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Quadro-pulse stimulation is more effective than paired-pulse stimulation for plasticity induction of the human motor cortex

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Abstract

Objective: Repetitive paired-pulse transcranial magnetic stimulation (TMS) at I-wave periodicity has been shown to induce a motor-evoked potential (MEP) facilitation. We hypothesized that a greater enhancement of motor cortical excitability is provoked by increasing the number of pulses per train beyond those by paired-pulse stimulation (PPS).

Methods: We explored motor cortical excitability changes induced by repetitive application of trains of four monophasic magnetic pulses (quadro-pulse stimulation: QPS) at 1.5-ms intervals, repeated every 5 s over the motor cortex projecting to the hand muscles. The after-effects of QPS were evaluated with MEPs to a single-pulse TMS, motor threshold (MT), and responses to brain-stem stimulation. These effects were compared to those after PPS. To evaluate the QPS safety, we also studied the spread of excitation and after discharge using surface electromyograms (EMGs) of hand and arm muscles.

Results: Sizes of MEPs from the hand muscle were enhanced for longer than 75 min after QPS; they reverted to the baseline at 90 min. Responses to brain-stem stimulation from the hand muscle and cortical MEPs from the forearm muscle were unchanged after QPS over the hand motor area. MT was unaffected by QPS. No spreads of excitation were detected after QPS. The appearance rate of after discharges during QPS was not different from that during sham stimulation.

Conclusions: Results show that QPS can safely induce long-lasting, topographically specific enhancement of motor cortical excitability.

Significance: QPS is more effective than PPS for inducing motor cortical plasticity.

Keywords: Repetitive transcranial magnetic stimulation; LTP; Motor cortex

1. Introduction

Repetitive transcranial magnetic stimulation (rTMS), a noninvasive method to activate cortical neurons focally and transsynaptically in the human brain, has the potential to modulate motor cortical excitability. During the past decade, high-frequency rTMS has been shown to induce facilitatory effects on the human motor cortex (Pascual-Leone et al., 1994; Chen et al., 1997b; Berardelli et al., 1998; Maeda et al., 2000; Baumer et al., 2003; Peinemann et al., 2004). Other methods using direct current (transcranial direct current stimulation, tDC; Nitsche and Paulus, 2001), ischemic nerve block (INB; Ziemann et al., 1998), paired associative stimulation (PAS; Stefan et al., 2000; Wolters et al., 2003; Quartarone et al., 2006), and theta-burst stimulation (TBS; Huang et al., 2005) have also been shown to induce long-lasting aftereffects. Thickbroom et al. (2006) showed recently that repetitive, monophasic paired-pulse rTMS (paired-pulse stimulation; PPS) at 1.5-ms

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interval induces motor cortical excitability enhancement lasting up to 10 min after cessation of the intervention. In our previous investigation, the aftereffects of PPS continued as long as those described by Thickbroom et al. (2006) (Hamada et al., 2007), although their effects were smaller than those of Thickbroom et al. (2006). A few reports have shown that the site of action of PPS is the motor cortex (Hamada et al., 2007; Di Lazzaro et al., 2007).

The mechanism of these aftereffects is suggested to be synaptic modification similar to that of long-term potentiation (LTP) or long-term depression (LTD) in animal experiments (Chen et al., 1997b; Ziemann et al., 1998; Stefan et al., 2000; Siebner et al., 2000; Wolters et al., 2003; Lee et al., 2003; Huang et al., 2005; Quartarone et al., 2006; Cooke and Bliss, 2006). Thickbroom et al. (2006) speculated that PPS might reinforce transsynaptic events within the motor cortex because 1.5 ms corresponds to I-wave periodicity. This idea was also supported by results of paired-pulse TMS (Tokimura et al., 1996; Hanajima et al., 2002) and quadro-pulse TMS (Amassian and Deletis, 1999). The lack of invasive recording of synaptic responses in conscious humans renders any inference of precise neuronal mechanisms underlying these aftereffects speculative (Cooke and Bliss, 2006), but scientific knowledge of synaptic plasticity can provide important information for plasticity induction in the human motor cortex (Cooke and Bliss, 2006).

In animal experiments, the threshold for LTP induction is a complex function of the intensity and pattern of tetanic stimulation (Malenka, 1991; Bliss and Collingridge, 1993). The number of pulses per train is known to be a very potent factor of tetanic stimulation to influence the level of synaptic plasticity in the hippocampus (Nakao et al., 2004). These lines of evidence lead us to presume that a greater enhancement of motor cortical excitability can be provoked by increasing the number of pulses per train. The present study investigated the effects of repetitive application of four monophasic magnetic pulses (quadro-pulse stimulation, QPS) on motor cortical excitability.

Unfortunately, no safety guidelines for complex rTMS protocols such as QPS, PPS (Thickbroom et al., 2006), or TBS (Huang et al., 2005) exist. According to established guidelines of rTMS (Chen et al., 1997c; Wassermann, 1998), seizure occurrence, post-TMS electromyogram (EMG) activity, and the spread of excitation (SE) to proximal muscles are considered unsafe. Post-TMS EMG activity referred to continuation of EMG activity after cessation of rTMS; it was thought to be a possible correlate to an after discharge. In addition, SE was thought to indicate a breakdown of lateral inhibition in the cortex (Pascual-Leone et al., 1993, 1994). For these reasons, we have also studied the safety of QPS by monitoring SE or occurrence of post-TMS EMG activity after QPS. This information will facilitate development of further clinical applications of QPS.

2. Subjects and methods

2.1. Subjects

Subjects were 16 healthy volunteers (3 women, 13 men; mean ± SD age, 37.0 ± 6.0) who gave their written informed consent to participate in the experiments. No subjects had neurological, psychiatric disorders or other medical problems or had any contraindication to TMS (Wassermann, 1998). All were right-handed according to the Oldfield handedness inventory (Oldfield, 1971). The protocol was approved by the Ethics Committee of the University of Tokyo and was carried out in accordance with the ethical standards of the Declaration of Helsinki.

2.2. Recordings

Subjects were seated on a comfortable chair and told to keep the target muscle relaxed. Motor-evoked potentials (MEPs) were recorded from the right first dorsal interosseous (FDI) muscle. Pairs of Ag/AgCl surface electrodes (9 mm diameter) were placed over the muscle belly (active electrode) and over the metacarpophalangeal joint of the index finger (reference). Responses were amplified (Biotop; GE Marquette Medical Systems, Japan) through filters set at 100 Hz and 3 kHz, digitized with an A/D converter at a sampling rate of 20 kHz, and then stored on a computer for offline analysis (TMS bistim tester; Medical Try System, Japan).

2.3. Stimulation

Transcranial magnetic stimulation (TMS) was administered through a figure-of-eight coil (9 cm external diameter at each wing; The Magstim Co. Ltd., Whitland, Dyfed, UK). Single monophasic TMS pulses were delivered using a magnetic stimulator (Magstim 200; The Magstim Co. Ltd.). Paired- or quadro-pulse stimuli were delivered by two or four stimulators (Magstim 200^2; The Magstim Co. Ltd.) connected by a specially designed combining module (The Magstim Co. Ltd.). This device combines the outputs from four stimulators to allow a train of 2–4 monophasic magnetic pulses to be delivered through a single coil.

The coil was placed tangentially over the scalp to induce currents in the brain flowing at about 45 deg in an anterior-medial direction, which is almost perpendicular to the central sulcus. This was the optimal orientation for activating the corticospinal tracts transsynaptically via horizontal cortical connections (Sakai et al., 1997).

The optimal site for eliciting MEPs in the right FDI muscle (i.e., the *hot spot*) was first determined before each experiment. We stimulated several positions 1 cm apart from each other using the same intensity. The *hot spot* was defined as the site where the largest responses were elicited. The position was marked using a red pen on the scalp for repositioning the coil. Then we determined the thresholds. The resting motor threshold (RMT) was defined as

the lowest intensity that evoked a response of at least 50 μV in the relaxed FDI in at least five of ten consecutive trials (Rossini et al., 1994). The active motor threshold (AMT) was defined as the lowest intensity that evoked a small response (>100 μV), when the subjects maintained a slight contraction of the right FDI (5–10% of the maximum voluntary contraction) with the aid of an oscilloscope monitor in more than five of ten consecutive trials. The stimulus intensity was changed in steps of 1% of the maximum stimulator output of each magnetic stimulator.

Brain-stem electrical stimulation was performed using a method described in a previous study (Ugawa et al., 1991). The anode (right) and cathode (left) were attached to the skin overlying the mastoids. Stimulation was performed using a high-voltage electrical stimulator (maximal output 1.2 A, 1200 V, D180A; Digitimer Ltd., Welwyn Garden City, UK).

2.4. Conditioning

2.4.1. Quadro-pulse stimulation (QPS) and paired-pulse stimulation (PPS)

Quadro-pulse stimulation (QPS) and paired-pulse stimulation (PPS) were applied over the *hot spot* for FDI. One train consisted of four pulses at the same intensity separated by 1.5 ms, repeatedly given at 0.2 Hz in QPS; it consisted of paired pulses of equal intensity separated by 1.5 ms, repeatedly given at 0.2 Hz in PPS. Because there was no aftereffect of single-pulse rTMS at 0.2 Hz (Thickbroom et al., 2006), we did not perform this conditioning.

2.4.2. Realistic sham conditioning

Realistic sham conditioning was described previously (Okabe et al., 2003). Four electric pulses (each electric pulse: duration, 0.2 ms; intensity, twice sensory threshold) were given to the skin of the head at 0.2 Hz using a conventional electric peripheral nerve stimulator to mimic the skin sensation of TMS. Electric pulses were applied through the electrodes placed over the left-hand motor area and Fz. A coil, which was disconnected with the stimulator, was placed over the left-hand motor area for mimicking real TMS. Another coil, which was connected to a combining module with four Magstim 200^2 stimulators, was held off the scalp but placed near the subject; it was discharged simultaneously with the scalp electrical stimulation to produce a similar sound to that associated with real QPS.

2.5. Timelines of experiments and measurement parameters (Fig. 1)

Seven different experiments were performed. For each subject, two successive experiments were separated by at least a one-week interval. The order of experiments was pseudorandomized and counterbalanced among subjects. Timelines of all experiments, except for the one studying safety, are shown in Fig. 1. We defined "0" min as the end of each conditioning (Fig. 1).

2.5.1. Experiment 1: Comparison between QPS and PPS with the same number of trains

In this experiment, 12 subjects participated. To compare the effects of QPS with those of PPS, 360 trains of QPS, PPS, or sham stimulation were applied at 0.2 Hz for 30 min. The stimulation intensity was set to elicit MEPs as large as 0.4 mV by each train of quadro pulses or paired pulses. The mean (±SD) stimulus intensity of each pulse was 130 ± 24% AMT (82 ± 7% RMT; 74–98% RMT) in QPS conditioning; the mean intensity of each pulse was 148 ± 27% AMT (98 ± 10% RMT) in PPS conditioning. These characteristic conditioning protocols are designated as QPS-supra-360 and PPS-supra-360 for subsequent discussion. We also performed sham conditioning for 30 min (QPS-sham-360) in four subjects.

2.5.1.1. MEPs recorded from FDI muscle during conditioning. Conditioning was started from "−30" min (Fig. 1). During QPS-supra-360 and PPS-supra-360, we obtained a total of 360 MEPs (each MEP elicited by one train of quadro pulses or paired pulses every 5 s). The peak-to-peak amplitude of each MEP was measured. Every 60 MEP responses were averaged to obtain the mean MEP amplitudes at 5-min intervals. During the experiments, EMG activity of the FDI was monitored using an oscilloscope monitor. Trials contaminated with voluntary EMG activity were discarded from analyses.

2.5.1.2. MEPs recorded from FDI muscle before and after conditioning. The aftereffects of QPS-supra-360, PPS-supra-360, or QPS-sham-360 were evaluated according to changes in the peak-to-peak amplitude of MEPs to single-pulse TMS (cortical MEPs) in the right FDI muscle at rest. In all, 20 cortical MEPs were collected every 14.5–15.5 s before conditioning and during six epochs following each conditioning: 0–5, 5–10, 10–15, 15–20, 20–25, and 25–30 min. We used this long interval to match that of brain-stem electrical stimulation (see below; Experiment 5). The stimulus intensity of single-pulse TMS was adjusted to elicit MEP of approximately 0.4 mV before conditioning and was kept constant during all experiments. The excitability of each period was estimated by averaging 20 MEPs; it was expressed as the size ratio relative to the baseline average MEP.

2.5.2. Experiment 2: Effects of the intensity and the number of trains of QPS on aftereffects

In this experiment, 12 subjects participated. Two subjects not included in Experiment 1 were also enrolled. We performed three conditioning types to elucidate the relative contribution of intensity and number of trains to the QPS aftereffects: (1) QPS-sub-360, 360 trains of QPS with 90% AMT (each pulse at 90% AMT); (2) QPS-supra-180, 180 trains of QPS with 130% AMT; and (3) QPS-sub-180, 180 trains of QPS with 90% AMT. During QPS-sub-360 and QPS-sub-180 conditioning, we were unable to obtain any MEPs to one train of quadro pulses. Before and after

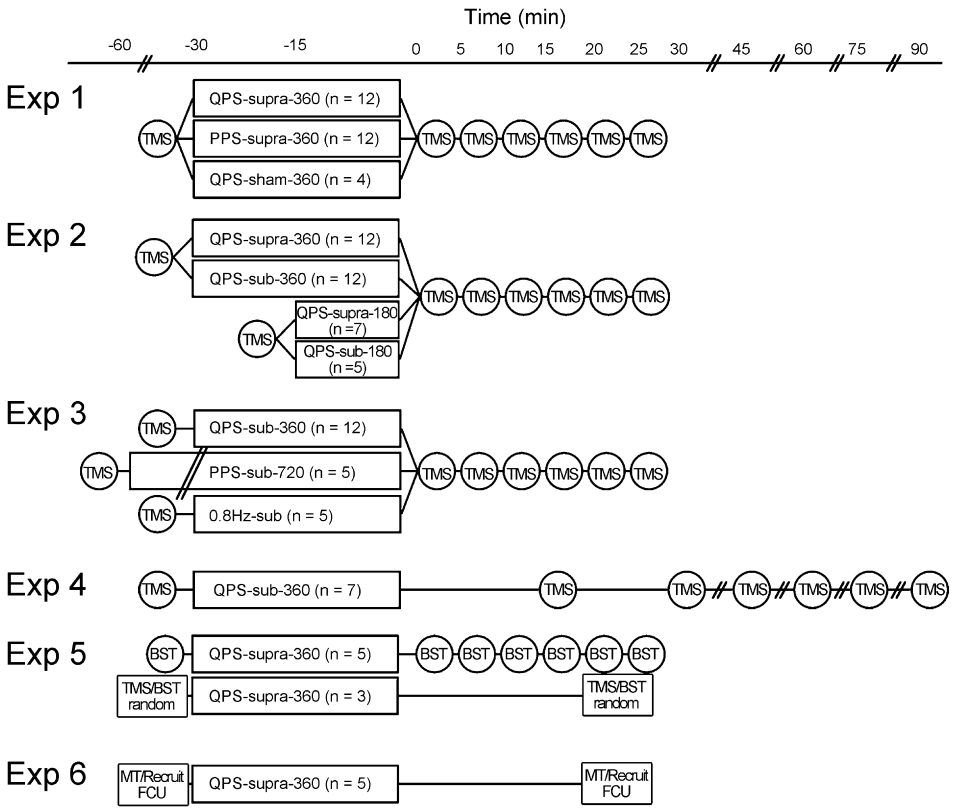

Fig. 1. Timelines of experiments (see Section 2.5).

each conditioning, cortical MEPs were recorded in the same manner as that described for Experiment 1. Results of QPS-supra-360 obtained in Experiment 1 were also used for comparison with those of Experiment 2.

2.5.3. Experiment 3: Comparison between QPS and PPS with the same total number of pulses

Five subjects participated in this experiment. One subject, who was not included in Experiments 1 and 2, was enrolled. Because we should consider the effects of the total number of pulses when we compare the aftereffects of QPS to those of PPS, we performed the following conditioning protocols with the same total number of pulses as that of QPS-sub-360 (1440 pulses): (1) PPS-sub-720, 720 trains of PPS with 90% AMT and (2) 0.8 Hz-sub, continuous single-pulse TMS at 0.8 Hz with 90% AMT for 30 min. The latter one (0.8 Hz-sub) contains the same number of pulses with QPS in 1 s. During these conditionings, we were unable to obtain any MEPs. Before and after each conditioning, cortical MEPs were measured in the same manner as that described for Experiment 1.

2.5.4. Experiment 4: Duration of QPS aftereffects

Seven subjects participated in this experiment. One subject, who did not participate in Experiment 1, 2, or 3, was enrolled in this experiment. We explored QPS-sub-360 for conditioning in this experiment. Every 15 min for 90 min, 20 cortical MEPs (collected every 14.5–15.5 s) were measured.

2.5.5. Experiment 5: Effects of QPS on brain-stem MEPs

Five subjects who enrolled in Experiment 1 participated in this experiment. We performed brain-stem electrical stimulation to clarify the origins of facilitation after QPS (BST). We explored QPS-supra-360 for conditioning in this experiment.

2.5.5.1. Brain-stem MEPs before and after conditioning. Before QPS-supra-360, eight MEPs to single-pulse BST (brain-stem MEPs) were obtained every 14.5–15.5 s. The stimulation intensity was set to elicit brain-stem MEPs as large as cortical MEPs to a single TMS in resting FDI (about 0.4 mV). The intensity was about 50% of the maximal electrical stimulator output. Then, QPS-supra-360 was applied over the *hot spot* for FDI. After QPS-supra-360, 8 brain-stem MEPs (each brain-stem MEP obtained every 14.5–15.5 s) were collected every 5 min for 30 min.

2.5.5.2. Cortical and brain-stem MEPs obtained in a random order before and after conditioning. Eight cortical MEPs and eight brain-stem MEPs were obtained in a random order for baseline measurement to exclude the effect of prediction of pain associated with BST (i.e., 16 stimuli were given every 14.5–15.5 s in a random order). We measured cortical and brain-stem MEPs in the same manner (random order stimulation) as that in baseline measurement 20 min after QPS-supra-360.

2.5.6. Experiment 6: Effects of QPS on motor threshold, recruitment curves and somatotopy

2.5.6.1. Motor threshold and recruitment curves. Five subjects who had enrolled in Experiment 1 participated in this experiment. Before and after QPS-supra-360, AMT, RMT and recruitment curves were studied. Stimuli were applied at the optimal site for eliciting cortical MEPs from FDI. After AMT and RMT measurement, eight stimuli were applied at an intensity of 10% below RMT every 7.5–8.5 s. The stimulus intensity was then increased by 5%; another eight stimuli were applied. This process was repeated until the intensity reached 135% RMT. The timing of evaluation after QPS-supra-360 was 20 min post conditioning.

2.5.6.2. Cortical MEPs from the FCU muscle. We recorded cortical MEPs from the right flexor calpi ulnaris muscle (FCU) in these five subjects to confirm topographically specific modulation of the motor cortex after QPS. The optimal site to elicit cortical MEPs from FCU (*hot spot* for FCU, about 1–3 cm medial to the *hot spot* for FDI) was first determined. Then 20 cortical MEPs from the right FCU were obtained by delivering a single-pulse TMS. The stimulus intensity for FCU was set to elicit MEPs with peak-to-peak amplitude of 0.2–0.3 mV. To obtain cortical MEPs from the right FDI, 20 single-pulse TMS were then applied to the *hot spot* for FDI. The QPS-supra-360 was applied over the *hot spot* for FDI, as described previously. At 20 min after QPS-supra-360, 20 cortical MEPs were obtained from both FCU and FDI when TMS was given over the *hot spot* for each muscle.

2.5.7. Experiment 7: Safety study

Three subjects who had participated in Experiment 1 were enrolled in this experiment. Surface electromyograms (EMGs) were recorded from the right FDI, FCU, and biceps brachii (BB) muscles. The EMGs were monitored continuously for the spread of excitation (SE) to proximal muscles and post-TMS EMG activity. According to previous safety studies (Pascual-Leone et al., 1993; Chen et al., 1997c), SE was defined as a progressive increase in four consecutive MEPs of FCU or BB muscle by more than 100% of the baseline (average MEP amplitude of the first 12 MEPs evoked by the first 12 QPS trains) because it was not possible to evoke MEPs from the FDI muscle without inducing small MEPs in FCU or BB muscle in our subjects. In many instances, it was difficult to distinguish post-TMS EMG activity from poor muscle relaxation (Chen et al., 1997c). As a safety precaution, post-TMS EMG activity was defined as isolated EMG activity of shorter than 50 ms and larger than 100 μV in the 1000 ms following a QPS train. We explored QPS-supra-360 or QPS-sham-360 for conditioning to compare the appearance rates of post-TMS EMG activity for these types of conditioning.

2.6. Data analysis and statistics

Data were analyzed using software (SPSS ver. 13.0 for Windows; SPSS Inc.). The effect during conditioning was analyzed using two-way repeated-measures analysis of variance (ANOVA) (between-subject factor, conditioning; within-subject factor, time). The aftereffect of different conditioning was analyzed using two-way repeated ANOVA (between-subject factor, conditioning; within-subject factor, time). For analysis of conditioning parameters, we conducted three-way factorial repeated-measures ANOVA to compare time courses of the four conditioning types (QPS-supra-360, QPS-sub-360, QPS-supra-180, and QPS-sub-180). The intensity (supra-threshold and sub-threshold), the number of trains of QPS (360 and 180 trains), and times of seven levels (baseline, 0–5, 5–10, 10–15, 15–20, 20–25, and 25–30 min) were independent variables. The dependent variable was the normalized amplitude of cortical MEPs. The aftereffect of each conditioning type on MEP sizes was analyzed using one-factor ANOVA (within-subject factor, time).

The effect of the stimulation pattern (single-pulse TMS and brain-stem electrical stimulation) after QPS-supra-360 on MEP sizes was evaluated using two-way repeated-measures ANOVA (within-subject factors: stimulation pattern, time). Recruitment curves before and after QPS-supra-360 were compared using two-way repeated-measures ANOVA (within-subject factors: pre and post QPS-supra-360, and intensity). Paired *t*-tests were used to compare variables (RMT, AMT, and the absolute MEP amplitudes recorded from FCU) before and after QPS-supra-360. The Greenhouse-Geisser correction was used as necessary to correct nonsphericity. Bonferroni's post hoc method was used for further analyses: p values less than 0.05 were considered significant. All figures depict group data.

3. Results

No subject reported any adverse effect during or after any intervention.

3.1. Experiment 1: Comparison between QPS and PPS with the same number of trains

3.1.1. MEPs recorded from FDI muscles during conditioning

Marked MEP facilitation was apparent throughout QPS-supra-360 (Fig. 2a and b). In contrast, PPS-supra-360 showed a slight increase in the MEP amplitude (Fig. 2b) (two-way repeated ANOVA: effect of conditioning, $F[1,22]=8.415$, $p=0.008$; effect of time, $F[2.313,50.892]=4.179$, $p=0.012$; conditioning × time interaction, $F[2.305,50.700]=2.104$, $p=0.125$). Post hoc analysis revealed a significant facilitation of MEP to one QPS train at the end of QPS-supra-360, although no significant facilitation of MEP to one PPS train at the end of PPS-supra-360 (Fig. 2b) was found.

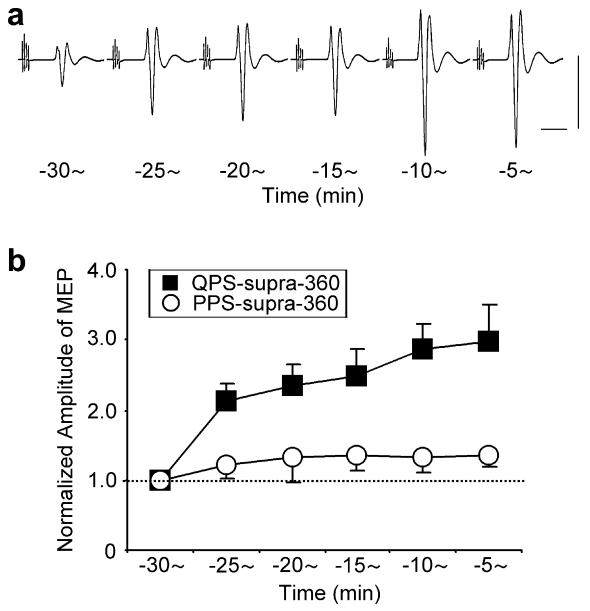

Fig. 2. Comparison between QPS and PPS with the same number of trains: during conditioning. (a) Waveforms of MEPs for 30 min during QPS-supra-360 in a representative subject. Each MEP was elicited by one train of quadro stimuli every 5 s and 360 MEPs were obtained in all. Average waveforms at 5-min intervals were obtained by averaging 60 consecutive responses (started from "−30" min; see Fig. 1). Marked MEP facilitation existed throughout QPS-supra-360. Calibration bars: 20 ms, 0.5 mV. (b) Time courses of normalized MEP amplitudes (mean ± SE) during QPS-supra-360 (filled squares, $n = 12$) and PPS-supra-360 (circles, $n = 12$). During QPS-supra-360 and PPS-supra-360, we obtained a total of 360 MEPs (one MEP elicited by one train every 5 s); the peak-to-peak amplitude of each MEP was measured. A series of 60 MEP amplitudes was averaged to obtain the mean MEP amplitudes at 5 min periods (started from "−30" min, see Fig. 1). Significant effects of conditioning (QPS-supra-360 and PPS-supra-360) ($p < 0.01$) and time ($p < 0.05$) were found, but no significant interaction was found between conditioning and time ($p > 0.05$) using two-way repeated ANOVA, with significant post hoc facilitation of cortical MEPs at the end of QPS-supra-360 (mean ± SE; 281 ± 49%, $t = -2.548$, $p = 0.027$). No significant increase was found in MEPs at the end of PPS-supra-360 (132 ± 15%, $t = -2.047$, $p = 0.062$).

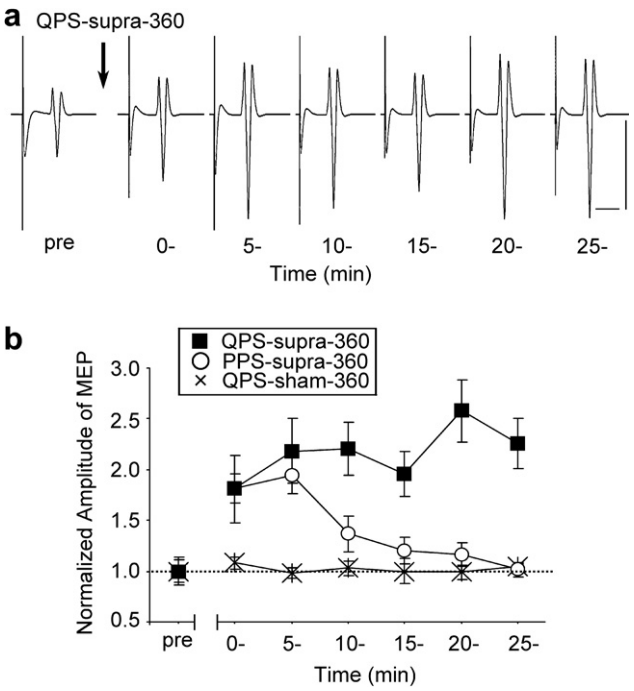

Fig. 3. Comparison between QPS and PPS with the same number of trains: aftereffects. (a) Representative cortical MEPs (i.e., MEPs to single-pulse TMS) before and after QPS-supra-360 from one subject. Averaged waveforms were made from 20 consecutive responses. A marked cortical MEP facilitation was identified after QPS-supra-360 (arrow) for 30 min. Calibration bars: 20 ms, 0.5 mV. (b) Time courses of normalized amplitudes of cortical MEPs (mean ± SE) after three conditioning: QPS-supra-360 (filled squares, $n = 12$), PPS-supra-360 (circles, $n = 12$) and QPS-sham-360 (crosses, $n = 4$). The abscissa shows the time with respect to the conditioning (before conditioning = "pre", immediately after conditioning = "0"). The ordinate shows the normalized amplitude of cortical MEPs. Average amplitudes were calculated from those of 20 consecutive responses obtained every 5 min and normalized to that of baseline measurements. Significant effects of the conditioning ($p < 0.005$) and time ($p < 0.005$) were found; also, a significant interaction between them ($p < 0.001$) was found using two-way repeated ANOVA, with significant post hoc differences between QPS-supra-360 and PPS-supra-360 ($p < 0.05$), and QPS-sham-360 ($p < 0.01$). Cortical MEPs after QPS-supra-360 were facilitated for 30 min after conditioning (post hoc analysis using Bonferroni's method: each time point, $p < 0.05$). After PPS-supra-360, cortical MEPs were facilitated for only 10 min and then reverted to baseline level (post hoc analysis using Bonferroni's method: 0–5 min, $p < 0.05$; 5–10 min, $p < 0.001$; 10–30 min, $p > 0.05$). No significant cortical MEP changes were found after QPS-sham-360 (one-factor ANOVA: effect of time, $p > 0.05$).

3.1.2. MEPs recorded from FDI muscle before and after conditioning

Cortical MEPs were facilitated significantly for 30 min after QPS-supra-360 (Fig. 3a and b), but for only 10 min after PPS-supra-360 (Fig. 3b). No significant cortical MEP changes were found after QPS-sham-360 (Fig. 3b) (two-way repeated ANOVA: effect of conditioning, $F[2, 25] = 8.084$, $p = 0.002$; effect of time, $F[6, 150] = 4.098$, $p = 0.001$; conditioning × time interaction, $F[12, 150] = 4.664$, $p < 0.001$; post hoc analysis: QPS-supra-360 vs. PPS-supra-360, $p = 0.017$; QPS-supra-360 vs. QPS-sham-360, $p = 0.005$).

3.2. Experiment 2: Effect of the intensity and the number of trains of QPS on aftereffects

Three-way repeated ANOVA revealed that QPS aftereffects were affected significantly by the number of trains rather than by the intensity (Fig. 4a) (effect of the number of trains, $F[1, 32] = 9.290$, $p = 0.005$; effect of the intensity, $F[1, 32] = 0.007$, $p = 0.934$; effect of time, $F[6, 192] = 7.585$, $p < 0.001$; the number of trains × time interaction, $F[6, 192] = 3.194$, $p = 0.001$; the intensity × time interaction, $F[6, 192] = 0.858$, $p = 0.527$; the number of trains × the intensity × time interaction, $F[6, 192] = 1.265$, $p = 0.275$). A significant cortical MEP facilitation was found for 30 min, even after QPS-sub-360, but for only 10 min after QPS-supra-180. No significant cortical MEP changes were found after QPS-sub-180 (Fig. 4a).

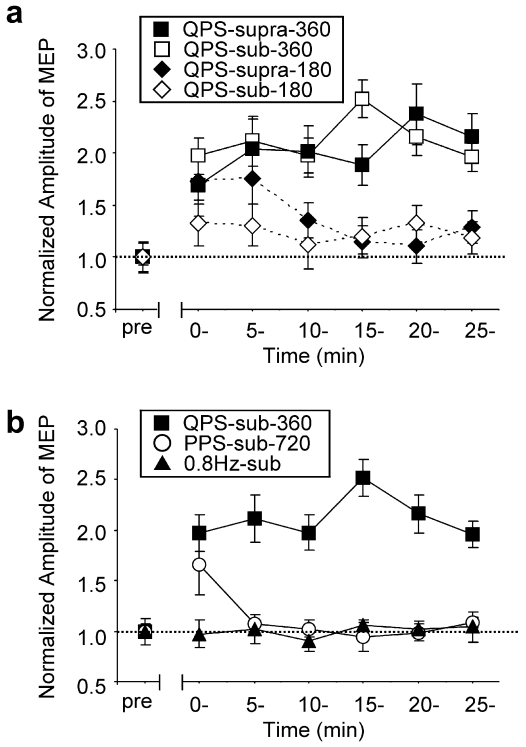

Fig. 4. (a) Effect of the intensity and the number of trains of QPS on aftereffects. Time courses of normalized amplitudes of cortical MEPs (mean ± SE) after four conditioning: QPS-supra-360 (filled squares, $n = 12$), QPS-sub-360 (squares, $n = 12$), QPS-supra-180 (filled diamonds, $n = 7$), and QPS-sub-180 (diamonds, $n = 5$). After both QPS-supra-360 and QPS-sub-360 conditioning, cortical MEPs were facilitated for at least 30 min. Significant effects of the number of trains ($p < 0.01$) and time ($p < 0.001$) were found, as well as a significant interaction between the number of trains and time ($p < 0.005$) using three-way repeated ANOVA. Cortical MEPs after both QPS-sub-360 and QPS-supra-360 were significantly facilitated for 30 min post conditioning (post hoc analysis using Bonferroni's method: each period, $p < 0.05$). After QPS-supra-180, cortical MEPs were facilitated significantly for only 10 min (post hoc analysis using Bonferroni's method: 0–5 min, $p < 0.05$; 5–10 min, $p < 0.05$; 10–30 min, $p > 0.05$). No significant cortical MEP changes were found after QPS-sub-180 (one-factor ANOVA: effect of time, $p > 0.05$). (b) Comparison between QPS and PPS with the same total number of pulses. Time courses of the normalized amplitudes of cortical MEPs (mean ± SE) after three conditioning: QPS-sub-360 (filled squares, $n = 12$), PPS-sub-720 (circles, $n = 5$), and 0.8 Hz-sub (filled triangles, $n = 5$). Even with the same total number of pulses (1440 pulses), only QPS-sub-360 induced long-lasting MEP facilitation after conditioning (two-way repeated ANOVA: conditioning × time, $p < 0.05$; effect of conditioning, $p < 0.005$; effect of time, $p > 0.05$; post hoc analysis, difference between QPS-sub-360 and PPS-sub-720 ($p < 0.05$), or 0.8 Hz-sub-30 ($p < 0.01$)). Cortical MEPs after PPS-sub-720 were facilitated only for 5 min (post hoc analysis using Bonferroni's method: 0–5 min, $p < 0.05$; 5–30 min, $p > 0.5$). No significant cortical MEP changes were found after 0.8 Hz-sub (one-factor ANOVA: effect of time, $p > 0.05$).

3.3. Experiment 3: Comparison between QPS and PPS with the same total number of pulses

The effects of the total number of pulses should be examined when we compare the aftereffects of QPS with those of PPS. Indeed, the time course of normalized amplitude of cortical MEPs after QPS-supra-180 (180 trains, total 720 pulses; Fig. 4a) seemed to be similar to that after PPS-supra-360 (360 trains, total 720 pulses; Fig. 3b) (two-way repeated ANOVA: effect of time, $p < 0.001$; effect of conditioning, $p > 0.05$; conditioning × time interaction, $p > 0.05$). Hence, QPS, PPS or 0.8 Hz conditioning with 1440 pulses in all were performed. Even with the same total number of conditioning pulses, cortical MEPs were significantly facilitated for only 5 min after PPS-sub-720 (Fig. 4b) (two-way repeated ANOVA: effect of conditioning, $F[2,19] = 8.153$, $p = 0.003$; effect of time, $F[6,114] = 2.160$, $p = 0.080$; conditioning × time interaction, $F[8.177, 77.677] = 2.648$, $p = 0.012$; post hoc analysis: QPS-sub-360 vs. PPS-sub-720, $p = 0.020$; QPS-sub-360 vs. 0.8 Hz-sub, $p = 0.008$). No significant MEP changes were found after 0.8 Hz sub (Fig. 4b).

3.4. Experiment 4: Duration of QPS aftereffects

The duration of the cortical MEP amplitude enhancement was studied by obtaining MEPs for 90 min following QPS-sub-360 (Fig. 5). Cortical MEPs were facilitated significantly for 75 min, and returned to the baseline level at 90 min (effect of time, $F[6,36] = 6.964$, $p < 0.001$). Cortical MEPs after QPS-supra-360 also returned to the baseline level at 90 min ($n = 3$, data not shown).

3.5. Experiment 5: Effects of QPS on brain-stem MEPs

The QPS-supra-360 did not affect the brain-stem MEPs for 30-min post conditioning (Fig. 6a). Fig. 6b shows the effects of QPS-supra-360 on cortical and brain-stem MEPs obtained in a randomized order. Only cortical MEPs were facilitated significantly, although brain-stem MEPs remained unchanged (Fig. 6b) (two-way repeated ANOVA: effect of stimulation pattern, $F[1,2] = 9.944$, $p = 0.088$; effect of time, $F[1,2] = 54.954$, $p = 0.018$; time × stimulation pattern interaction, $F[1,2] = 87.116$, $p = 0.011$; post hoc analysis: cortical MEPs after

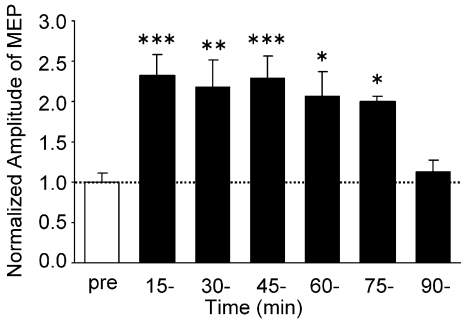

Fig. 5. Duration of QPS aftereffect. Time courses of normalized amplitudes of cortical MEPs (mean ± SE) after QPS-sub-360 ($n = 7$). The period of measurement was extended to 90 min after the conditioning to study when the effects of QPS-sub-360 returned to the baseline level. Cortical MEPs were facilitated for 75 min post conditioning and returned to the baseline level at 90 min post conditioning (one-factor ANOVA: effect of time, $p < 0.001$). Asterisks $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$, by post hoc analysis using Bonferroni's method.

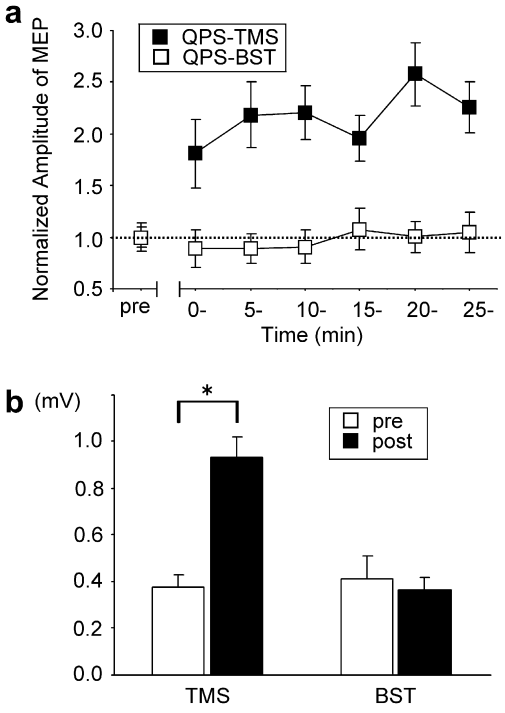

Fig. 6. Effects of QPS on brain-stem MEPs. (a) Time courses of normalized amplitudes of brain-stem MEPs (mean ± SE) after QPS-supra-360 (squares, $n = 5$). Normalized amplitude of brain-stem MEPs did not change significantly for 30 min after conditioning (one-factor ANOVA: effect of time, $p > 0.05$). For comparison, cortical MEPs after QPS-supra-360 (filled squares) are also depicted. (b) Cortical and brain-stem MEPs obtained before (pre, bars) and after (post, filled bars) QPS-supra-360. The responses were sampled in a pseudorandom order to avoid the effect of anticipation of the uncomfortable brain-stem stimulation ($n = 3$). The bars show absolute amplitudes of MEPs (mV, mean ± SE). Only cortical MEPs (i.e., MEPs to single-pulse TMS) were facilitated, whereas brain-stem MEPs (i.e., MEPs to BST electrical stimulation) remained unchanged (two-way repeated ANOVA: effect of time, $p < 0.05$; effect of stimulation pattern, $p > 0.05$; stimulation pattern × time, $p < 0.05$). Asterisks, $p < 0.05$, by post hoc paired t-test.

QPS-supra-360, $t = -8.888$, $p = 0.012$; brain-stem MEPs, $t = 4.009$, $p = 0.057$).

3.6. Experiment 6: Effects of QPS on motor threshold, recruitment curves and somatotopy

3.6.1. Motor threshold and recruitment curve

The MTs (RMT and AMT) did not change significantly after QPS-supra-360 (Fig. 7a). However, the recruitment curve after QPS-supra-360 became steeper (Fig. 7b) (two-way repeated ANOVA: effect of time, $F[1,4] = 87.481$, $p = 0.001$; effect of intensity, $F[1.355, 5.421] = 19.324$, $p = 0.005$; intensity × time interaction, $F[1.328, 5.311] = 11.376$, $p = 0.015$).

3.6.2. Cortical MEPs from the FCU muscle

The QPS-supra-360 showed topographically specific modulation of the motor cortex (Fig. 8). Paired t-tests showed a significant facilitation of cortical MEPs from FDI muscle ($t = -4.492$, $p = 0.011$), although no significant changes were evoked in cortical MEPs from FCU muscle ($t = -1.119$, $p = 0.326$).

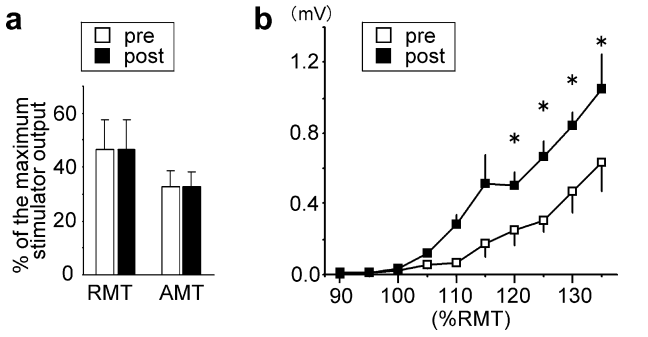

Fig. 7. Effects of QPS on motor threshold and recruitment curves. (a) RMT and AMT before (pre, bars) and after (post, filled bars) QPS-supra-360 ($n = 5$). The bars show the mean ± SD threshold as a percentage of the maximum stimulator output (MSO). No changes in RMT and AMT were found after QPS conditioning (RMT pre: 46.6 ± 11.1%, RMT post: 46.4 ± 11.2%, $p > 0.05$; AMT pre: 32.8 ± 5.9%, AMT post: 32.6 ± 5.7%, $p > 0.5$, by paired t-test). (b) Recruitment curves before (pre, squares) and after (post, filled squares) QPS-supra-360 ($n = 5$). The abscissa shows the stimulus intensity relative to the motor threshold. The ordinate shows absolute peak-to-peak MEP amplitudes (mV, mean ± SE). The recruitment curve after QPS-supra-360 became steeper than that before (two-way repeated ANOVA: intensity × time (pre and post), $p < 0.05$; effect of time, $p < 0.005$; effect of intensity, $p < 0.01$). Asterisks, $p < 0.05$, by post hoc analysis using Bonferroni's method.

3.7. Experiment 7: Safety study

Post-TMS EMG activity was observed in two of three studied subjects (Fig. 9), but SE was not observed during either QPS-supra-360 or QPS-sham-360 in any subject. The occurrence rate of post-TMS EMG activity during QPS-supra-360 was not different from that during QPS-sham-360 (Table 1).

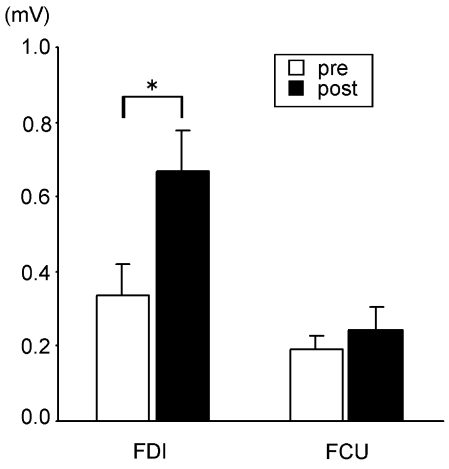

Fig. 8. Effects of QPS on somatotopy. Absolute amplitudes of cortical MEPs recorded from FDI and FCU muscles before (pre, bars) and after (post, filled bars) QPS-supra-360 applied over the *hot spot* for FDI ($n = 5$). Each bar shows the MEP amplitude (mV, mean ± SE). Only cortical MEPs from FDI muscle were facilitated after QPS conditioning. Asterisks, $p < 0.05$, by paired t-test.

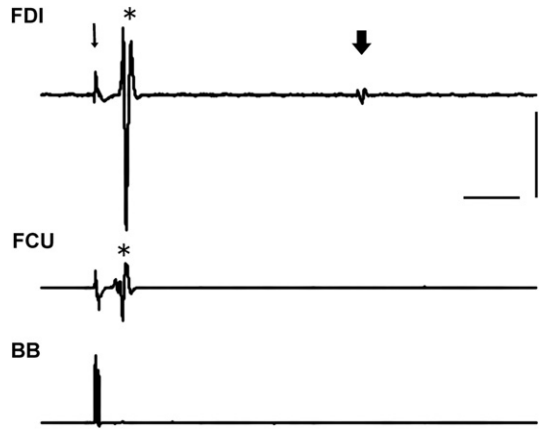

Fig. 9. Representative example of post-TMS EMG activity. Surface EMGs were recorded simultaneously from the right first dorsal interosseus (FDI), flexor calpi ulnaris (FCU) and biceps brachii (BB) muscles during QPS-supra-360. They are single EMGs from three muscles. Post-TMS EMG activity (arrow) was observed following a QPS train. The thin arrow indicates an artifact of stimulation. Asterisks, MEP elicited by one train of four magnetic pulses. Calibration bars, 50 ms, 0.5 mV.

Table 1
Rate of post-TMS EMG activity during QPS-supra-360 and QPS-sham-360

Subject	Post-TMS EMG activities/trains	
	QPS-supra-360	QPS-sham-360
A	8/360	18/360
B	0/360	0/360
C	7/360	8/360

4. Discussion

4.1. Comparison between QPS and PPS

The main finding of this study was that a long-lasting cortical MEP facilitation was induced by QPS. Results showed that the total number of pulses or the number of trains was unable to account for the disparity of duration of aftereffects between QPS and PPS. Therefore, the number of pulses per train might be critical for determining the duration of aftereffects, similar to the well-known results of animal experiments (Nakao et al., 2004).

The effects on MEPs during conditioning differed between QPS and PPS (Fig. 2), although MEPs to single-pulse TMS immediately after each conditioning were similarly facilitated (Fig. 3b). More specifically, we observed no marked facilitation of MEPs to paired TMS during PPS-supra-360, but MEPs to single-pulse TMS were enhanced immediately after conditioning. In addition, MEPs to QPS at the end of QPS-supra-360 were more enhanced than MEPs to single-pulse TMS immediately after conditioning. A possible explanation for this discrepancy in MEP size between the last part of conditioning and the first part of aftereffects is that different populations of descending volleys contribute to MEP generation in single-pulse, paired-pulse, and quadro-pulse TMS. At the end of both conditioning types, the motor cortical excitability might be similarly enhanced because the MEPs obtained immediately after the QPS and PPS conditioning were almost equivalent. However, the MEPs collected at the end of conditioning were different in size between the two conditioning types because one was MEP to paired-pulse TMS and the other to quadro-pulse TMS. In any case, a similar amount of facilitation was induced by both QPS and PPS when we evaluated motor cortical excitability, as indexed by MEP amplitude to single-pulse TMS, whereas it continued longer after QPS than PPS. These results support the hypothesis that the number of pulses per train plays an important role in determining the duration of the aftereffects and not so for the degree of size enhancement. Another implication from results of the present investigation is that single-pulse TMS should be used for correctly probing motor cortical excitability.

4.2. Possible origin of QPS aftereffects

In fact, QPS did not enhance the brain-stem MEPs. This finding strongly suggests that cortical MEP facilitation after QPS occurs at the motor cortex. Several possible explanations exist for cortical MEP facilitation during and after QPS.

First, excitability changes of the postsynaptic neuronal membrane of corticospinal neurons might occur after QPS (Woody et al., 1991; Aou et al., 1992). Several precedent pharmacological studies have proposed that motor thresholds reflect postsynaptic neuronal membrane excitability and a part of synaptic efficacy within the motor cortex (Mavroudakis et al., 1994, 1997; Inghilleri et al., 1996; Ziemann et al., 1996a,b; Chen et al., 1997a; Di Lazzaro et al., 2003). Because motor thresholds (both AMT and RMT) were unchanged after QPS (Fig. 7a), the neuronal membrane excitability changes might not have contributed to cortical MEP facilitation after QPS.

Second, an enhancement of cortico-cortical synaptic efficacy within the motor cortex might explain the aftereffects of QPS. Although motor thresholds were unchanged, QPS made the recruitment curves steeper (Fig. 7b). These results are probably attributable to the induction of an enhanced synaptic efficacy, which contributes much to the generation of MEPs, but little to the threshold determination (threshold depends on synapses for small sized MEPs). The enhancement of synaptic efficacy might occur either at the synapses between the corticospinal neurons and interneurons or between interneurons within the motor cortex. Whichever synaptic efficacy enhancement occurs, the spatial distribution of excitable elements can change, and more motor cortical neurons might be activated by stimuli at the same intensity, which in turn would cause a steeper recruitment curve.

Third, the aftereffect might be explained by a decrease of synaptic efficacy at the inhibitory interneurons. For example, rTMS, such as TBS, elicited a mixture of facilitatory and inhibitory effects on synaptic transmission (Huang

et al., 2005). Similarly, the magnitude of aftereffects of QPS presumably depends on the balance between inhibition and facilitation.

Fourth, a recent study examined the direct recording of descending volleys before, during, and after PPS at I-wave periodicity in a single subject (Di Lazzaro et al., 2007). The authors concluded that the facilitation produced by PPS at I-wave periodicity might involve circuits different from those involved in I-wave generation (Di Lazzaro et al., 2007). A similar mechanism might underlie the facilitatory aftereffects by QPS.

Despite the lack of any experimental evidence, the properties shown here might provide some clues for speculation on the mechanism of action of QPS-induced plasticity.

4.3. Properties and possible mechanisms for QPS aftereffects

Sustained and long-lasting modulation of MEP amplitudes has been proposed to reflect LTP-like plasticity of the human motor cortex by several rTMS interventions (Chen et al., 1997b; Ziemann et al., 1998; Stefan et al., 2000; Wolters et al., 2003; Huang et al., 2005; Quartarone et al., 2006). The long duration of the aftereffects of QPS (longer than 60 min) is comparable to the durations described in those studies.

According to previous knowledge of synaptic plasticity, one characteristic of LTP is cooperativity, which implies the existence of a threshold for LTP induction, depending on the stimulus intensity, the total number of pulses, and the pattern of tetanic stimulation (Malenka, 1991; Trepel and Racine, 1998). We found a threshold of QPS train numbers for inducing long-lasting motor cortical enhancement. More specifically, 360 trains of QPS induced a long-lasting MEP facilitation, although 180 trains of QPS did not.

Another characteristic of LTP is input specificity (Bliss and Collingridge, 1993), which means that LTP occurs only along the pathways activated by the conditioning stimulation: not along other pathways. We confirmed that QPS applied to the left-hand motor area for FDI induced facilitation of the right FDI, but not FCU.

These properties of QPS (the duration of facilitation, *cooperativity* and *input specificity*) led us to conjecture that QPS induces LTP or closely related phenomena in the human motor cortex. However, a limitation of our investigation is that we showed no other basic properties of LTP (i.e., *N*-methyl-D-aspartate (NMDA) dependency and associativity; Bliss and Collingridge, 1993). Notwithstanding, the present results provide at least some clues indicating the mechanism of action of rTMS. Further studies are necessary to clarify the mechanism of plasticity induction by QPS.

4.4. Safety issues

No subject reported any adverse effect during or after an intervention. Moreover, the spread of excitation to proximal muscles was not observed. The occurrence rate of post-TMS EMG activity during QPS-supra-360 was not different from that during QPS-sham-360. In many cases, it is difficult to distinguish post-TMS EMG activity from poor muscle relaxation (Chen et al., 1997b). Consequently, occurrence of post-TMS EMG activity during QPS-sham-360 might be a false positive indicating some voluntary activities. The present interpretation of these as after discharges is a kind of conservative approach we adopted for safety monitoring. These findings provide evidence that QPS can safely induce motor cortical plasticity. However, in the safety guideline of rTMS (Wassermann, 1998), a safe number of pulses at high-frequency stimulation (1–25 Hz) were determined based on the stimulus intensity normalized to RMT. Consequently, the establishment of new safety guidelines for complex rTMS protocols such as QPS, PPS (Thickbroom et al., 2006), or TBS (Huang et al., 2005) awaits further investigation. Although we never observed SE or any significant increment of after discharges during QPS, adequate EMG monitoring is necessary to recognize early signs of serious side effects during future monophasic rTMS studies.

4.5. Conclusion

We conclude that QPS can induce long-lasting, topographically specific enhancement of motor cortical excitability safely, which might be a consequence of the induction of LTP or closely related phenomena in the human motor cortex. Although we cannot define mechanisms of the QPS aftereffects, the present results provide useful information for further studies addressing the mechanism of plasticity induction by QPS.

Acknowledgements

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