows mean ± standard errors (SE) of systolic BP (sBP) (A), diastolic BP (dBP) (B), and HR (C) before and after QPS-5 (upper) or QPS-50 (lower). QPS-TYPE and TIME did not have significant main effects or interaction on sBP, dBP, or HR. Figure 1D shows PRL levels (mean ± SE) before and after QPS-5 (upper) or QPS-50 (lower). Neither QPS-TYPE nor TIME had significant main effect or interaction on PRL level ($P > 0.4$). Figure 1E shows percent EEG power (mean ± SE) at each frequency band recorded at C3 and C4 (filled bars, pre-QPS; bars, post-QPS). Three-ways ANOVA showed no significant main effect of QPS-TYPE, TIMING, or SIDE on any frequency band (δ, θ, α, β, γ, $P > 0.4$). None of the interactions were significant (TIMING × SIDE, TIMING × QPS-TYPE, TIMING × QPS-TYPE, SIDE × QPS-TYPE, TIMING × QPS-TYPE × SIDE, $P > 0.1$).

In summary, none of our subjects had subjective complaints as well as physical and neurologic changes.

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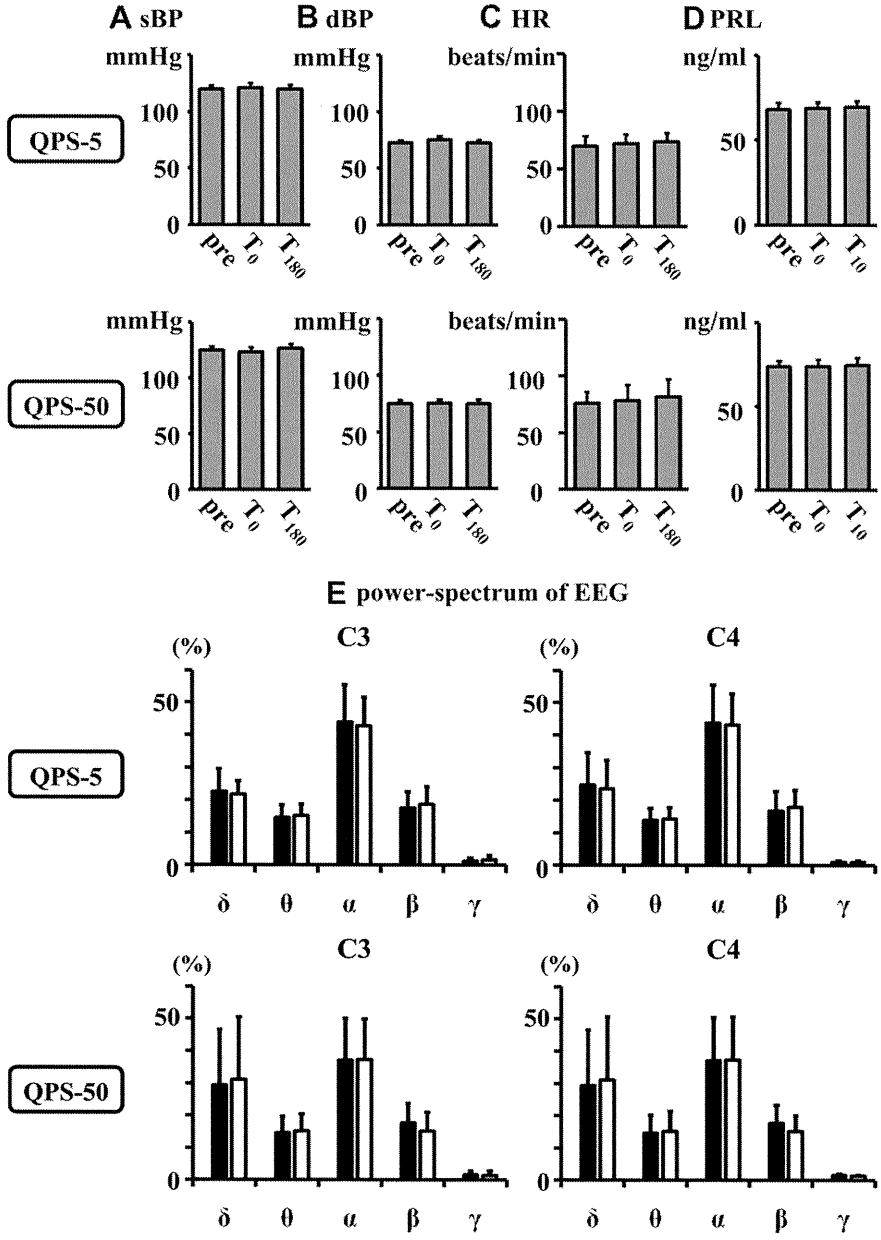

Figure 1 (**A–C**) shows mean ± SE of systolic blood pressure (**A**), diastolic blood pressure (**B**) and heart rate (**C**) before (pre), right after (T0) and 3 minutes after (T180) QPS-5 or QPS-50. There were not significant changes after QPSs in any physical parameters. Figure 1**D** shows mean ± SE of serum prolactin level before (pre), right after (T0) and 10 minutes after (T10) QPS-5 or QPS-50. They did not differ significantly. Figure 1E shows power spectrum of electroencephalogram recorded at C3 and C4 (filled bars, pre-QPS; bars, post-QPS). No any percent EEG powers were affected by QPS-5 or QPS-50.

QPS did not alter serum PRL levels or EEG frequency patterns. The results support the safety of QPS applied on the motor cortex of normal subjects with our recommended stimulation parameters.

High frequency rTMS has 1.4% crude risk estimate in epileptic patients and less than 1% in normal subjects.[4] Furthermore, in previous reports, high-frequency rTMS using suprathreshold pulses affected BP, HR,[5] or EEG.[6,7] One of 9 normal subjects experienced seizure with increases of serum PRL level after rTMS when the stimulus intensity was extremely high.[6] The lack of these changes in the present experiment may be explained by the subthreshold stimulus intensity (90% AMT) that we used.

There are a few limitations in the current study. First, we tested the null hypothesis that QPS has no effect on our measurements with a relatively small sampling population. Although the probability of incorrectly accepting the null hypothesis was kept below 5%, the power to reject the null hypothesis will increase with more subjects examined. For example, Griškova et al.[7] examined 18 subjects and found changes of EEG power in delta band with 10 Hz rTMS. Assuming the similar effect size as in the study by

Griškova et al.,[7] the power to reject the null hypothesis would increase from 63% to 97% by increasing the subject size from 8 to 18. Thus, the safety of QPS needs to be confirmed with a study of larger number of subjects. Second, the safety of QPS on pathologic brain remains uncertain. Even with these limitations, we can conclude that our current results partly support the safety of QPS. As suggested by the safety guideline, it is advisable that licensed physician supervise the experiments with careful monitoring and life-support system during QPS experiments.[4]

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RESEARCH ARTICLE

Triad Stimulation Frequency for Cortical Facilitation in Cortical Myoclonus

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ABSTRACT: Background: Abnormally enhanced cortical rhythmic activities have been reported in patients with cortical myoclonus. We recently reported a new triad-conditioning transcranial magnetic stimulation (TMS) method to detect the intrinsic rhythms of the primary motor cortex (M1). Triad-conditioning TMS revealed a 40-Hz intrinsic rhythm of M1 in normal subjects. In this investigation, we study the motor cortical facilitation induced by rhythmic triple TMS pulses (triad-conditioning TMS) in patients with cortical myoclonus.

Methods: Subjects were 7 patients with cortical myoclonus (28–74 years old) and 13 healthy volunteers (30–71 years old). Three conditioning stimuli over M1 at the intensity of 110% active motor threshold preceded the test TMS at various interstimulus intervals corresponding to 10–200 Hz. The resulting amplitudes of conditioned motor evoked potentials recorded from the contralateral hand muscle were compared with those evoked by the test stimulus alone.

Results: The facilitation at 25 ms (40 Hz) observed in normal subjects was absent in patients with cortical myoclonus. Instead, triad-conditioning TMS induced facilitation at a 40 ms interval (25 Hz) in cortical myoclonus.

Discussions: This change in the timing of facilitation may be explained by a shift of the most preferential intrinsic rhythm of M1, or by some dysfunction in the interneuronal network in cortical myoclonus. © 2011 *Movement* Disorder Society

Key Words: transcranial magnetic stimulation; motor evoked potentials; cortical intrinsic rhythm; gamma band; beta band

Cortical myoclonus is a brief muscle jerk generated by abnormal activation of the sensorimotor cortex. Some patients with cortical myoclonus show rhythmic electromyographic (EMG) bursts. These rhythmic myoclonus jerks consist of EMG bursts with frequencies of 10,[1] 20,[2] or 50 Hz.[3,4] Jerk-locked back averaging (JLA)[5,6] and electroencephalography (EEG)-EMG coherence analyses[4,7] have revealed the same rhythmic cortical activities. Brown et al. (1999)[4] considered that the cortical rhythmic activities at frequencies of around 20, 40, and up to 175 Hz play important roles in the generation of cortical myoclonus. Of all these frequencies, 20 Hz has been considered especially important in cortical myoclonus.[2,8–10] However, the physiological significance of 20 Hz is still unclear. We have recently shown the presence of a 40-Hz intrinsic motor cortical rhythm in normal subjects using a newly developed triad-conditioning transcranial magnetic stimulation (TMS) method.[11] In this article, we applied this triad-conditioning TMS method to patients with cortical myoclonus.

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TABLE 1. Patients' data

No.	Gender	Age	Diagnosis	AED
1	M	74	Benign myoclonus epilepsy	Phenytoin sodium
2	M	64	Benign myoclonus epilepsy	Clonazepam, valproic acid, phenytoin sodium
3	M	42	Benign myoclonus epilepsy	Clonazepam, valproic acid, primidone
4	F	59	Benign myoclonus epilepsy	Valproic acid
5	F	51	Benign myoclonus epilepsy	Clonazepam
6	F	51	Benign myoclonus epilepsy	Phenytoin sodium
7	M	28	Autoimmune-mediated encephalopathy	Carbamazepine, valproic acid

M: male, F: female, AED: antiepileptic drug.

Methods

Subjects

Seven patients with myoclonus epilepsy (4 men and 3 women; mean age 56.8 ± 11.3 years; age range 28–74 years; Table 1) and 13 age-matched healthy volunteers (7 men and 6 women, mean age 49.2 ± 13.6 years; age range 32–75 years) participated in this study. Six patients were diagnosed with benign myoclonus epilepsy because lysosomal enzyme activities, analyses of mitochondrial genes, and brain MRI were all normal. Another patient had autoimmune encephalitis. All the patients were on some antiepileptic drugs (AEDs: clonazepam, valproic acid, phenytoin sodium, phenobarbital, and primidon) during our study. The diagnoses of cortical myoclonus were made based on clinical features and electrophysiological studies. All patients had spontaneous, reflex, and action myoclonus in their hands, but none of them had rhythmic myoclonus. They all had paroxysmal abnormal EEG discharges and abnormally enhanced median nerve somatosensory evoked potentials (SEPs) (giant SEPs: the amplitude of P25–N33 was 17.9–37.5 μV) with enhanced long-loop reflexes. No patients in the present study were participants in our previous study.[12] Healthy volunteers had neither neurological disorders nor episodes of seizure. Written informed consent to participate in this study was obtained from all the subjects. The experiments were performed according to the Declaration of Helsinki; the procedures were approved by the Ethics Committee of the University of Tokyo. No adverse effects were noted in any individuals.

Electromyographic Recordings

Surface EMG signals were recorded from the right first dorsal interosseous muscle using 9-mm-diameter Ag-AgCl surface cup electrodes. The active electrode was placed over the muscle belly and the reference electrode over the metacarpophalangeal joint of the index finger. Responses were amplified (Biotop; GE Marquette Medical Systems Japan, Japan) through filters set at 100–3 kHz, digitized at a sampling rate of 20 kHz, and stored on a computer (TMS bistim tester; Medical Try System, Japan) with which we performed a randomized conditioning test paradigm and off-line averaging. Because muscle relaxation was very important in this experiment, EMG activities were monitored on an oscilloscope during the experiments. Subjects kept the right first dorsal interosseous muscle relaxed throughout the experiments by monitoring EMG activity on the oscilloscope. Trials in which EMG activity appeared during data collection were not used in the analysis.

Transcranial Magnetic Stimulation

Four Magstim 200[2] magnetic stimulators (The Magstim Company, UK) were used to deliver TMS. We placed a figure-8-shaped coil (7-cm external diameter at each wing; The Magstim Company, UK) over the primary hand motor area (M1) of the left hemisphere. Induced currents in the brain flowed in the posterior to anterior direction. To determine the hot spot for the first dorsal interosseous muscle in each subject, we changed the stimulation site in 1-cm steps starting at a point 5 cm lateral to the vertex and determined the location at which the largest responses were elicited by the same intensity stimulation. This position was marked on the scalp using a red pen to guide repositioning of the coil throughout the experiments. Outputs from four magnetic stimulators were connected with a special device (Combine module, The Magstim Company, UK) that enabled us to deliver four monophasic pulses through the same coil.

We determined the threshold for evoking EMG activities in the active target muscle (active motor threshold: AMT) when the subject contracted the target muscle at 5–10% of maximum contraction. The stimulation intensity was changed in steps of 1% of the maximum stimulator output until we determined the lowest intensity that evoked a small response at the amplitude of 200 μV, compared with the prestimulus background activity in half of the trials.

Short Interval Intracortical Inhibition and Intracortical Facilitation

To evaluate the cortical excitability changes, we studied the short interval intracortical inhibition (SICI)

and intracortical facilitation (ICF) using the paired pulse TMS method first reported by Kujirai et al.[13] In the SICI experiment, we used the conditioning stimulus at 90% AMT and interstimulus intervals (ISIs) of 3, 4, and 5 ms. We set the conditioning stimulus at 110% AMT and used ISIs of 7, 8, and 10 ms in the ICF experiment.

Triad-Conditioning TMS

We used the same method as has been reported previously.[11] Here, we describe it briefly. Three subthreshold conditioning TMS pulses were set at 110% AMT. The test stimulus was set to elicit an MEP as large as 0.3 mV in the relaxed muscle when given alone. The final pulse of the conditioning triad preceded the test pulse by the same interval as that between the conditioning pulses. The ISIs between the pulses were 5, 7, 8, 10, 15, 20, 25, 30, 40, 50, and 100 ms (corresponding, respectively, to 200, 143, 125, 100, 66, 50, 40, 33, 25, 20, and 10 Hz). We used a randomized conditioning test paradigm. In one session, several conditioned trials in which a triad of conditioning pulses with several ISIs preceded the test stimulus were intermixed randomly with control trials in which the test stimulus was given alone. The intertrial interval was set at 12–15 s. Two blocks of trials were performed to investigate all intervals. In the first block, the ISIs were 5, 7, 8, and 10 ms; in the second block, the ISIs were 15–100 ms. Ten responses were collected and averaged for each condition in which four stimuli were given; 20 responses were collected for the control condition. For each subject, we calculated the ratio of the mean amplitude of the conditioned response to that of the control response at each ISI. The time course of the effect of the conditioning triad was plotted with this ratio on the ordinate and the ISI on the abscissa.

Single Pulse Conditioning TMS

To study whether a single-pulse conditioning stimulus is able to elicit the same effect evoked by the triad-conditioning stimulus, we performed a single-pulse conditioning TMS experiment in 6 patients, in which only a single TMS pulse was given instead of triad conditioning. The intensity of the conditioning stimulus was fixed at 110% AMT.

Statistical Analysis

Statistical analyses were performed using SPSS v. 14.0 for Windows (SPSS, Chicago, USA). To compare the effects of triad-conditioned MEPs between patients with cortical myoclonus and healthy volunteers, we used two-way repeated measures analysis of variance (ANOVA) for two experimental blocks separately (first block: 5–10 ms; second block: 15–100 ms) [Group (myoclonus and normal); ISI (control, ISIs of 5, 7, 8, and 10 ms or control and ISIs of 15, 20, 25, 30, 40, 50, and 100 ms)].

The dependent variable was the MEP size. When necessary, the Greenhouse-Geisser correction was used to correct for nonsphericity. Tukey's test was used for multiple comparisons in post hoc analyses; *P*-values less than 0.05 were considered significant. If there was significant interaction between two factors, we used a paired *t*-test to compare MEP sizes at two intervals judged as having some physiological meaning (ISIs of 25 or 40 ms).

To compare the amount of SICI or ICF between patients with myoclonus and healthy volunteers, we used two-way repeated measures ANOVA [Group (myoclonus and normal); ISI: 3, 4 and 5 ms for SICI and 7, 8, and 10 ms for ICF]. The dependent variable was the MEP size ratio.We compared the MEP size ratios between the triad and single-pulse conditioning stimulus experiments at ISI of 40 ms using a paired *t*-test.

Data are described as mean ± standard error (SE) in the following presentations, unless otherwise indicated.

Results

The mean AMT was 35.8 ± 3.36% (SE) in cortical myoclonus, which was not significantly different from that of healthy volunteers (37.2 ± 1.75%). The amount of SICI[12] was abnormally reduced in all of them and significantly different from that of healthy volunteers [Group F (1, 48) =12.5 $P < 0.05$; ISI F (2, 48) = 5.25, $P < 0.05$; ISI × Group F(2, 48) = 3.43, $P < 0.05$]. The mean average size ratio (3–5 ms) was 1.03 ± 0.14 in cortical myoclonus patients and 0.59 ± 0.06 in healthy volunteers. There were no significant differences in the ICF between cortical myoclonus patients and healthy volunteers [Group F (1, 32) = 2.35, $P > 0.05$; ISI F (2, 32) = 0.729, $P > 0.05$; ISI × Group F (2, 32) = 0.184, $P > 0.05$]. The mean average-size-ratio (7–10 ms) was 1.54 ± 0.45 in cortical myoclonus patients and 1.29 ± 0.13 in healthy volunteers.

Figure 1 depicts how the MEP amplitude to a test TMS was modulated by triad-conditioning stimuli in patients with cortical myoclonus and healthy volunteers.

For the first interval block of 5–10 ms, two-way repeated measures ANOVA revealed a significant effect of ISI, [ISI 5–10 ms ISI – F (4, 72) = 8.62, $P < 0.01$] but no interaction between the subject group and ISI [(ISI × Group)—ISI 5–10 ms F (4, 72) = 0.961]. For the second interval block of 15–100 ms, two factor ANOVA revealed a significant interaction between ISI and group (F (7, 126) = 4.995, $P < 0.01$] and a significant effect of ISI [F (7, 126) = 2.203, $P < 0.05$]. At ISI of 5–10 ms, MEPs were significantly

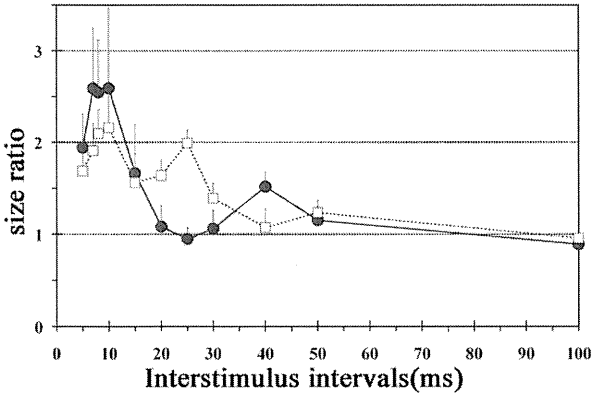

FIG. 1. Time courses of the size ratio of conditioned MEP to control MEP for the triad-conditioned TMS experiment in normal subjects (squares) and patients with cortical myoclonus (dots). The ordinate displays the size ratio of conditioned MEP to control MEP; the abscissa shows the ISIs between successive pulses. Two peaks demonstrate facilitation at ~ 7–8 ms and at 25 ms in healthy volunteers. In patients with cortical myoclonus, the facilitation was absent at 25 ms but present at 40 ms.

larger than control MEPs in both subject groups. Significant enlargement was seen at 20 and 25 ms in healthy volunteers but not in cortical myoclonus patients. In contrast, MEP enlargement was seen at 40 ms ($P < 0.05$) in cortical myoclonus.

To confirm the necessity of triad-conditioning stimulus for inducing the facilitation, we compared the facilitation at a 40-ms interval between the triad and single-pulse conditioning stimulus experiments (Fig. 2). The size ratio at an ISI of 40 ms was significantly larger in the triad-conditioning experiment than in the single-pulse experiment. Significant facilitation was evoked by the triad-conditioning stimulus but not by the single-pulse conditioning stimulus.

Discussion

The triad-conditioning stimulus facilitated test MEPs at two intervals (7–10 ms and 25 ms) in healthy volunteers, as shown in our previous report.[11] In cortical myoclonus, the early peak at ISIs of 7–10 ms was normally present, but the later facilitation at 25 ms was absent. Instead, MEP was significantly enlarged at an ISI of 40 ms in patients with cortical myoclonus.

All patients took their daily AED regimens during this study. AEDs must alter cortical excitability and may affect the amount of inhibition or facilitation studied by TMS. In fact, many articles have shown that AEDs reduce motor cortical excitability or enhance motor cortical inhibition,[14–17] but none reported any enhancement of the motor cortical excitability. We therefore consider that AEDs may explain neither the facilitation at an ISI of 40 ms shown by triad-conditioning TMS nor the reduced SICI in cortical myoclonus.

Reduction of SICI in Cortical Myoclonus

Consistent with previous reports,[18,19] the amount of SICI was smaller in patients with cortical myoclonus than in normal subjects. The reduction of SICI is most likely due to dysfunction of the inhibitory interneuronal network because pathological studies have revealed involvement of GABAergic interneurons of the motor cortex.[20] We used ISIs of 3–5 ms to study SICI based on our previous report.[21] Peurala et al.[22] suggested that short interval intracortical facilitation could overlap with SICI at these ISIs and may affect the results of SICI experiment. However, this overlap is usually seen when using a stronger conditioning stimulus (greater than 95% AMT).[22] Based on these arguments, we consider that this possibility less likely explains the reduced SICI because lower conditioning stimuli were used in the present experiments.

Facilitation at 7–8 ms

We[11] have previously proposed that the facilitation at 7–10 ms is produced by the same mechanism as the intracortical facilitation (ICF) in paired pulse magnetic stimulation.[23,24] This 7–10 ms facilitation is unrelated to rhythmic activities because even a single conditioning stimulus can evoke this facilitation.[11] In patients with cortical myoclonus, both the ICF and the 7–8 ms facilitation in the triad-conditioning stimulus experiment were normally elicited. This is consistent with normal ICF in cortical myoclonus reported previously.[24]

Facilitation at 40 ms without 25 ms Facilitation in Cortical Myoclonus

Instead of facilitation at 25 ms (40 Hz), the triad-conditioning stimulus elicited facilitation at 40 ms (25 Hz) in cortical myoclonus. Several mechanisms

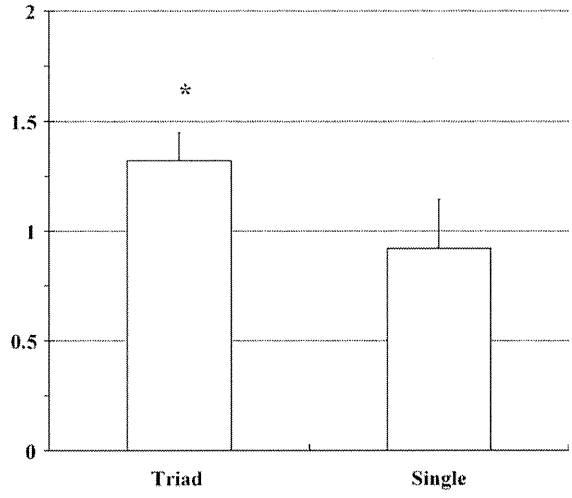

FIG. 2. Comparison of MEP size ratio at the 40-ms interval between triad and single-pulse conditioning stimulus experiments. The ratio in the triad-conditioning experiment differs significantly from that of the single-pulse conditioning experiment.

that may explain these findings correlate strongly with which mechanism produces the facilitation shown by triad conditioning experiments.

Following our previous hypothesis,[11] the motor cortical intrinsic rhythm and its changes may explain the present whole results. The rhythmic triad conditioning stimulus is originally considered to enhance a certain intrinsic rhythm generated by interneurons or some neuronal loops. The simple explanation for the present results directly following our hypothesis is that the normally undetectable 25 Hz intrinsic rhythm is enhanced and the normally present 40 Hz intrinsic rhythm is reduced in cortical myoclonus because several previous reports have revealed these rhythmic activities in cortical myoclonus.[2,6]

Another possibility is that the triad-conditioning TMS may change the frequency of the cortical intrinsic rhythm to a new rhythm synchronizing with the stimulation frequency. This rhythm after modulation by the triad conditioning stimulus (triad modulated rhythm) may be detected by our experiment. The triad modulated rhythm is 40 Hz in normal subjects and 25 Hz in cortical myoclonus. Even in this case, the motor cortex must tend to synchronize to 25 Hz for some reason. Several previous papers support this explanation of the intrinsic rhythm. Some patients with cortical myoclonus have rhythmic EEG activities at a frequency of 20 Hz coupled with myoclonic jerks.[2] In such patients, single conditioning stimuli of 100% RMT were able to induce facilitation at 50 ms. We also revealed 20 Hz oscillatory EEG potentials preceding the myoclonic jerk in jerk-locked averaging methods in patients with rhythmic myoclonus.[6] Some animal data also support this hypothesis. Animal studies have revealed that the beta rhythm (including 25 Hz) is produced in layer V of the cortex,[25] the gamma rhythm (including 40 Hz) originates from cortical layer II/III,[26,27] and both layers mutually modulate each other.[28] In cortical myoclonus, dysfunction of cortical inhibitory interneurons at layer II/III may reduce the gamma rhythm,[29] and the preferential rhythm may shift to the usually masked beta rhythm.

A nonintrinsic rhythm mechanism may explain our results. The motor cortical excitability is simply enhanced at 40 ms in cortical myoclonus, independent of changes in the intrinsic cortical rhythm. The triad-conditioning stimuli may be strong enough to induce facilitation at 40 ms even though a single stimulus is not strong enough. Some interneuronal dysfunction of the motor cortex may cause this facilitation.

Either or both of the above-mentioned intrinsic rhythm and nonintrinsic rhythm mechanisms may explain the lack of 25 ms facilitation and the occurrence of 40 ms facilitation. Whichever mechanism explains our results, we conclude that the motor cortex tends to be activated by multiple stimuli at a certain interval. Our new stimulation method must be useful to investigate some motor circuit abnormalities, including intrinsic rhythmic changes in humans. ■

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RESEARCH ARTICLE

On-line effects of quadripulse transcranial magnetic stimulation (QPS) on the contralateral hemisphere studied with somatosensory evoked potentials and near infrared spectroscopy

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Abstract To evaluate on-line effects of quadripulse stimulation (QPS) over the primary motor cortex (M1) on cortical areas in the contralateral hemisphere. QPS consisted of 24 bursts of transcranial magnetic stimulation (TMS) pulses with an inter-burst interval of 5 s for 2 min (for on-line effect study) or 360 bursts for 30 min (for after-effect study). Each burst consisted of four TMS pulses (i.e. QPS) separated by an interstimulus interval of 5 or 50 ms (QPS-5 or QPS-50). QPSs were delivered over the left M1. Experiment 1 [on-line effect on somatosensory evoked potential (SEP)]: Left median nerve SEPs were recorded before, during and after QPS. Experiment 2 (after effect on SEP): After-effects of QPS were evaluated by following up SEPs after the QPS sessions. Experiment 3 (on-line effect on NIRS): Near infrared spectroscopy (NIRS) was also recorded at the right hemisphere during all QPS paradigms. Both QPS-5 and QPS-50 enlarged a cortical component of the contralateral SEP during stimulation. On the other hand, concerning the after effects, QPS-5 over M1 potentiated the contralateral SEP and QPS-50 tended to depress it. In NIRS study, both QPS-5 and QPS-50 induced a significant oxy-Hb decrease (deactivation pattern) at the right hemisphere during stimulation whereas sham stimulations unaffected them. We have shown the unidirectional on-line effects evoked by QPS-5 and QPS-50 on both SEP and NIRS, and bidirectional after effects on SEP at the contralateral hemisphere. The discrepancy between on-line effect and after effect may be explained by the differences in the underlying mechanisms between them. The former may be mainly explained by pure electrophysiological property changes in the membrane or synapses. The latter may be explained by synaptic efficacy changes which need some protein syntheses at least partly. Another discrepancy shown here is the direction of on-line effects. Electrophysiological (SEP) function was potentiated by both QPSs whereas hemodynamic (NIRS) function was depressed. This may be explained by which sensory areas contribute to NIRS or SEP generation.

Keywords Near infrared spectroscopy · Optical recording · Interhemispheric connection · Transcranial magnetic stimulation · Somatosensory evoked potential

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Introduction

Repetitive transcranial magnetic simulation (rTMS) is one of the methods to modulate brain activity and sometimes give some benefits to patients with neurological or psychiatric diseases; Parkinson's disease, epilepsy, depression and so on (Epstein et al. 2007; Hamada et al. 2008b; Kimiskidis 2010). Regularly given, conventional high frequency rTMS (i.e. above 5 Hz) over the primary motor cortex (M1) usually potentiates M1 (Pascual-Leone et al. 1994), and low frequency rTMS (i.e. under 1 Hz) depresses it (Chen et al. 1997). These effects are often weak and highly variable from one individual to another (Maeda et al. 2000).

Several patterned rTMSs have been reported to have more powerful modulation effects than regularly given rTMS (Huang et al. 2005, 2009). In the theta burst stimulation (TBS), one of patterned rTMSs, bursts of three TMS pulses [50 Hz; inter-stimulus interval (ISI) = 20 ms] are delivered at 5 Hz in a few special patterns. Intermittent TBS potentiates the stimulated area (M1, somatosensory cortex or premotor area) whereas continuous TBS depresses it (Huang et al. 2005; Mochizuki et al. 2005; Ishikawa et al. 2007). In 2008, quadripulse stimulation (QPS), a newly developed patterned rTMS protocol was introduced to produce a broad range of motor cortical plasticity ranging from depression to potentiation depending on an interval of the TMS pulses (Hamada et al. 2008a). QPS at an interval of 5 ms (QPS-5) induced most powerful potentiation and QPS at 50 ms interval (QPS-50) most powerful depression.

The purpose of this study is to investigate whether or not the on-line effects of QPS-5 and QPS-50 are oppositely directed similarly to the after effects. We selected QPS-5 and QPS-50 here since they had the most prominent potentiation and depression after effects, respectively. We evaluated the on-line effect and after effect of QPS-5/QPS-50 over M1 on the contralateral somatosensory evoked potentials (SEPs).

Neuroimaging studies have been used to assess the impact of rTMS on the brain. Because of their temporal resolution, only a few methods are applicable to study the brain activity changes during or just after rTMS (Siebner et al. 2009). The on-line effects of single pulse TMS or rTMS were evaluated by functional magnetic resonance imaging method (Bestmann et al. 2008), electroencephalography (Ilmoniemi and Kičić 2010) or near-infrared spectroscopy (NIRS) (Noguchi et al. 2003; Mochizuki et al. 2006). NIRS recording estimates hemoglobin (Hb) concentration changes by measuring reflected light and is not interfered with magnetic fields associated with TMS. NIRS is one of noninvasive neuroimaging methods studying rTMS on-line effects but is not a suitable method for investigation of after effect since it needs to record repeatedly at least a few times. In this paper, we used multi-channel NIRS to evaluate on-line effects of QPS on the contralateral hemisphere.

Subjects and methods

Subjects

Ten healthy volunteers (three women and seven men; age, 27–57 years old, mean ± SD, 39 ± 9 years old) participated in this study. None reported a history of neurological disorders or episodes of seizure. All subjects were right handed based on the Edinburgh Handedness Inventory (Oldfield 1971) and they all gave written informed consent to participate in the study. The experimental procedures used here were approved by the Ethics Committee of Fukushima Medical University and were carried out in accordance with Declaration of Helsinki. No side effects were noted in any individuals. Subjects sat with earplugs in a comfortable reclining chair during the experiments.

Methods

In this study, we performed three experiments as follows.

Experiment 1: On-line effects of 2 min QPS over M1 on the contralateral SEPs.
Experiment 2: After effects of 30 min QPS over M1 on the contralateral SEPs.
Experiment 3: On-line effects of 2 min QPS over M1 on the contralateral NIRS.

Electromyogram (EMG) recordings

Surface EMGs were recorded from the bilateral first dorsal interosseous muscles (FDIs) with 9 mm diameter, Ag–AgCl surface cup electrodes. The active electrode was placed over the muscle belly and the reference electrode over the metacarpophalangeal joint of the index finger. Responses were amplified with an amplifier (MA1116; Digitex Laboratory, Japan) through filters set at 20 Hz and 3 kHz, digitized at a sampling rate of 20 kHz and stored by a computer (TMS bistim tester; Medical Try System, Japan) that was used to perform the randomized conditioning test paradigm and off-line averaging in each condition.

TMS

Magstim 200^2 magnetic stimulators (The Magstim Company Ltd., UK) were used to deliver TMS. We placed a figure 8-shaped coil (7 cm external diameter at each wing; The Magstim Company Ltd., UK) over the M1 of the left hemisphere. The induced currents in the brain were set in the anteromedial direction at a 45 degree angle from the midline (Fig. 1a). M1 was defined as the "hot spot" where stimulation evoked the largest motor evoked potential (MEP) in the contralateral FDI. In two of the subjects, that position was confirmed to be over the primary motor cortex by the neuronavigation system (Spetzger et al. 1995; Boroojerdi et al. 1999). Outputs from four magnetic stimulators were connected with a special device (The Magstim Company Ltd., UK) that enabled us to deliver four monophasic pulses through the same coil.

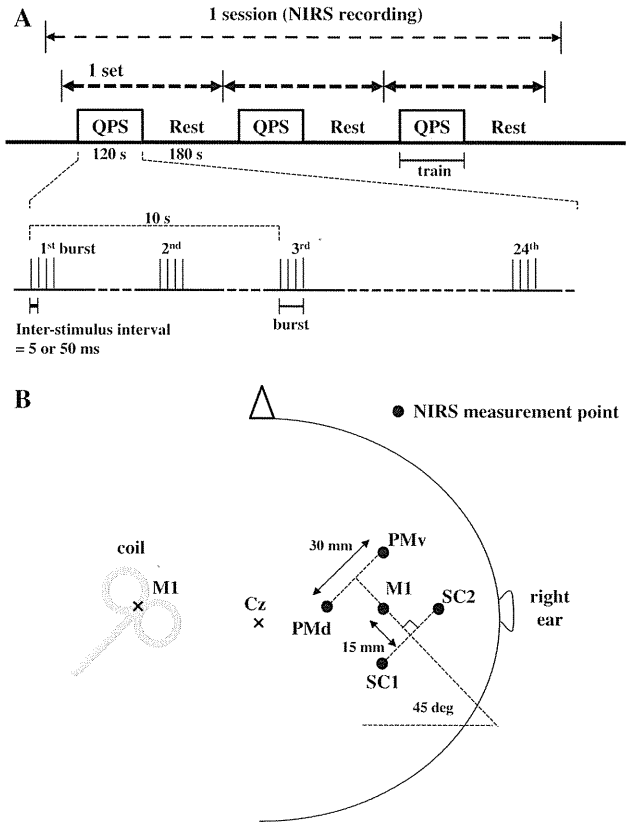

Fig. 1 **a** The paradigm for NIRS on-line effect study (Experiment 3). Two types of QPS (QPS-5 and QPS-50) over left M1 or two types of sham QPS (SHAM-5 and SHAM-50) were delivered for 120 s (1 train, 24 burst, 96 pulses). Rest time of 180 s followed the end of each QPS. This time course (120 s QPS and 180 s rest; 1 set) was repeated three times in one session (total 3 trains, 72 bursts, 288 pulses). **b** The allocations of TMS coil and NIRS measurement points. TMS coil was positioned at the left primary motor cortex (M1). The induced currents in the brain were set in the anteromedial direction at a 45 degree angle from the midline. The five measurement points were the right M1 and four points around the M1. The four measurement points were named here as the right dorsal premotor area (PMd), ventral premotor area (PMv), sensory cortex 1 (SC1) and sensory cortex 2 (SC2)

Repetitive TMSs used here were two types of QPS. QPS was reported to have stable good modulation effects on the stimulated site (Hamada et al. 2008a). In that paper (Hamada et al. 2008a), each burst consisted of four magnetic pulses (i.e. QPS) separated by an ISI of 5 or 50 ms with an inter-burst interval of 5 s (i.e. 0.2 Hz) (Fig. 1a). These QPS types designated as QPS-5 or QPS-50. QPS-5 for 30 min (360 bursts, 1,440 pulses) has a long-term potentiation (LTP) like effect (facilitatory after effect) and QPS-50 a long-term depression (LTD) like effect (inhibitory after effect) on the stimulated site (Hamada et al. 2008a).

The intensity was adjusted at 110% (for on-line effect studies; experiments 1 & 3) or 90% (for after effect study; experiment 2) of active motor threshold (AMT) at M1. We determined the AMT at that to evoke EMG activities in the active target muscle when a subject contracted the target muscles at 5–10% of maximum contraction (about 50 μV). The stimulation intensity was changed in steps of 1% of the maximum stimulator output until we determined the lowest intensity that evoked a small response (about 200 μV) as compared to the pre-stimulus background activity in half of the trials.

For the on-line effect experiments (Experiments 1 & 3), the ISI and inter-burst interval were the same as the previous paper (Hamada et al. 2008a). To produce a considerable effect, a duration of rTMS should be longer than 2 min (Thickbroom et al. 2006), and a few times averaging is necessary for getting reliable NIRS results. To get rid of subjects' fatigue, one session must be within 20 min. Taking consideration of all the above factors together, we made one session of three trains of 24 QPS bursts (96 pulses) for 120 s which were interrupted by 180 s rest (total 72 bursts, 288 pulses; Fig. 1a). For the after effects experiment (Experiment 2), QPS was given for 30 min the same as our previous paper (Hamada et al. 2008a).

Experiment 1: on-line effect of QPS on the contralateral SEPs

Eight (three women and five men; age, mean ± SD, 40 ± 10 years old) out of 10 subjects took part in this experiment. To evaluate electrophysiological changes in the sensory cortex, we measured the left median nerve somatosensory evoked potential (SEP) during 2 min QPS.

Left median nerve SEP was recorded before, during and after QPS-5 or QPS-50 sessions. Brief electrical stimuli (0.2 ms duration) were delivered to the left median nerve at the wrist 2 s after the every onset of QPS burst (0.2 Hz). The stimulus intensity was fixed at about 1.2 times the motor threshold, which was strong enough to evoke tingling sensation radiating to the tip of the index finger. To confirm that electric stimulus delivered on median nerve constantly, we monitored antidromic sensory never action potential (SNAP) at the left index finger and averaged them. For cortical SEPs, electrodes were placed on C4′ (2 cm behind C4) and Fz according to the International 10–20 system. Before and after the QPS session, 180 responses (15 min) amplified with filters set at 1 and 1,500 Hz were averaged. During the QPS session, 72 responses were averaged for 6 min.

Experiment 2: after effect of QPS on the contralateral SEPs

Six subjects (one woman and five men; age, mean ± SD, 37 ± 5 years old) were studied in this experiment. QPS-5

or QPS-50 (360 bursts, 1,440 pulses) was delivered to the left M1 for 30 min. The recording and stimulation methods are all the same as Experiment 1. The simulation rate was 2 Hz and 500 responses were averaged for SEP.

Experiment 3: on-line effect on NIRS at the contralateral hemisphere

Nine subjects (three women and six men; age, mean ± SD, 40 ± 10 years old) took part in this experiment.

We used a NIRS system (ETG-4000; Hitachi Medical Corporation, Tokyo, Japan) having five pairs of emitter and detector. The distance between emitter and detector was 3 cm. The five measurement points (midpoints between emitters and detectors) were placed on the right M1 and four points around the M1. Those measurement points were named here as the right dorsal premotor area (PMd), ventral premotor area (PMv), sensory cortex 1 (SC1) and sensory cortex 2 (SC2) (indicated in Fig. 1b). Near-infrared laser diodes with two wavelengths, 695 and 830 nm, were used as the light sources, and transmittance data of the light beams were obtained every 100 ms. We calculated oxyhemoglobin (oxy-Hb) and deoxyhemoglobin (deoxy-Hb) concentrations from the transmittance data.

In this study (paradigm was indicated in Fig. 1a), each event period ranged from 30 s before the QPS train onset to 120 s after the end of the train. Under each condition, the average Hb concentration changes were obtained from the results of two sessions, namely 6 trains, and they were used in statistical analyses. We mostly neglected an accumulative effect during one session because the concentrations returned to the baseline before the next train, even though we cannot completely exclude a small accumulation.

Sham stimulation was performed with two coils. One non-discharging coil was positioned at the left M1, and the other was positioned 10 cm above the head and the same currents as real stimulation (QPS-5 or QPS-50) were induced in it to make sounds. Two types of sham QPS (SHAM-5 and SHAM-50) were applied.

Four different stimulation conditions (two real and two sham stimulation) were done in all the subjects. Each stimulation condition consisted of two sessions. Then, in total, eight sessions (two sessions × four conditions) were done in one subject. The subjects kept both hand muscles relaxed and no MEPs were induced by TMS during all QPS sessions. The inter-session interval was set at 20 min or longer, which was long enough for the Hb concentration changes to return to the baseline. The order of sessions was counterbalanced within and across subjects. Four sessions were done on one experimental day (two QPS and two sham sessions). Any experiments of Experiment 1, 2 and 3 were separated by 1 week or more in the same subject.

Statistical analyses

In SEP studies, repeated-measures ANOVAs (factor, TIME; Experiment 1, before, during and after QPS; Experiment 2, before and 0, 15, 30, 45, 60, 75 and 90 min after QPS) were performed for each SEP component. When an ANOVA test showed significant effects, we further performed post-hoc analyses with Bonferroni's method for multiple comparisons compensation. In the NIRS experiment (Experiment 3), we obtained a representative value of Hb concentration changes by averaging Hb data from 60 to 180 s after the onset of QPS in each condition, and used these average values (mean Hb change) in statistical comparisons. The comparisons were performed by *t* test between QPS-5 and SHAM-5 and between QPS-50 and SHAM-50 at each channel. The statistical significance was set at $P = 0.05$.

Results

None of the subjects reported any adverse effects in our experiments.

SEP studies (Experiments 1 & 2)

Experiment 1: on-line effect

Typical waveforms of SEP before, during and after QPS-5 and QPS-50 for 2 min are shown in Fig. 2. SNAP showed no changes (less than 5%), but P25 peak–N33 peak amplitudes during QPS-5 or QPS-50 were larger than those before or just after each QPS (more than 130%). Before, during and just after QPS-5 or QPS-50, the mean amplitudes of SNAP, N20 onset–peak, N20 peak–P25 peak and P25 peak–N33 peak were shown in Fig. 3. Repeated-measures ANOVA revealed no significant TIME effect on SNAP (QPS-5, $F = 0.313$, $P = 0.736$; QPS-50, $F = 0.020$, $P = 0.981$). This finding means that the left median nerve was stimulated constantly in any conditions. For amplitudes of N20 onset–peak and N20 peak–P25 peak, in either QPS-5 or QPS-50, ANOVA revealed no significant TIME effects on the amplitude (N20 onset–peak, QPS-5, $F = 3.567$, $P = 0.056$, QPS-50, $F = 2.948$, $P = 0.085$; N20 peak–P25 peak, QPS-5, $F = 2.722$, $P = 0.100$, QPS-50, $F = 1.912$, $P = 0.184$). For P25 peak–N33 peak, in contrast, repeated-measures ANOVA revealed significant TIME effects in both QPS types (QPS-5, $F = 9.157$, $P = 0.003$; QPS-50, $F = 8.440$, $P = 0.004$). Post-hoc analyses with Bonferroni method showed that the amplitudes of P25 peak–N33 peak during QPS-5 or QPS-50 were significantly larger than those before or after QPS-5 or QPS-50 ($P < 0.05$).