$p = 0.05$. Then, the coordinates that reached the significance level in a MNI standard brain model were transformed into the Talairach and Tournoux coordinates (Talairach and Tournoux, 1988).

3. Results

3.1. SEP

3.1.1. Experiment 1 (phase)

Fig. 2 shows the grand-averaged waveforms obtained from 8 subjects. Because of the variations of latencies across the subjects, grand-averaged waveforms were constructed by adjusting the time to coincide the P14 peaks of each average. At all electrodes, the subcortical far-field P14 component was the first activity detected in all subjects. Table 1 shows the peak amplitudes of each component before and after application of monophasic 0.2 Hz, biphasic 1 Hz, biphasic 0.2 Hz and sham rTMS. In the analysis of SEP amplitudes, only those of N30 component at F3 reached a significance level in both one-way repeated measures ANOVA [$F(3,28) = 3.156$, $p = 0.046$] and post-hoc test; the ratio in monophasic 0.2 Hz rTMS condition was significantly larger than that in sham condition ($p = 0.035$) (Fig. 2 and Table 1). Biphasic 0.2 and 1 Hz rTMS conditions showed no significant differences with sham condition.

As for the duration of after-effects, only monophasic 0.2 Hz rTMS condition was studied here, one-way repeated-measures AN-OVA reached a significance level [$F(2,18) = 14.408$, $p = 0.001$]. Post-

hoc test showed that N30 amplitude increased immediately after rTMS than before rTMS ($p = 0.001$), whereas 30 min after rTMS was not different with that before rTMS (Fig. 3).

3.1.2. Experiment 2 (frequency)

In the analysis of SEP amplitudes, only those of N30 component at F3 reached a significance level in both one-way repeated-measures ANOVA [$F(2,24) = 6.328$, $p = 0.009$] and post-hoc test; the ratio in monophasic 0.2 Hz ($p = 0.003$) or 0.8 Hz ($p = 0.009$) rTMS condition was significantly larger than that in sham condition (Fig. 4 and Table 2).

3.2. SPECT (rCBF)

The analyses of SPECT images revealed significant increases of cerebral blood flow in several regions after the application of monophasic 0.2 Hz and biphasic 1 Hz rTMS over premotor cortex, whereas no regions showed significant decreases. Fig. 5 shows the areas with significant increases in rCBF after rTMS, and Table 3 shows their exact coordinates. In monophasic 0.2 Hz rTMS condition, areas with significant increases in blood flow included the left frontal gyrus (Brodmann area 6), which were under or near the magnetic coil, and in addition the left thalamus (Fig. 5 and Table 3). In biphasic 1 Hz rTMS, significant increases were found in the left occipital and the right parietal lobes (Fig. 5 and Table 3), but there was no increase in the motor cortices.
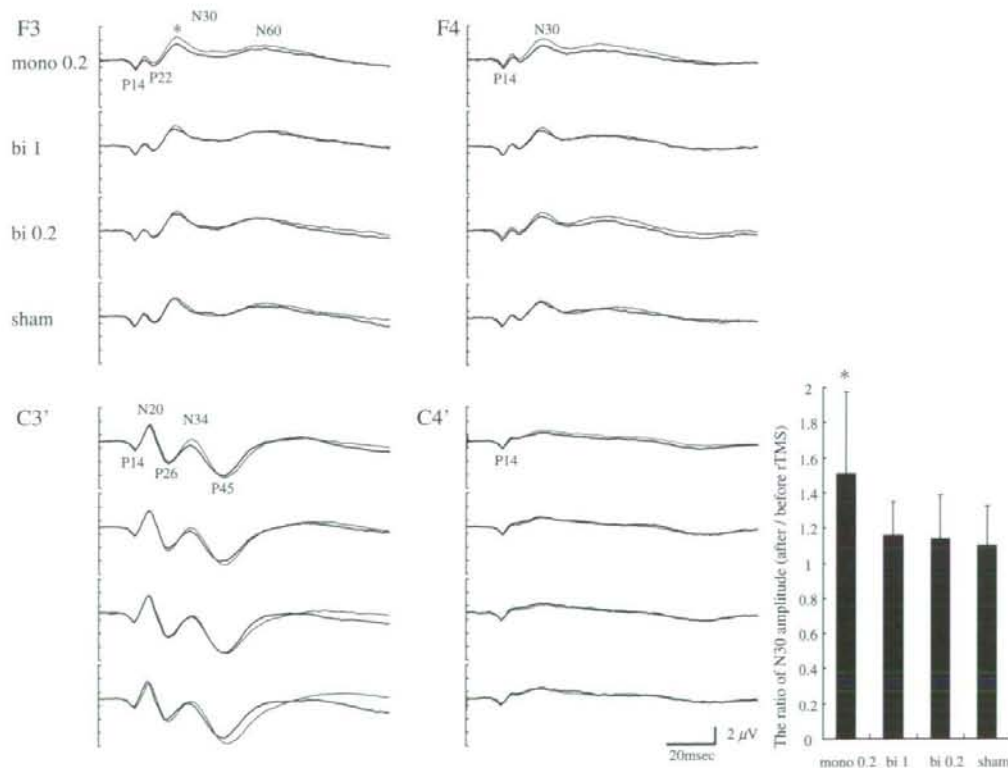

Fig. 2. Grand-averaged SEPs waveforms are recorded from the electrodes at F3, C3', F4 and C4' before (thick wave) and after (thin wave) monophasic 0.2 Hz, biphasic 1 Hz, biphasic 0.2 Hz and sham rTMS. The ratios of N30 amplitude (after/before rTMS) at F3 in all four stimulus conditions (mean ± SD). Asterisks show significant difference with the value in sham condition by Dunnett's multiple-comparison test (*$p < 0.05$). Mono 0.2, monophasic 0.2 Hz condition; bi 1, biphasic 1 Hz condition; bi 0.2, biphasic 0.2 Hz condition; and sham, sham condition.

Table 1
Peak amplitudes of each SEP component before and after the application of monophasic 0.2 Hz, biphasic 1 Hz, biphasic 0.2 Hz and sham rTMS

F3

	P14 Before	P14 After	P14 Δ	P22 Before	P22 After	P22 Δ	N30 Before	N30 After	N30 Δ	N60 Before	N60 After	N60 Δ
Mono 0.2	1.47 ± 0.41	1.22 ± 0.34	0.87 ± 0.24	1.22 ± 0.54	0.75 ± 0.83	0.37 ± 0.78	−2.66 ± 1.31	−3.70 ± 1.38	**1.51 ± 0.47**	−2.00 ± 1.16	−2.50 ± 1.70	1.36 ± 0.76
Bi 1	1.41 ± 0.37	1.46 ± 0.39	1.04 ± 0.15	0.94 ± 0.77	1.17 ± 0.81	−3.28 ± 12.84	−2.74 ± 1.62	−3.13 ± 1.82	1.16 ± 0.19	−2.41 ± 1.22	−2.46 ± 1.17	1.16 ± 0.53
Bi 0.2	1.45 ± 0.45	1.38 ± 0.41	0.97 ± 0.16	1.23 ± 0.68	1.46 ± 0.96	2.62 ± 4.28	−2.95 ± 1.56	−3.23 ± 1.62	1.13 ± 0.25	−2.26 ± 1.38	−2.29 ± 0.85	1.15 ± 0.37
Sham	1.47 ± 0.56	1.41 ± 0.54	1.21 ± 0.94	1.19 ± 1.08	1.18 ± 0.87	2.71 ± 4.63	−2.60 ± 1.30	−2.89 ± 1.78	1.10 ± 0.22	−2.00 ± 0.73	−2.02 ± 1.71	0.92 ± 0.55

F4

	P14 Before	P14 After	P14 Δ	N30 Before	N30 After	N30 Δ
Mono 0.2	1.36 ± 0.53	0.97 ± 0.41	0.76 ± 0.48	−2.39 ± 1.01	−3.44 ± 1.31	1.61 ± 0.81
Bi 1	1.37 ± 0.32	1.42 ± 0.44	1.04 ± 0.24	−2.49 ± 1.25	−2.84 ± 1.17	1.32 ± 0.79
Bi 0.2	1.56 ± 0.29	1.12 ± 0.55	0.69 ± 0.23	−2.40 ± 1.26	−3.00 ± 1.18	1.38 ± 0.48
Sham	1.26 ± 0.60	1.30 ± 0.84	1.81 ± 2.80	−2.53 ± 1.10	−2.61 ± 1.41	1.04 ± 0.25

C3'

	P14 Before	P14 After	P14 Δ	N20 Before	N20 After	N20 Δ	P26 Before	P26 After	P26 Δ	N34 Before	N34 After	N34 Δ	P45 Before	P45 After	P45 Δ
Mono 0.2	1.45 ± 0.32	1.26 ± 0.36	0.89 ± 0.22	−2.64 ± 1.18	−2.93 ± 1.30	1.11 ± 0.25	3.67 ± 2.23	3.54 ± 2.29	3.78 ± 7.88	0.37 ± 1.30	−0.51 ± 1.09	−3.67 ± 6.67	5.53 ± 2.93	5.72 ± 2.94	1.07 ± 0.25
Bi 1	1.35 ± 0.38	1.40 ± 0.37	1.05 ± 0.18	−2.91 ± 1.05	−2.87 ± 1.12	0.98 ± 0.11	3.49 ± 2.22	4.03 ± 3.12	1.18 ± 0.43	0.41 ± 0.70	−0.07 ± 1.09	−3.09 ± 27.19	5.53 ± 3.05	5.85 ± 3.85	1.04 ± 0.24
Bi 0.2	1.48 ± 0.31	1.36 ± 0.33	0.93 ± 0.15	−2.95 ± 1.27	−3.04 ± 1.28	1.04 ± 0.09	3.88 ± 2.28	4.05 ± 2.96	0.97 ± 0.39	0.28 ± 1.05	0.14 ± 1.59	−4.32 ± 19.03	6.32 ± 3.57	6.33 ± 3.09	1.03 ± 0.17
Sham	1.40 ± 0.45	1.48 ± 0.36	1.11 ± 0.29	−2.65 ± 1.34	−2.49 ± 1.39	0.90 ± 0.13	3.55 ± 2.54	3.79 ± 2.62	4.72 ± 10.62	0.36 ± 0.95	0.08 ± 1.11	2.42 ± 3.93	5.92 ± 3.83	6.86 ± 4.31	1.17 ± 0.09

C4'

	P14 Before	P14 After
Mono 0.2	1.16 ± 0.62	1.12 ± 0.23
Bi 1	1.15 ± 0.31	1.32 ± 0.49
Bi 0.2	1.18 ± 0.25	1.18 ± 0.65
Sham	1.03 ± 0.51	1.15 ± 0.55

Values are expressed as means ± SD. Δ indicates the ratios of amplitudes in each SEP component before and after rTMS were calculated. Bold figures show the significantly increased value than sham rTMS by Dunnett's multiple comparison test. Mono 0.2, monophasic 0.2 Hz condition; bi 1, biphasic 1 Hz condition; bi 0.2, biphasic 0.2 Hz condition; and sham, sham condition.
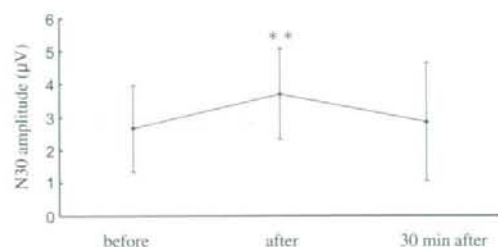
Fig. 3. The amplitudes of the N30 component were recorded at F3 before, immediately after and 30 min after the end of monophasic 0.2 Hz rTMS in 7 of 8 subjects (means ± SD). Asterisks show significant difference with the value before rTMS by Dunnett's multiple-comparison test ($^{**}p < 0.01$).

4. Discussion

We compared the effects of rTMS on SEPs and rCBF under different stimulus conditions: phase (monophasic *versus* biphasic 0.2 Hz) or frequency (0.2 *vs.* 0.8 Hz monophasic). These analyses disclosed that the monophasic rTMS was more effective than biphasic rTMS, whereas the effects of 0.2 and 0.8 Hz monophasic rTMS were similar. For SPECT studies, monophasic 0.2 Hz rTMS increased rCBF at premotor cortex, whereas biphasic 1 Hz rTMS had no effect on the motor cortices.

Differences between monophasic and biphasic stimulation were reported in the previous studies with rTMS over MC (Sommer et al., 2002; Arai et al., 2005; Tings et al., 2005). The authors reasoned that monophasic rTMS tends to activate predominately one kind of neurons such as facilitatory interneuron in particular direction and summate such effects, whereas biphasic rTMS tends to activate several kinds of neurons including interneuron in random direction simultaneously and leads to

cancellation of effects. Our study of SEPs provided further evidence that monophasic rTMS has higher efficacy than biphasic rTMS over premotor cortex as well as MC.

The different areas were activated for rCBF between monophasic and biphasic stimulation. After the application of biphasic 1 Hz rTMS over premotor cortex, blood flow was increased on the distant regions from the coil, such as the left occipital and right parietal lobes. Siebner et al. (2003) reported that these areas remote from the stimulus sites were activated by rTMS. They reasoned that biphasic 1 Hz rTMS had a remote effect on distant areas through intercortical pathways. Another study has suggested that these remote effects were produced from activation of transcallosal pathways (Okabe et al., 2003). Alternative possibility is that the difference in stimulus frequency might affect mental activities, which could account for the activation of non-motor cortices. By contrast, monophasic 0.2 Hz rTMS in our study significantly increased rCBF under or near the stimulation sites, which most likely represent the direct effect of rTMS.

The rCBF reflects cerebral metabolism, and both excitatory and inhibitory synaptic activities consume energy and therefore involve metabolic activity. Loo et al. (2003) proposed that the effect of rTMS is a complex mix of activity at excitatory and inhibitory synapses as well as neuronal firing. An increase in blood flow could be consistent with an overall reduction in physiological activity if this is caused by an increase of inhibition.

It is conceivable that an increase in N30 also reflects inhibition rather than facilitation, the opposite to the N30 decreasing effect of motor imagery (Cheron and Borenstein, 1992). Therefore, increases in inhibitory interneuronal activities could increase rCBF while decreasing the cortical excitability.

The frequency-dependent effect was reported in the previous study using biphasic stimulation of 1–5 Hz frequencies (Siebner et al., 2001). In this study, however, monophasic 0.2 and 0.8 Hz rTMS were not different in the ratios of N30 amplitude. This result
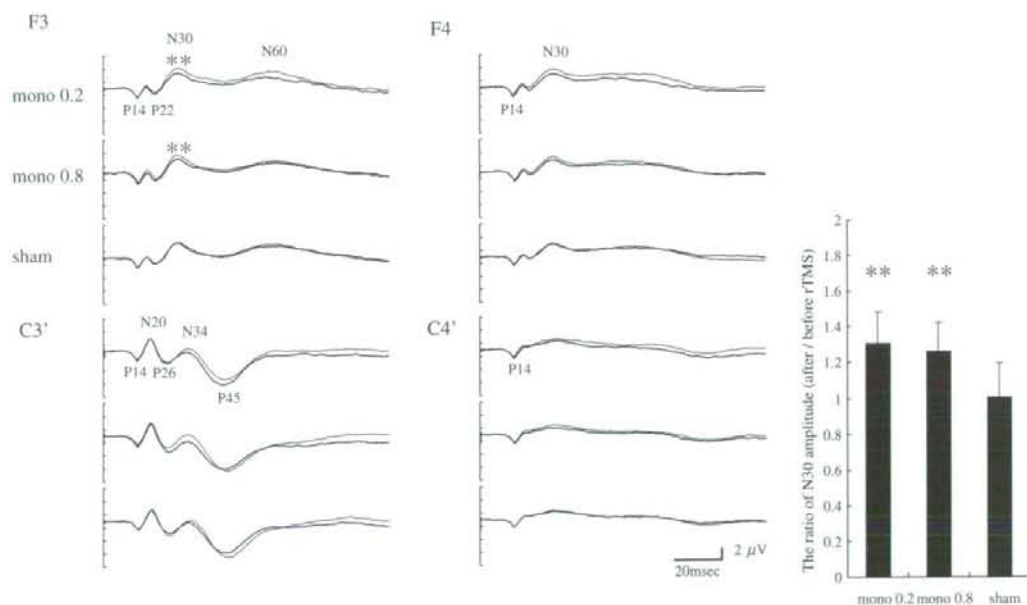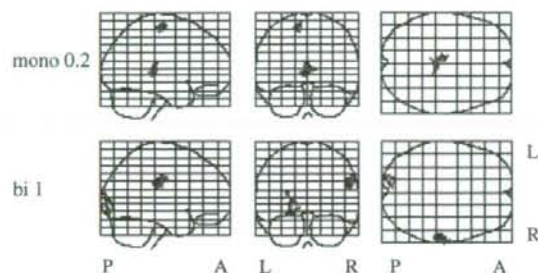

Fig. 4. Grand-averaged SEPs waveforms are recorded from the electrodes at F3, C3′, F4 and C4′ before (thick wave) and after (thin wave) monophasic 0.2 Hz, monophasic 0.8 Hz and sham rTMS. The ratios of N30 amplitude (after/before rTMS) at F3 in all three stimulus conditions (means ± SD). Asterisks show significant difference with the value in sham condition by Dunnett's multiple-comparison test ($^{**}p < 0.01$). Mono 0.2, monophasic 0.2 Hz condition; mono 0.8, monophasic 0.8 Hz condition; and sham, sham condition.

Table 2
Peak amplitudes of each SEP component before and after the application of monophasic 0.2 Hz, monophasic 0.8 Hz and sham rTMS

F3

	P14			P22			N30			N60		
	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ
Mono 0.2	1.51 ± 0.40	1.41 ± 0.42	0.98 ± 0.38	1.26 ± 1.27	0.99 ± 1.16	1.33 ± 1.03	−2.76 ± 1.34	−3.53 ± 1.62	**1.31 ± 0.18**	−2.02 ± 1.19	−2.92 ± 1.34	4.67 ± 9.83
Mono 0.8	1.70 ± 0.43	1.36 ± 0.31	0.83 ± 0.23	1.42 ± 1.14	1.07 ± 1.21	0.73 ± 0.54	−2.43 ± 1.17	−3.01 ± 1.42	**1.27 ± 0.16**	−1.87 ± 0.78	−2.19 ± 1.27	1.35 ± 0.99
Sham	1.56 ± 0.53	1.41 ± 0.39	1.13 ± 0.86	1.28 ± 1.26	1.15 ± 0.98	1.85 ± 4.44	−2.54 ± 1.40	−2.71 ± 1.91	1.00 ± 0.19	−1.98 ± 0.66	−2.38 ± 1.34	1.22 ± 0.50

F4

	P14			N30		
	Before	After	Δ	Before	After	Δ
	1.51 ± 0.48	1.20 ± 0.37	0.82 ± 0.19	−2.48 ± 1.00	−3.13 ± 1.35	1.28 ± 0.26
	1.45 ± 0.43	1.26 ± 0.41	0.89 ± 0.26	−2.07 ± 0.90	−2.55 ± 1.26	1.24 ± 0.27
	1.29 ± 0.54	1.39 ± 0.58	1.83 ± 2.54	−2.23 ± 1.14	−2.36 ± 1.43	1.05 ± 0.23

C3′

	P14			N20			P26			N34			P45		
	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ
Mono 0.2	1.56 ± 0.57	1.33 ± 0.52	0.88 ± 0.25	−2.47 ± 1.27	−2.68 ± 1.36	1.09 ± 0.17	3.08 ± 2.89	2.80 ± 3.26	0.79 ± 0.65	−0.67 ± 1.71	−1.05 ± 1.26	0.98 ± 4.00	5.53 ± 3.90	4.83 ± 3.40	0.97 ± 0.36
Mono 0.8	1.69 ± 0.68	1.39 ± 0.49	0.90 ± 0.33	−2.31 ± 1.20	−2.69 ± 1.71	1.13 ± 0.20	3.37 ± 3.52	2.80 ± 2.67	0.84 ± 0.28	0.27 ± 1.46	−0.84 ± 1.10	0.99 ± 4.70	5.66 ± 4.26	5.82 ± 4.57	1.00 ± 0.28
Sham	1.41 ± 0.38	1.41 ± 0.47	1.02 ± 0.34	−2.31 ± 1.34	−2.18 ± 1.28	0.95 ± 0.20	2.67 ± 2.82	2.78 ± 2.75	0.97 ± 0.67	−0.42 ± 0.98	−0.53 ± 1.07	0.40 ± 1.21	5.37 ± 3.96	6.03 ± 4.41	1.21 ± 0.35

C4′

	P14		
	Before	After	Δ
	1.40 ± 0.58	1.16 ± 0.43	0.93 ± 0.44
	1.39 ± 0.52	1.25 ± 0.33	0.99 ± 0.41
	1.17 ± 0.59	1.16 ± 0.51	4.26 ± 10.03

Values are expressed as means ± SD. Δ indicates the ratios of amplitudes in each SEP component before and after rTMS were calculated. Bold figures show the significantly increased value than sham rTMS by Dunnett's multiple-comparison test. Mono 0.2, monophasic 0.2 Hz condition; mono 0.8, monophasic 0.8 Hz condition; and sham, sham condition.

Fig. 5. Parametric statistical MIP maps show the blood flow increase areas after the application of monophasic 0.2 Hz (mono 0.2) and biphasic 1 Hz (bi 1) rTMS over premotor cortex (see also Table 3). A, anterior; P, posterior; R, right; and L, left.

Table 3
Regional CBF increase areas after monophasic 0.2 Hz and biphasic 1 Hz rTMS over premotor cortex

Condition	Brain region	x	y	z	Z score
Monophasic 0.2 Hz	Left thalamus	0	−23	12	5.03
	Left frontal gyrus (Brodmann area 6)	−8	−11	58	4.27
Biphasic 1 Hz	Left occipital lobe (Brodmann area 17)	−16	−84	−1	4.51
	Right parietal lobe (Brodmann area 1)	63	−16	−6	3.99

The panel shows the Talairach coordinates and Z scores of maximal peaks of regions (see also Fig. 5).

suggested that the frequency-dependent effect could not be approved after very low-frequency ($\leqslant 1$ Hz) monophasic rTMS.

In conclusion, our data indicated that monophasic has more profound physiological effect on premotor cortex than biphasic stimulation.

Acknowledgement

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Bidirectional long-term motor cortical plasticity and metaplasticity induced by quadripulse transcranial magnetic stimulation

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Repetitive transcranial magnetic stimulation (rTMS) has emerged as a promising tool to induce plastic changes that are thought in some cases to reflect N-methyl-D-aspartate-sensitive changes in synaptic efficacy. As in animal experiments, there is some evidence that the sign of rTMS-induced plasticity depends on the prior history of cortical activity, conforming to the Bienenstock–Cooper–Munro (BCM) theory. However, experiments exploring these plastic changes have only examined priming-induced effects on a limited number of rTMS protocols, often using designs in which the priming alone had a larger effect than the principle conditioning protocol. The aim of this study was to introduce a new rTMS protocol that gives a broad range of after-effects from suppression to facilitation and then test how each of these is affected by a priming protocol that on its own has no effect on motor cortical excitability, as indexed by motor-evoked potential (MEP). Repeated trains of four monophasic TMS pulses (quadripulse stimulation: QPS) separated by interstimulus intervals of 1.5–1250 ms produced a range of after-effects that were compatible with changes in synaptic plasticity. Thus, QPS at short intervals facilitated MEPs for more than 75 min, whereas QPS at long intervals suppressed MEPs for more than 75 min. Paired-pulse TMS experiments exploring intracortical inhibition and facilitation after QPS revealed effects on excitatory but not inhibitory circuits of the primary motor cortex. Finally, the effect of priming protocols on QPS-induced plasticity was consistent with a BCM-like model of priming that shifts the crossover point at which synaptic plasticity reverses from depression to potentiation. The broad range of after-effects produced by the new rTMS protocol opens up new possibilities for detailed examination of theories of metaplasticity in humans.

Repetitive transcranial magnetic stimulation (rTMS), which activates cortical-output neurons trans-synaptically (Day *et al.* 1989), is a promising tool to induce lasting plastic changes in humans that are thought in some cases to reflect N-methyl-D-aspartate (NMDA)-sensitive changes in synaptic efficacy such as long-term potentiation (LTP) and long-term depression (LTD) (Stefan *et al.* 2002; Huang *et al.* 2007). As in animal experiments, in which prior history of neuronal activity determines the sign and magnitude of subsequent synaptic plasticity (i.e. metaplasticity; Abraham & Bear, 1996), some evidence indicates that the direction of the rTMS-induced plasticity depends on prior cortical activity (e.g. Iyer *et al.* 2003; Ziemann *et al.* 2004). The implication is that non-invasive methods might be able to probe the homeo-

static mechanisms which prevent neuronal circuits from becoming destabilized and which maintain synapses within a dynamic range of modifiability (Abbott & Nelson, 2000).

Previous experiments explored the effects of priming stimulation on only a limited number of rTMS protocols, often using designs in which the effects of priming protocols alone were larger than those of the principle conditioning protocol. For example, Lang *et al.* (2004) and Siebner *et al.* (2004) used a priming protocol that itself produced lasting increase of motor cortical excitability whereas the primary conditioning alone had no effect on MEP size. More recently, Müller *et al.* (2007) employed two sessions of paired associative stimulation, both of which could modulate MEP amplitudes alone. Although

the results were in accord with the BCM model of synaptic metaplasticity, data from animal studies show that prior induction of LTP or LTD is not essential for changing subsequent synaptic plasticity (Huang et al. 1992; Abraham & Tate, 1997; Abraham, 2008). Even if the priming protocol was below threshold for LTP (i.e. it induced only transient increase of basic synaptic efficacy), subsequent tetanic stimulation, which was able to induce LTP when given alone, failed to elicit LTP (Huang et al. 1992; Abraham & Tate, 1997). In the present experiments we tested whether it was possible to produce similar effects in human primary motor cortex using a priming protocol that itself was unable to evoke any lasting effects on cortical excitability.

The experiments use a new rTMS protocol termed quadripulse stimulation (QPS). In their original description of QPS, Hamada et al. (2007b) applied four equal monophasic TMS pulses at an interstimulus interval (ISI) of 1.5 ms. When repeated at a rate of 0.2 Hz for 30 min, this led to significant increases in MEP amplitude lasting more than an hour. Here we employ QPS with a range of ISIs from 1.5 to 1250 ms and show that increasing the ISI leads to a gradual shift in the sign of the after-effect from facilitation to suppression. We then test how the threshold ISI at which this transition occurs is affected by priming. This allows for a direct comparison with the *sliding* modification threshold (θ_M) of the Bienenstock–Cooper–Munro (BCM) theory (Bienenstock et al. 1982).

Methods

Subjects

Subjects were 10 healthy volunteers (three women, seven men; 27–53 years old, mean ± S.D., 38.6 ± 6.9 years) who gave their written informed consent to participate in the experiments. No subjects had neurological, psychiatric, or other medical problems, or had any contra-indication 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*.

Recordings

Subjects were seated on a comfortable chair. Motor-evoked potentials (MEPs) were recorded from the right first dorsal interosseous muscle (FDI). Pairs of Ag/AgCl surface cup electrodes (9 mm diameter) were placed over the muscle belly (active) and the metacarpophalangeal joint of the index finger (reference). We also recorded surface electro-

myogram (EMG) from the abductor digiti minimi (ADM) and flexor carpi ulnaris (FCU) muscles on the same side in the experiment to study the input specificity and spread of excitation. Responses were input to an amplifier (Biotop; GE Marquette Medical Systems, Japan) through filters set at 100 Hz and 3 kHz; they were then digitized and stored on a computer for later offline analyses (TMS bistim tester; Medical Try System, Japan).

Stimulation

First, TMS was given over the hand area of the motor cortex using a hand-held figure-of-eight coil (9 cm external diameter at each wing; The Magstim Co. Ltd, Whitland, Dyfed, UK) placed tangentially over the scalp with the handle pointing backwards at about 45 deg laterally, which is perpendicular to the central sulcus. Single monophasic TMS pulses were delivered using a magnetic stimulator (Magstim 200; The Magstim Co. Ltd). Quadripulse stimuli were delivered using four magnetic stimulators (Magstim 200²; The Magstim Co. Ltd) connected with a specially designed combining module (The Magstim Co. Ltd). This device combines the outputs from four stimulators to allow a train of four monophasic magnetic pulses to be delivered through a single coil.

The optimal site for eliciting MEPs in the right FDI muscle (i.e. hot spot) was determined before each experiment. In this determination, we stimulated several positions 1 cm distant from each other using the same intensity. The hot spot was defined as the site at which the largest responses were elicited. This position was marked using a red pen on the scalp for repositioning the coil. Placing the coil over this position, 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 10 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), as observed using an oscilloscope monitor, in more than 5 of 10 consecutive trials. The stimulus intensity was changed in steps of 1% of the maximum stimulator output.

Experiment 1. ISI dependency of QPS-induced plasticity

Conditioning protocols consisted of 360 trains of TMS pulses with an inter-train interval (ITI) of 5 s (i.e. 0.2 Hz) over 30 min (total 1440 magnetic pulses) (Fig. 1A). Each train consisted of four magnetic pulses (i.e. quadripulse stimulation: QPS) separated by a certain interstimulus interval (ISI) 1.5 ms, 5 ms, 10 ms, 30 ms, 50 ms, 100 ms,

or 1250 ms. These conditioning types were designated as QPS-1.5 ms, QPS-5 ms, ···, QPS-1250 ms. The stimulus intensity of each pulse was set to 90% AMT. The trains were applied over the hot spot for FDI. During the conditioning, no MEPs were observed. The subjects participated in different protocols at least 1 week after the preceding experiment. The numbers of subjects participating in each experimental condition are presented in the Results section. The order of the experiments was randomized and counter-balanced among all subjects.

Motor cortical excitability was assessed by measuring the peak-to-peak amplitude of MEPs from the relaxed right FDI muscle for all experiments. During the experiments, the EMG activity of the FDI was monitored using an oscilloscope monitor. Trials contaminated with voluntary EMG activity were discarded from analyses. Before QPS, 20 MEPs were obtained every 14.5–15.5 s using single-pulse TMS at a fixed intensity. The stimulus intensity was adjusted to produce MEPs of about 0.4–0.5 mV in the right FDI muscle at baseline; the intensity was kept constant throughout the same experiment. After QPS conditioning, MEPs were measured every 5 min for 30 min and then every 15 min for 90 min. For one subject, we extended MEP measurements beyond 180 min. At each time point of the measurements, MEPs were collected in the same manner as baseline measurements. For each subject and time point, MEP amplitudes were averaged and normalized to the MEP amplitude measured at the baseline (i.e. baseline 1 in Fig. 1).

Experiment 2. Basic properties of QPS-induced plasticity

Experiment 2a. Motor thresholds and recruitment curves.
The AMT and RMT were measured; recruitment curves were constructed before and after QPS-5 ms ($n = 5$) or QPS-50 ms ($n = 5$). Stimuli were applied at the hot spot for FDI. After the AMT and RMT determination, eight stimuli were applied every 7.5–8.5 s at an intensity of 10% below RMT. The stimulus intensity was then increased by 5% and another eight stimuli were applied. This process was repeated until the intensity reached 135% RMT. The time of evaluation after QPS was 30 min after conditioning.

Experiment 2b. MEP from FDI, ADM and FCU muscles.
The MEPs from the right FDI and FCU were simultaneously recorded before and after QPS-5 ms ($n = 5$) or QPS-50 ms ($n = 5$) over the hot spot for FDI, in order to see the topographically specific modulation of QPS. They were collected in separate trials with 20 stimuli every 14.5–15.5 s. The stimulus intensity was set to elicit MEPs of 0.2–0.3 mV in the FCU muscle at the baseline. At 30 min after QPS, MEPs from target muscles were measured in the same manner as that used for baseline

measurements. Additionally, we also recorded MEPs from the right FDI and ADM muscles before and after QPS-5 ms or QPS-50 ms in same subjects in separate experiments. The stimulus intensity was set to elicit MEPs of 0.4–0.5 mV in the ADM muscle.

Experiment 2c. Motor cortical excitability accessed by paired-pulse TMS.
In 10 subjects, short-interval intra-cortical inhibition (SICI) and intracortical facilitation (ICF) were evaluated using the paired-pulse TMS technique described by Kujirai et al. (1993) to clarify the effects of QPS on excitatory and inhibitory circuits of the primary motor cortex.

First, SICI was examined at an interstimulus interval (ISI) of 3 and 4 ms using a conditioning stimulus (CS) intensity of 70%, 80% and 90% AMT and ICF at an ISI of 10 and 15 ms with a CS intensity of 90% AMT. The intensity of the test stimulus (TS) was adjusted to elicit MEPs of 0.4–0.5 mV in relaxed FDI. Second, short-interval intracortical facilitation (SICF) was examined at an ISI of 1.2, 1.3, 1.5, 1.7, 1.9 and 2.1 ms (Tokimura et al. 1996; Ziemann et al. 1998; Hanajima et al. 2002). The intensity of the first stimulus (S1) was adjusted to elicit MEPs of 0.4–0.5 mV and that of second stimulus (S2) was set at 10% below AMT.

In both studies, 15 trials were recorded for each condition and randomly intermixed with 15 trials with the TS alone. Inter-trial intervals were 6.5–7.5 s. Each experiment was performed in one session using the four magnetic stimulators (i.e. three stimulators produced the different CS intensities and one gave the TS). Because AMT did not change significantly after QPS-5 ms or QPS-50 ms (see Results), the CS intensity was kept constant. However, the TS intensity was adjusted to evoke test MEPs with amplitudes of 0.4–0.5 mV after QPS-5 ms or QPS-50 ms.

Measurements of SICI, ICF and SICF were performed in blocks immediately before (baseline) and just after QPS-5 ms or QPS-50 ms to the left primary motor cortex (M1) (post 1) as well as 30–60 min (post 2) after QPS-5 ms or QPS-50 ms. Each block lasted for approximately 30 min (20 min for SICI and ICF, and 10 min for SICF).

Experiment 3. Priming-induced effects on QPS-induced plasticity

To investigate metaplasticity of the human motor cortex, priming stimulation was performed prior to QPS conditioning (Figs 5 and 7).

Experiment 3a. QPS conditioning with QPS-5 ms priming.
Main experiments. Six subjects participated in this study. QPS-5 ms for 10 min (i.e. four pulses at an ISI of 5 ms with an ITI of 5 s for 10 min) was selected as the priming stimulation because LTP is occluded by high-frequency priming, which transiently enhances synaptic efficacy (i.e.

using a protocol below the threshold for LTP induction) (Huang *et al.* 1992). QPS-5 ms (at 90% AMT) for 10 min induces no substantial effects on MEP sizes (see Results). We presumed that even this priming stimulation, which did not induce an LTP-like phenomenon by itself, would have considerable effects on subsequent QPS-induced plasticity according to precedent animal studies, which showed that prior induction of LTP is not a requisite for metaplasticity (Abraham & Tate, 1997).

Before the priming stimulation (baseline 0 in Fig. 5A), 20 MEPs were collected every 14.5–15.5 s using single-pulse TMS at a fixed intensity, which was adjusted to elicit MEPs of about 0.4 mV in the right FDI muscle at baseline 0 and kept constant throughout the experiment. After priming, 20 MEPs were again obtained in the same manner as measurements at baseline 0 (baseline 1 in Fig. 5A). Following this measurement at baseline 1 (i.e. immediately after priming), QPS conditioning of various types (QPS-1.5 ms, QPS-5 ms, QPS-10 ms, QPS-30 ms, QPS-50 ms, and QPS-100 ms) was then performed for 30 min. The stimulus intensity of each pulse of QPS conditioning was set to 90% AMT. After each QPS, MEPs were measured every 5 min for 30 min.

Control experiments. In the first control experiment (Fig. 5B), priming stimulation (i.e. QPS-5 ms) was followed by sham conditioning stimulation (i.e. sham with priming) to examine whether the priming alone affects motor cortical excitability. In the second control experiment (Fig. 5C), sham priming stimulation was followed by QPS-10 ms to confirm that sham priming did not affect QPS-induced plastic changes (QPS-10 ms with sham priming). In fact, QPS-10 ms was chosen because this protocol induced mild facilitatory after-effects (see Results) rendering it more susceptible, if at all, to the effects of sham priming.

The sham stimulation procedure used for these control experiments was identical to those described in a previous report (Okabe *et al.* 2003). In brief, four electric pulses (each electric pulse was of 0.2 ms duration with an intensity of twice the sensory threshold) were given to the head skin at 0.2 Hz at an ISI of 10 ms for sham conditioning (first control experiment) and of 5 ms for sham priming (second control experiment) with 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 the vertex (Cz of international 10–20 system). A coil, which was not connected to the stimulator, was placed over the left-hand motor area to mimic real TMS. Another coil, which was connected to a combining module with four Magstim (2002) stimulators, was held off the scalp but placed near the subject. This coil was discharged simultaneously with the scalp electrical stimulation to produce a similar sound to that associated with real QPS. For each subject and time

point (including baseline 0), the MEP amplitudes were averaged and normalized to the MEP amplitude measured at baseline 1 (Fig. 5A).

Supplementary experiments. As shown in Results, QPS-5 ms priming for 10 min followed by 30 min of QPS-5 ms produced no lasting changes in MEP size. To clarify whether a pause between priming and conditioning is necessary to induce metaplastic changes, we performed two additional experiments.

First, we performed an experiment using QPS-5 ms for 40 min with an ITI of 5 s in 5 subjects (Fig. 6A). The stimulus intensity of each pulse was set to 90% AMT. We measured MEPs before and after conditioning in a manner similar to Experiment 1. Second, QPS-5 ms for 20 min with an ITI of 5 s at 90% AMT was applied to the hot spot for FDI in 5 subjects to verify whether QPS-5 ms for 30 min can be regarded as QPS-5 ms for 20 min with 10 min QPS-5 ms priming. MEPs were measured before and after this conditioning in the same manner as Experiment 1.

Experiment 3b. Effects of QPS-5 ms priming on SICI, ICF, SICF and LICI.

Nine subjects participated in this experiment. To explore the effects of QPS-5 ms priming alone on either excitatory or inhibitory circuits of the primary motor cortex, SICI, ICF, SICF and long-interval intracortical inhibition (LICI) (Valls-Sole *et al.* 1992; Wassermann *et al.* 1996) were all measured before and after QPS-5 ms priming (i.e. four pulses at an ISI of 5 ms with an ITI of 5 s for 10 min; the stimulus intensity of each pulse, 90% AMT).

SICI was examined at an ISI of 3 ms using a CS intensity of 80% AMT. ICF was measured at an ISI of 10 ms with a CS intensity of 90% AMT. SICF was measured at an ISI of 1.5 ms with a S2 intensity of 90% AMT. LICI was measured at an ISI of 100 ms with a CS intensity of 110% RMT. The intensity of TS was adjusted to elicit MEPs of 0.4–0.5 mV in relaxed FDI at baseline. Twelve trials were recorded for each condition and randomly intermixed with 12 trials of TS alone with an ITI of 7.5–8.5 s. The SICI, ICF, SICF and LICI were all studied simultaneously in one session (about 8 min). Measurements of these values were performed in blocks immediately before (baseline) and just after QPS-5 ms priming (post 1) as well as 20–28 min (post 2). Conditioning intensities and test intensity were not changed after priming.

Experiment 3c. QPS conditioning with QPS-50 ms priming.

Six subjects participated in this study. According to the BCM model, the crossover point can shift horizontally in either direction, so that priming stimulation that reduced neuronal activity might shift the crossover point leftward. Thus, QPS-50 ms for 10 min (i.e. four pulses at an ISI of 50 ms with an ITI of 5 s for 10 min; the stimulus intensity

of each pulse, 90% AMT) was selected as another priming stimulation because QPS-50 ms for 30 min produced significant MEP and SICF suppression (see Results). We expected that QPS-50 ms priming might shift the stimulus–response curve of QPS-induced plasticity to the left. Amplitudes of MEPs were measured in the same manner as Experiment 3a before and after QPS-10 ms, QPS-30 ms, or QPS-100 ms with the priming stimulation of QPS-50 ms for 10 min.

Data analyses

Experiment 1. The after-effects of different conditioning protocols were analysed with absolute MEP amplitudes using two-way repeated-measures analysis of variance (ANOVA) (between-subject factor, CONDITION (QPS-1.5 ms, QPS-5 ms, ···, QPS-1250 ms); within-subject factor, TIME (baseline 1 and following six time points)) because of the different numbers of subjects among conditions. If the factors CONDITION and TIME showed significant interaction, *post hoc* paired *t* tests (two-tailed) with Bonferroni's corrections for multiple comparisons were used for further analyses. The time course of after-effects of each conditioning type on absolute MEP sizes was analysed using one-way repeated measures ANOVA (within-subject factor, TIME). Dunnett's *post hoc* test was used for further analyses. The Greenhouse–Geisser correction was used if necessary to correct for non-sphericity; P values less than 0.05 were considered significant.

Experiment 2a. Recruitment curves before and after QPS were compared using two-way repeated-measures ANOVA (within-subject factors: TIME and INTENSITY). Bonferroni's *post hoc* test was used for further analyses. Paired *t* tests (two-tailed) were also used to compare variables (RMT and AMT) before and after QPS.

Experiment 2b. Absolute MEP amplitudes from FDI, ADM and FCU before and after QPS were pooled and compared using two-way repeated-measures ANOVA (within-subject factors: TIME and MUSCLE). *Post hoc* paired *t* tests (two-tailed) were used for further analyses.

Experiment 2c. The ratio of the mean amplitude of the conditioned response to that of the control response was calculated for each condition in each subject. These individual mean ratios were then averaged to give a grand mean ratio. The values of SICI (individual mean ratios) were entered in three-way repeated-measures ANOVA with conditioning INTENSITY (70% AMT, 80% AMT and 90% AMT), ISI (3 ms and 4 ms), and TIME (baseline, post 1, and post 2) as within-subject factors. For ICF and SICF, the ratios were compared using two-way repeated measures ANOVA (within-subject factors; ISI (10 ms and 15 ms for ICF; 1.2 ms, 1.3 ms, ···, 2.1 ms for SICF), and

TIME (baseline, post 1, and post 2)). Dunnett's *post hoc* test was used for further analyses.

Experiment 3a. *Main experiments.* Absolute values of MEPs at baseline 0 and baseline 1 (Fig. 5A) were compared using paired *t* tests in each experiment. To evaluate priming effects on subsequent QPS-induced plasticity, the absolute amplitudes of MEPs collected in Experiment 1 (i.e. without priming) and Experiment 3a (i.e. with QPS-5 ms priming) were entered in three-way repeated-measures ANOVA with PRIMING (with and without priming), CONDITION (QPS-1.5 ms, QPS-5 ms, ···, and QPS-100 ms), and TIME (baseline 1, and following six time points) as within-subject factors to match the measurement time points relative to QPS conditioning between Experiments 1 and 3a. Additionally, it might be valid to evaluate the effect of priming stimulation on subsequent QPS-induced plasticity using these values because the absolute amplitudes obtained at baseline 0 and 1 were not significantly different (see Results).

Control experiments. The time course of after-effects for the first control experiment (i.e. sham conditioning with real priming) on absolute MEP sizes was analysed using one-way repeated measures ANOVA (within-subject factor, TIME (baseline 1 and following six time points)). For the second control experiment, the after-effects of QPS-10 ms with sham priming were compared with those of QPS-10 ms without priming using two-way repeated-measures ANOVA (within-subject factors, CONDITION (QPS-10 ms with sham priming, QPS-10 ms without priming) and TIME (baseline 1, and following six time points)). *Post hoc* paired *t* tests (two-tailed) with Bonferroni's corrections for multiple comparisons were used for further analyses, if the factors PRIMING, CONDITION and TIME or PRIMING and CONDITION showed significant interaction.

Supplementary experiments. First, the after-effects of QPS-5 ms for 30 min with 10 min QPS-5 ms priming and QPS-5 ms for 40 min on absolute MEP sizes were compared using two-way repeated-measures ANOVA (within-subject factors, CONDITION (QPS-5 ms with priming, QPS-5 ms for 40 min) and TIME (baseline 1, and following six time points)). Second, the after-effects of QPS-5 ms with different durations (20 min, 30 min and 40 min) on absolute MEP sizes were compared using two-way repeated-measures ANOVA (within-subject factors, DURATION (QPS-5 ms for 20 min, 30 min and 40 min) and TIME (baseline, and following six time points)). *Post hoc* Bonferroni's method was used for further analysis. The time course of after-effects of each condition (QPS-5 ms for 20 min, 30 min and 40 min) on absolute MEP sizes was analysed using one-way repeated measures ANOVA (within-subject factor, TIME (baseline 1 and following six time points)).

Table 1. Physiological parameters (mean ± s.d.)

	RMT	AMT	MEP size (FDI)		Test MEP size (for Expts 2c and 3b)		
Experiment 1	Baseline 1	Baseline 1	Baseline 1		Baseline	Post 1	Post 2
QPS-1.5 ms	52.3 ± 11.7%	35.0 ± 8.6%	0.39 ± 0.08		—	—	—
QPS-5 ms	53.6 ± 12.7%	37.5 ± 8.8%	0.43 ± 0.10		—	—	—
QPS-10 ms	53.5 ± 10.7%	37.6 ± 8.0%	0.48 ± 0.11		—	—	—
QPS-30 ms	56.2 ± 19.0%	36.6 ± 7.9%	0.42 ± 0.16		—	—	—
QPS-50 ms	52.5 ± 10.3%	36.7 ± 7.9%	0.47 ± 0.12		—	—	—
QPS-100 ms	54.9 ± 12.1%	35.8 ± 9.2%	0.45 ± 0.14		—	—	—
QPS-1250 ms	52.5 ± 16.1%	34.9 ± 16.1%	0.44 ± 0.16		—	—	—
Experiments 2a & 2b							
QPS-5 ms	51.8 ± 12.2%	37.4 ± 6.3%			—	—	—
QPS-50 ms	50.3 ± 6.1%	38.8 ± 2.1%			—	—	—
Experiment 2c							
QPS-5 ms	59.6 ± 8.6%	39.0 ± 3.6%			0.45 ± 0.12	0.44 ± 0.13	0.47 ± 0.14
QPS-50 ms	56.2 ± 9.2%	39.0 ± 5.5%			0.46 ± 0.13	0.48 ± 0.12	0.43 ± 0.10
Experiment 3a	Baseline 0	Baseline 0	Baseline 0	Baseline 1			
Main experiments; with QPS-5 ms priming							
QPS-1.5 ms	55.5 ± 14.8%	37.2 ± 11.1%	0.45 ± 0.13	0.48 ± 0.25	—	—	—
QPS-5 ms	56.0 ± 7.3%	37.1 ± 5.1%	0.42 ± 0.08	0.45 ± 0.17	—	—	—
QPS-10 ms	58.0 ± 6.0%	39.7 ± 6.2%	0.46 ± 0.17	0.46 ± 0.17	—	—	—
QPS-30 ms	55.5 ± 12.6%	39.7 ± 11.7%	0.48 ± 0.11	0.43 ± 0.16	—	—	—
QPS-50 ms	53.2 ± 12.2%	37.8 ± 4.9%	0.49 ± 0.11	0.45 ± 0.15	—	—	—
QPS-100 ms	54.7 ± 13.0%	36.0 ± 7.6%	0.47 ± 0.10	0.48 ± 0.16	—	—	—
Control experiments							
Sham with priming	53.6 ± 9.2%	34.6 ± 6.2%	0.46 ± 0.06	0.51 ± 0.14	—	—	—
QPS-10 ms with sham priming	54.5 ± 12.1%	37.4 ± 10.1%	0.47 ± 0.12	0.48 ± 0.18	—	—	—
Supplementary experiments							
QPS-5 ms for 20 min	55.5 ± 12.5%	37.7 ± 7.7%	—	0.43 ± 0.15	—	—	—
QPS-5 ms for 40 min	55.8 ± 12.5%	35.8 ± 7.9%	—	0.47 ± 0.16	—	—	—
Experiment 3b							
QPS-5 ms priming	52.5 ± 16.1%	34.9 ± 16.1%	—	—	0.46 ± 0.12	0.48 ± 0.21	0.48 ± 0.22
Experiment 3c *with QPS-50 ms priming*							
QPS-10 ms	50.0 ± 10.0%	36.8 ± 9.3%	0.44 ± 0.11	0.42 ± 0.18	—	—	—
QPS-30 ms	52.8 ± 12.6%	36.6 ± 11.6%	0.40 ± 0.12	0.41 ± 0.15	—	—	—
QPS-100 ms	51.7 ± 12.2%	36.3 ± 7.5%	0.46 ± 0.07	0.49 ± 0.14	—	—	—

Experiment 3b. The time course of after-effects on each value of SICI, ICF, SICF and LICI was analysed using one-way repeated measures ANOVA (within-subject factor, TIME). Dunnett's *post hoc* test was used for further analyses.

Experiment 3c. Absolute values of MEPs at baseline 0 and 1 were compared using paired *t* tests in each experiment. To evaluate priming effects on subsequent QPS-induced plasticity, the absolute amplitudes of MEPs collected in Experiment 1 (i.e. without priming) and Experiment 3c (i.e. with QPS-50 ms priming) were entered in three-way repeated-measures ANOVA with PRIMING (with and without priming), CONDITION (QPS-10 ms, QPS-30 ms and QPS-100 ms), and TIME (baseline 1, and following six time points) as within-subject factors. *Post hoc* paired *t* tests (two-tailed) with Bonferroni's corrections for multiple comparisons were used for further analyses, if the factors PRIMING, CONDITION and TIME showed significant interaction.

Data were analysed using software (SPSS vers.13.0 for Windows; SPSS Inc.). All figures depict group data.

Results

No subject reported any adverse effect during or after any experiment. Baseline physiological data (Table 1) did not differ significantly among different experiments.
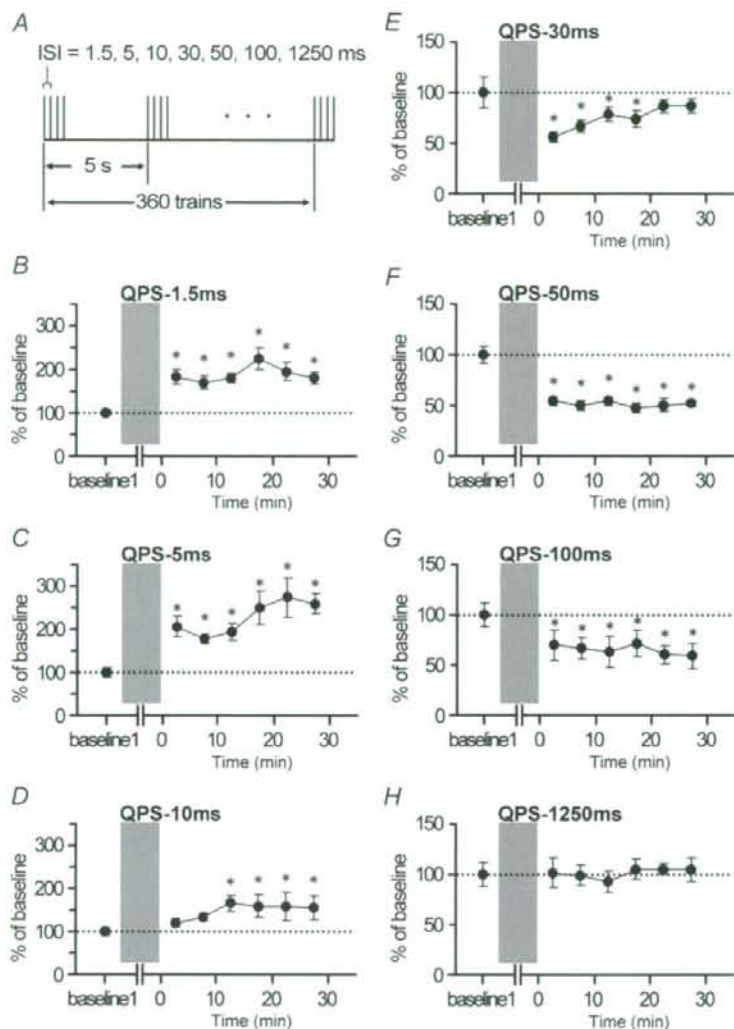
Figure 1. QPS protocol and bidirectional QPS-induced plasticity

A, the conditioning protocol comprises 360 trains of TMS pulses during 30 min. Each train comprises four mono-phasic magnetic pulses (i.e. quadripulse stimulation: QPS) delivered at interstimulus intervals of 1.5–1250 ms. One train was given once in 5 s. *B–H*, normalized amplitudes of MEPs (mean ± s.e.m.) for 30 min after QPS. *B–D*, QPS at short intervals provided significant and sustained MEP facilitation for at least 30 min compared to the baseline: *B*, QPS-1.5 ms, *n* = 10; *C*, QPS-5 ms, *n* = 10; *D*, QPS-10 ms, *n* = 7; *E–H*, QPS at long intervals yielded significant MEP suppression compared to the baseline: *E*, QPS-30 ms, *n* = 10; *F*, QPS-50 ms, *n* = 10; *G*, QPS-100 ms, *n* = 7; *H*, QPS-1250 ms, *n* = 8. QPS-30 ms produced a transient decrease of MEPs up to 20 min. A sustained MEP suppression (for 30 min) was found after QPS-50 ms and QPS-100 ms. No significant MEP changes were found after QPS-1250 ms. Asterisks denote *P* < 0.05 by *post hoc* Dunnett's test. *I*, normalized amplitudes of MEP measured at 30 min after QPS as a function of the reciprocal of ISI of QPS: left, individual results; right, group result, mean (± s.e.m.) of baseline, *n* = 7 for each point. Asterisks (*P* < 0.05, **P* < 0.001) denote significant difference from QPS-1250 ms. *J* and *K*, normalized amplitude of MEP for a longer period after QPS. *J*, QPS-1.5 ms (grey circles, *n* = 7), QPS-5 ms (open circles, *n* = 6), and QPS-10 ms (black circles, *n* = 7). In one subject, MEP measurements were extended beyond 180 min after QPS-5 ms (triangles). The MEP sizes returned to the baseline level at 240 min post QPS-5 ms. Asterisks denote *P* < 0.05 by *post hoc* Dunnett's test. *K*, QPS-50 ms (circles, *n* = 7) and QPS-100 ms (grey circles, *n* = 6). Asterisks denote *P* < 0.05 by *post hoc* Dunnett's test.

ISI-dependency of QPS-induced plasticity (Experiment 1)

Figure 1 shows time courses of MEP size following QPS conditioning. QPS at short ISIs produced an increase in the MEP amplitude (Fig. 1B–D), whereas QPS at long ISIs suppressed MEPs (Fig. 1E–H). Two-way repeated measures ANOVA revealed a significant CONDITION (QPS-1.5 ms, QPS-5 ms, ⋯, and QPS-1250 ms) × TIME interaction ($F_{21.090,193.321} = 3.794$, $P < 0.001$). The MEPs to single-pulse TMS were facilitated for at least 30 min after QPS at short ISIs (one-way repeated measures ANOVA: QPS-1.5 ms, effect of TIME, $F_{6.54} = 4.680$, $P < 0.001$; QPS-5 ms, effect of TIME, $F_{6.54} = 4.512$, $P < 0.001$; QPS-10 ms, effect of TIME, $F_{6.36} = 3.286$, $P = 0.011$)

(Fig. 1B–D). By contrast, QPS at long ISIs induced MEP suppression (one-way repeated measures ANOVA: QPS-30 ms, effect of TIME, $F_{6.54} = 4.509$, $P < 0.001$; QPS-50 ms, effect of TIME, $F_{6.54} = 27.073$, $P < 0.001$; QPS-100 ms, effect of TIME, $F_{6.36} = 3.987$, $P = 0.004$). The duration of suppression depended strongly on the ISI. In fact, QPS-30 ms induced significant MEP suppression up to 20 min (Fig. 1E). Marked MEP suppression was elicited for 30 min after QPS-50 ms and QPS-100 ms (Fig. 1F and G). No significant MEP changes were found after QPS-1250 ms (one-way repeated measures ANOVA: effect of TIME, $F_{6.42} = 0.717$, $P = 0.638$) (Fig. 1H).

Figure 1I presents the MEP amplitude normalized to the baseline MEP 30 min after QPS as a function of the reciprocal of the ISI used in each QPS
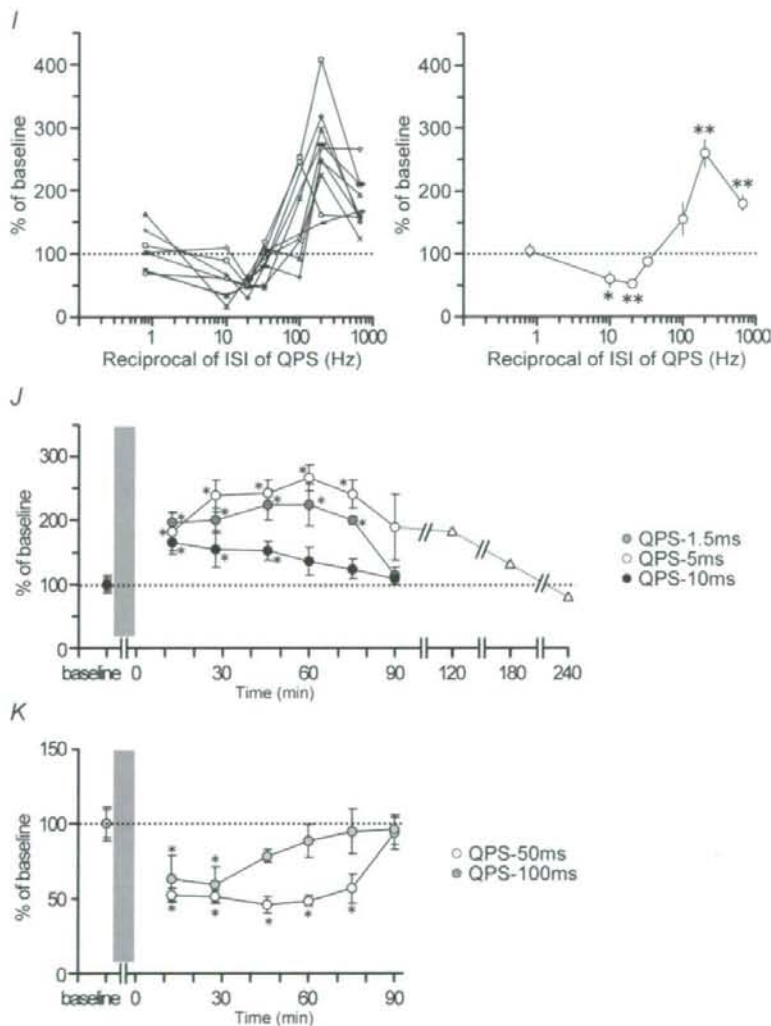

Figure 1. *Continued.*

burst. There was a non-linear relation between MEP excitability and ISI, which was similar to the BCM-like sigmoid curve (Bienenstock *et al.* 1982; Dudek & Bear, 1992). *Post hoc* analysis revealed that QPS-1.5 ms, QPS-5 ms and QPS-50 ms were significantly different from QPS-1250 ms (QPS-1.5 ms *versus* QPS-1250 ms, $P < 0.001$; QPS-5 ms *versus* QPS-1250 ms, $P < 0.001$; QPS-50 ms *versus* QPS-1250 ms, $P < 0.001$, QPS-100 ms *versus* QPS-1250 ms, $P = 0.021$).

We extended the period of MEP measurement to 90 min or longer to examine when the after-effects of QPS revert to the baseline level (Fig. 1*J*). Significant MEP facilitation was found for 75 min after QPS-1.5 ms and QPS-5 ms (QPS-1.5 ms, effect of TIME, $F_{6.36} = 7.807$, $P < 0.001$; QPS-5 ms, effect of TIME, $F_{6.30} = 5.887$, $P < 0.001$) (Fig. 1*J*). The mean MEPs remained larger than the baseline level ($189.9 \pm 57\%$) at 90 min after QPS-5 ms, although the difference was no longer statistically significant ($P = 0.085$). For one subject, we extended MEP

measurements beyond 180 min and confirmed that MEPs had returned to the baseline at 240 min post QPS-5 ms (Fig. 1*J*). Transient MEP facilitation was found after QPS-10 ms (effect of TIME, $F_{6.36} = 3.552$, $P = 0.007$), which lasted up to 45 min ($P < 0.05$), but not to 60 min ($P > 0.05$). For QPS at long intervals, MEPs returned to the baseline level at 90 min after QPS-50 ms (effect of TIME, $F_{6.36} = 13.965$, $P < 0.001$) (Fig. 1*K*). The after-effect did not last beyond 45 min after QPS-100 ms (effect of TIME, $F_{6.30} = 4.147$, $P = 0.004$) (Fig. 1*K*).

Basic properties of QPS-induced plasticity (Experiment 2)

Motor thresholds and recruitment curves (Experiment 2a). Motor thresholds and recruitment curves were compared before and after QPS-5 ms ($n = 5$; Fig. 2*A* and *B*) and QPS-50 ms ($n = 5$; Fig. 2*D* and *E*) to elucidate the basic properties of QPS-induced plasticity. These two
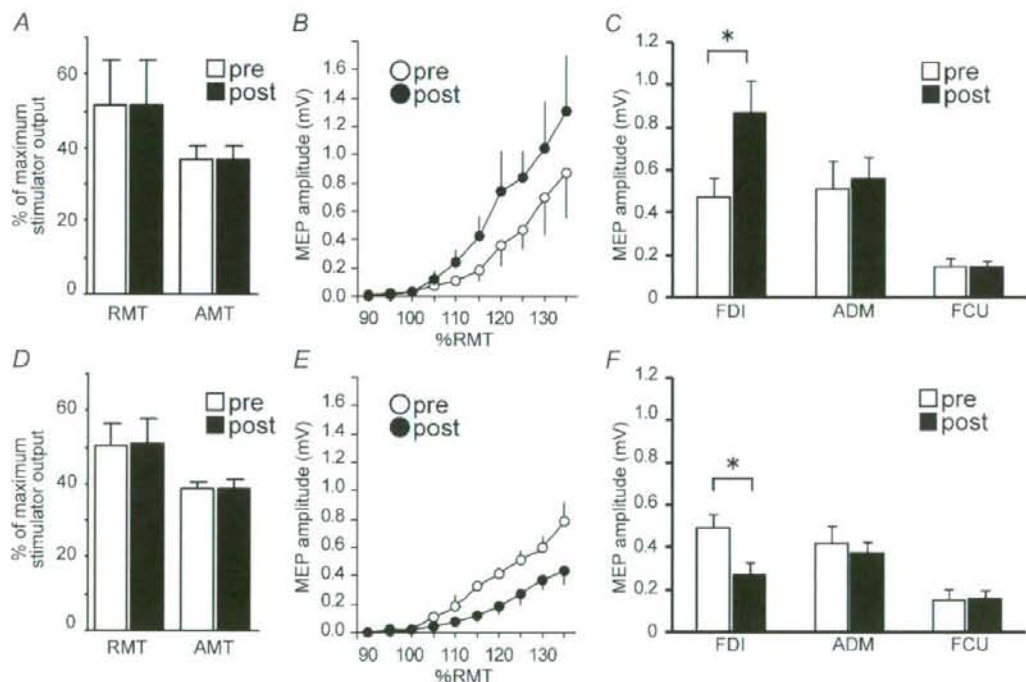
Figure 2. Basic properties of QPS-induced plasticity
A–C, effects of QPS-5 ms on variables. *A*, motor thresholds (mean ± s.d.). QPS-5 ms affected neither RMT nor AMT ($P > 0.5$). Pre-conditioning (open bars); post conditioning (30 min post conditioning) (filled bars). *B*, the recruitment curve (mean ± s.e.m.). The ordinate gives the MEP size in millivolts; the abscissa shows the stimulus intensity relative to RMT. Pre-conditioning (○); post conditioning (●). Significantly larger MEPs were elicited at intensities greater than 125% RMT after QPS-5 ms. *C*, MEPs from the FDI, ADM and FCU (mV, mean ± s.e.m., $n = 5$). *D–F*, effects of QPS-50 ms on variables. *D*, motor thresholds (mean ± s.d.). Neither RMT nor AMT was altered by QPS-50 ms. *E*, the recruitment curve (mean ± s.e.m.). Smaller MEPs were elicited at intensities greater than 115% RMT after QPS-50 ms. *F*, MEPs from the FDI, ADM and FCU (mV, mean ± s.e.m., $n = 5$). *$P < 0.05$.

conditioning types were chosen because they induced the strongest facilitatory or suppressive after-effects. Neither the resting motor threshold (RMT) nor the active motor threshold (AMT) was altered (Fig. 2A and D), although the recruitment curves were modulated by QPSs (Fig. 2B and E). After QPS-5 ms, the curve became steeper than before (two-way repeated-measures ANOVA: TIME × INTENSITY interaction, $F_{9,36} = 5.862$, $P < 0.001$; effect of TIME, $F_{1,36} = 12.728$, $P < 0.023$; effect of INTENSITY, $F_{9,36} = 9.026$, $P < 0.001$). *Post hoc* analysis with Bonferroni's adjustment showed significantly larger MEPs elicited at intensities greater than 125% RMT after QPS-5 ms than before (Fig. 2B). On the other hand, for QPS-50 ms (Fig. 2E), the recruitment curve became shallower after conditioning (two-way repeated-measures ANOVA: TIME × INTENSITY interaction, $F_{1,36} = 6.141$, $P < 0.001$; effect of TIME, $F_{1,36} = 33.939$, $P = 0.004$; effect of INTENSITY, $F_{9,36} = 30.388$, $P < 0.001$). *Post hoc* analysis using Bonferroni's adjustment showed significantly smaller MEPs elicited at intensities greater than 115% RMT after QPS-50 ms than before (Fig. 2E). These changes cannot be explained by the variability of measurements because the baseline recruitment curve showed good reproducibility. The curves before QPS-5 ms and QPS-50 ms did not differ significantly from each other (two-way repeated-measures ANOVA: CONDITION × INTENSITY interaction, $F_{9,72} = 0.301$, $P = 0.972$; effect of CONDITION, $F_{1,8} = 0.025$, $P = 0.879$; effect of INTENSITY, $F_{9,72} = 20.322$, $P < 0.001$).

MEPs from FDI, ADM and FCU muscle (Experiment 2b). The MEPs to single-pulse TMS from the right ADM and FCU were measured to confirm the topographical specificity of modulation by QPS. For QPS-5 ms (Fig. 2C), two-way repeated measures ANOVA showed a significant TIME × MUSCLE interaction ($F_{2,8} = 5.195$, $P = 0.036$). *Post hoc* paired *t* test results revealed that only MEPs from the FDI were facilitated after QPS-5 ms. For QPS-50 ms (Fig. 2F), similar results were obtained (two-way repeated measures ANOVA: TIME × MUSCLE interaction, $F_{2,8} = 6.579$, $P = 0.019$). Therefore, only MEPs from the FDI were suppressed after QPS-50 ms.

Motor cortical excitability accessed by paired-pulse TMS (Experiment 2c). To clarify the effects of QPS on excitatory and inhibitory circuits of the primary motor cortex, SICI, ICF and SICF were measured before and after QPS.

Figure 3 shows the effects of QPS-5 ms (Fig. 3A) or QPS-50 ms (Fig. 3B) on SICI. The test MEP sizes were adjusted before and after QPS conditioning (Table 1). For QPS-5 ms, three-way repeated measures ANOVA revealed a significant main effects of ISI ($F_{1,9} = 22.444$, $P = 0.001$)

and INTENSITY ($F_{2,18} = 28.731$, $P < 0.001$), but no significant effects of TIME ($F_{2,18} = 0.003$, $P = 0.997$), ISI × TIME interaction ($F_{2,18} = 0.012$, $P = 0.642$), INTENSITY × TIME interaction ($F_{4,36} = 0.328$, $P = 0.857$), nor TIME × ISI × INTENSITY interaction ($F_{4,36} = 0.679$, $P = 0.611$). Similar results were obtained for QPS-50 ms (Fig. 3B) (three-way repeated measures ANOVA: a main effect of ISI, $F_{1,9} = 16.616$, $P = 0.003$; effect of INTENSITY, $F_{2,18} = 28.936$, $P < 0.001$; effect of TIME, $F_{2,18} = 0.367$, $P = 0.698$; ISI × TIME interaction, $F_{2,18} = 0.181$, $P = 0.836$; INTENSITY × TIME interaction, $F_{4,36} = 0.095$, $P = 0.983$; TIME × ISI × INTENSITY interaction, $F_{4,36} = 0.286$, $P = 0.885$), indicating that QPS did not modulate inhibitory circuits within the primary motor cortex as evaluated by SICI.

In contrast, QPS significantly affected intracortical excitatory circuits. Figure 4A and B shows that ICF was significantly enhanced by QPS-5 ms (two-way repeated measures ANOVA: effect of TIME, $F_{2,18} = 4.641$, $P = 0.024$; ISI × TIME interaction, $F_{2,18} = 2.299$, $P = 0.129$) and suppressed by QPS-50 ms (two-way repeated measures ANOVA: effect of TIME, $F_{2,18} = 6.728$, $P = 0.007$; ISI × TIME interaction, $F_{2,18} = 1.358$, $P = 0.282$). *Post hoc* analysis revealed significant modulation of ICF at post 1 and post 2 compared with those at baseline, suggesting that the effects on ICF lasted longer than 60 min (Fig. 4A and B).

SICF was also modulated by QPS (Fig. 4C and D). After QPS-5 ms, SICF was enhanced (two-way repeated measures ANOVA: effect of TIME, $F_{2,18} = 20.828$, $P < 0.001$; ISI × TIME interaction, $F_{10,90} = 1.173$, $P = 0.319$). *Post hoc* analysis showed significant enhancement of SICF at post 1 and 2 ($P < 0.001$). By contrast, QPS-50 ms suppressed SICF (Fig. 4D). Two-way repeated measures ANOVA revealed significant ISI × TIME interaction ($F_{10,90} = 2.175$, $P = 0.026$). *Post hoc* analysis revealed significant suppression of SICF with 1.5 ms at post 1 and 2 ($P < 0.005$).

Priming-induced effects on QPS-induced plasticity (Experiment 3)

**QPS conditioning with QPS-5 ms priming (Experiment 3a).** *Control experiments.* First, the after-effects of sham conditioning with real priming were monitored to examine whether priming alone (i.e. QPS-5 ms for 10 min) affects motor cortical excitability. Figure 5B shows the time course of the normalized MEP amplitude following sham conditioning with real priming. No difference was found in MEP amplitudes at baseline 0 and 1 (paired *t* test, $P > 0.5$). Sham conditioning with real priming did not change the MEP amplitude for at least 30 min after

conditioning (one-way repeated measures ANOVA: effect of TIME, $F_{6,30} = 0.548$, $P = 0.767$).

Second, the after-effect of real conditioning (QPS-10 ms) with sham priming was compared to that of real conditioning without priming to confirm that sham priming does not affect motor cortical plasticity induced by real conditioning. Figure 5C shows the time courses of normalized amplitude of MEPs following real conditioning (QPS-10 ms) without priming and also with sham priming. No difference was found between MEPs of baseline 0 and 1 (paired t test, $P > 0.5$). Furthermore, MEP amplitudes following QPS-10 ms with sham priming were not different from those without priming (two-way repeated measures ANOVA: effect of CONDITION (QPS-10 ms with sham priming, QPS-10 ms without priming), $F_{1,5} = 0.046$, $P = 0.839$; CONDITION × TIME interaction, $F_{6,30} = 0.354$, $P = 0.902$).

Main experiments. Figure 5D–I shows the time courses of MEP amplitude following QPS at various intervals with and without priming. No difference in MEP amplitudes at baselines 0 and 1 was found in any condition (paired t test, $P > 0.5$).

Priming stimulation occluded MEP facilitation induced by QPS-1.5 ms and QPS-5 ms without priming (Fig. 5D and E). In fact, QPS-10 ms with priming induced lasting MEP suppression, whereas that without priming elicited MEP facilitation (Fig. 5F). Priming stimulation enhanced the suppression observed after QPS-30 ms (Fig. 5G). Both QPS-50 ms and QPS-100 ms with priming induced slightly stronger MEP suppression than that induced by QPS without priming (Fig. 5H and I). Three-way repeated measures ANOVA revealed a significant PRIMING × CONDITION interaction ($F_{2.764,13.813} = 9.624$, $P = 0.001$), but revealed
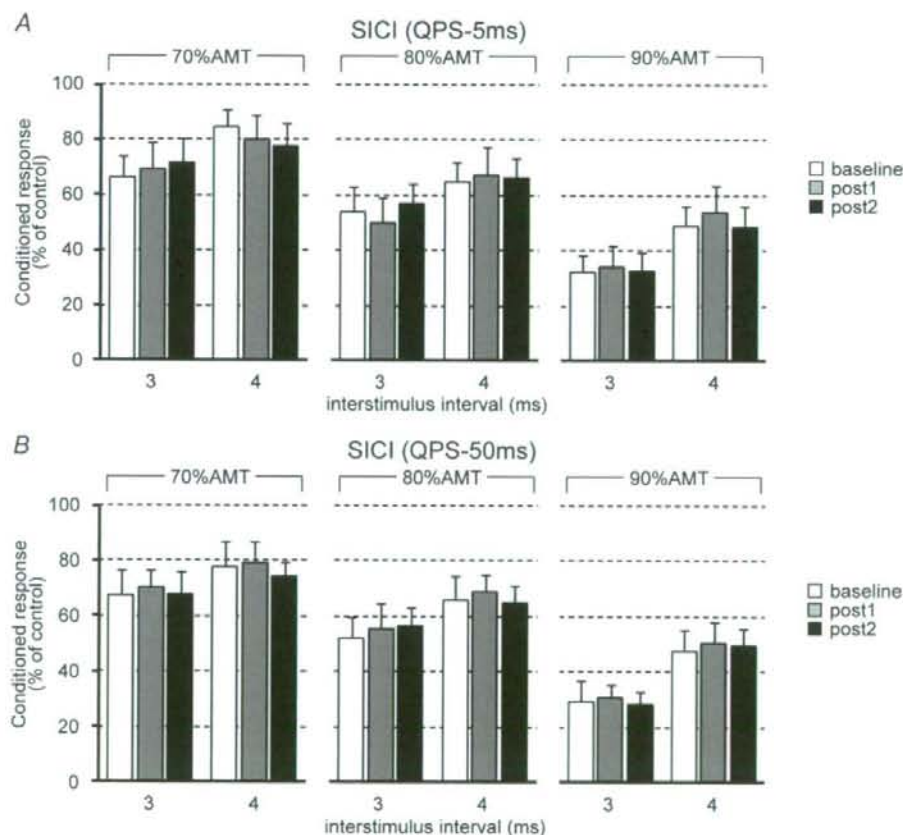
Figure 3. Effects of QPS on short-interval intracortical inhibition (SICI)
A and *B*, SICI before and after QPS-5 ms (*A*) or QPS-50 ms (*B*). Conditioning intensities were 70% active motor threshold (AMT; left), 80% AMT (middle), and 90% AMT (right). The abscissa shows conditioning–test intervals (3 ms and 4 ms). Baseline (open bars); post 1 (grey bars), 0–30 min after QPS; post 2 (black bars), 30–60 min after QPS.

no significant main effect of TIME ($F_{2.548, 12.738} = 1.662$, $P = 0.227$) nor a significant PRIMING × CONDITION × TIME interaction ($F_{3.384, 16.920} = 2.466$, $P = 0.092$). The results reveal that priming stimulation affected subsequent QPS-induced plasticity, irrespective of the time after QPS conditioning. *Post hoc* paired *t* tests revealed a significant effect of QPS-5 ms priming on the after-effects of QPS-1.5 ms, QPS-5 ms, QPS-10 ms and QPS-30 ms (Fig. 5D–G).

Supplementary experiments. Figure 5E shows that QPS-5 ms priming for 10 min followed by 30 min of QPS-5 ms produced no lasting changes in MEP sizes. To clarify whether the gap between priming and primary conditioning is necessary for inducing a metaplastic change, we performed an additional experiment using QPS-5 ms for 40 min (Fig. 6A and B). No significant difference was found between the after-effects of QPS-5 ms for 40 min and QPS-5 ms for 30 min with 10 min QPS-5 ms priming, indicating that the after-effects of QPS-5 ms for 30 min were altered by priming stimulation irrespective of the presence or absence of a gap (two-way repeated measures ANOVA: effect of

CONDITION (QPS-5 ms with priming, QPS-5 ms for 40 min), $F_{1.4} = 0.040$, $P = 0.851$; CONDITION × TIME interaction, $F_{6.24} = 0.299$, $P = 0.931$).

Since QPS-5 ms for 30 min can be regarded as QPS-5 ms for 20 min with 10 min QPS-5 ms priming, we also performed an experiment using QPS-5 ms for 20 min with an ITI of 5 s at 90% AMT to identify whether the first part of QPS-5 ms for 30 min conditioning 'primes' subsequent 20 min QPS-5 ms conditioning (Fig. 6C). The results showed that QPS-5 ms for 20 min did not produce any plastic changes. In terms of the duration of QPS-5 ms conditioning, two-way repeated measures ANOVA revealed a significant main effect of DURATION ($F_{2.8} = 5.923$, $P = 0.026$), but no significant effect of DURATION × TIME interaction ($F_{12.48} = 1.126$, $P = 0.370$), indicating that the effect of DURATION did not depend on the measurement time points. Separate one-way repeated measures ANOVA on the time course of each conditioning revealed no significant facilitation of MEPs after QPS-5 ms for 20 min or 40 min compared with baseline MEP sizes (effect of TIME, QPS-5 ms for 20 min, $F_{6.24} = 0.655$, $P = 0.686$; QPS-5 ms for 40 min, $F_{6.24} = 0.460$, $P = 0.831$). Figure 6D shows the grand
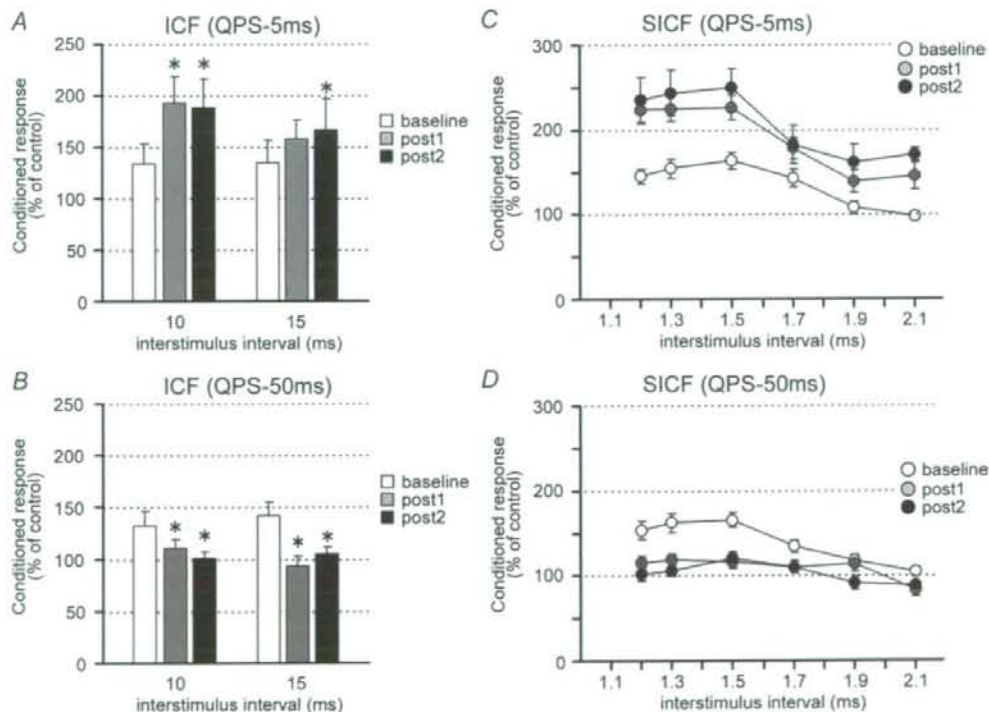

Figure 4. Effects of QPS on intracortical facilitation (ICF) and short-interval ICF (SICF)
A and B, ICF before and after QPS-5 ms (A) or QPS-50 ms (B). Conditioning intensity was 90% AMT. Conditioning–test intervals were 10 and 15 ms. C and D, SICF before and after QPS-5 ms (C) or QPS-50 ms (D). Conditioning intensity was 90% AMT. The abscissa shows conditioning–test intervals.

averages for 30 min post conditioning as a function of duration of QPS-5 ms. QPS-5 ms for 30 min significantly facilitated MEPs compared with those for 20 min and 40 min. *Post hoc* analysis of these data using Bonferroni's method revealed a significant difference in DURATION of QPS-5 ms (20 min *versus* 30 min, $P < 0.05$; 30 min *versus* 40 min, $P < 0.05$).

Effects of QPS-5 ms priming on SICI, ICF, SICF and LICI (Experiment 3b). Although the QPS-5 ms priming did not produce any lasting changes as indexed by MEP sizes (Table 1), Fig. 7 shows that SICF (but no other measures) was significantly enhanced after QPS-5 ms for 10 min (one-way repeated measures ANOVA: SICI, effect of TIME, $F_{2,14} = 0.916$, $P = 0.824$; ICF, effect
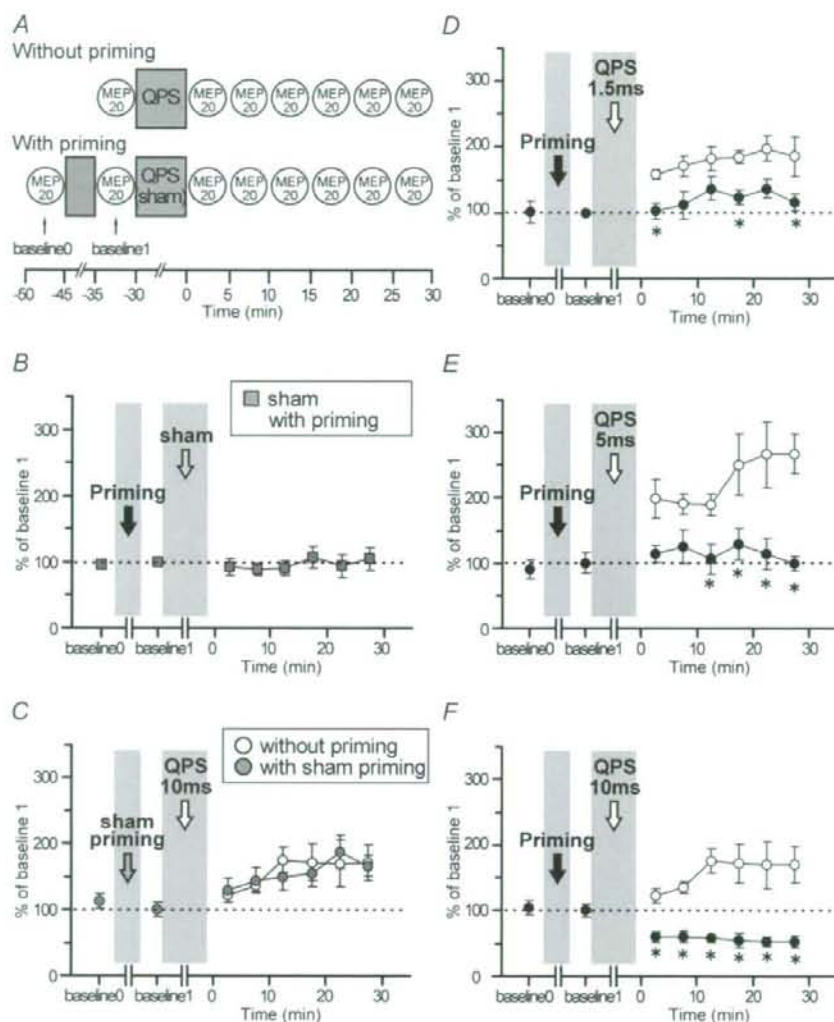
Figure 5. QPS-5 ms priming effects on QPS-induced plasticity ($n = 6$)
A, timeline of experiments (see Methods). The priming is QPS-5 ms for 10 min. *B*, sham conditioning with real priming did not modify motor cortical excitability. *C*, the after-effects of QPS-10 ms without priming (open circles) were not different from those of QPS-10 ms with sham priming (grey circles). *D–I*, time courses of MEP amplitude following QPS at various intervals with (•) and without priming (○). Asterisks denote significant difference of MEP sizes with priming from those without priming at each time point ($P < 0.05$ by *post hoc* paired *t* tests). *D* and *E*, priming stimulation induced by QPS-1.5 ms and QPS-5 ms. *F*, priming stimulation reversed the QPS-10 ms-induced long-lasting MEP facilitation to the lasting MEP suppression. *G*, when priming was applied before QPS-30 ms, MEP suppression did not revert to the baseline level at 30 min post conditioning. *H* and *I*, QPS-50 ms and QPS-100 ms: priming slightly enhanced MEP suppression after conditioning.

of TIME, $F_{2,14} = 1.193$, $P = 0.332$; SICF, effect of TIME, $F_{2,14} = 8.434$, $P = 0.004$; LICI, effect of TIME, $F_{2,14} = 0.236$, $P = 0.793$). However, the effects were only transient, being significant only at post 1 (Fig. 7).

QPS conditioning with QPS-50 ms priming (Experiment 3c).
Figure 8 shows significant facilitation of MEP sizes following QPS-50 ms priming in comparison to those without priming (three-way repeated measures ANOVA: PRIMING × CONDITION × TIME interaction, $F_{12,60} = 2.226$, $P = 0.021$). Post hoc paired t tests revealed a significant effect of QPS-50 ms priming on the after-effects of QPS-10 ms, QPS-30 ms and QPS-100 ms (Fig. 8A–C).
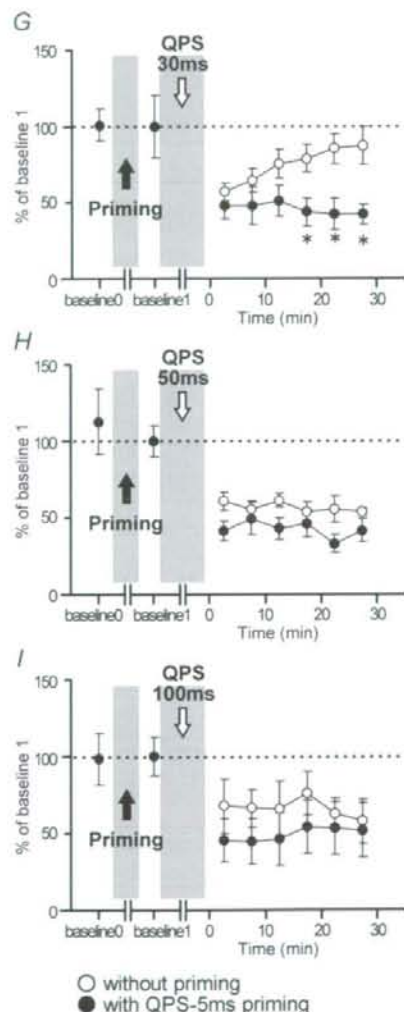

Figure 5. *Continued.*

Stimulus–response function with priming

Figure 9 shows the normalized MEP amplitudes at 30 min post conditioning as a function of the reciprocal of the ISI used in each QPS with and without priming. It appears as if the stimulus–response function can be shifted in either direction along the x-axis according to which priming stimulation was employed. *Post hoc* analysis with these data revealed that QPS-5 ms priming stimulation significantly reduced MEP sizes after QPS-1.5 ms, QPS-5 ms, QPS-10 ms and QPS-30 ms, whereas QPS-50 ms priming facilitated MEP sizes after QPS-10 ms, QPS-30 ms and QPS-100 ms (Fig. 9).

Discussion

Our study had three major findings. First, there was a non-linear relationship between the sign and duration of plastic changes induced by QPS and the ISI between each of the four pulses in the QPS burst. Second, QPS modulated intracortical excitatory circuits of the primary motor cortex, whereas inhibitory circuits remained unchanged. Third, QPS-induced plasticity was altered by priming stimulation in a manner consistent with the BCM rule.

Relationship to conventional rTMS studies

QPS at short intervals induced facilitatory after-effects with prolonged duration (Fig. 1). We hypothesized previously that I-wave periodicity (i.e. 1.5 ms), a basic property of the human motor cortex (Hanajima *et al.* 2002), is suitable for LTP-like plasticity induction (Thickbroom *et al.* 2006; Hamada *et al.* 2007*a,b*). However, QPS-5 ms, which does not correspond to I-wave periodicity, also yielded MEP facilitation lasting longer than 75 min. The fact that it is not necessary to use ISIs at an I-wave periodicity to induce prolonged changes in motor cortical excitability raises the intriguing possibility that I-wave periodicity is not the critical factor in the QPS protocol.

The sign and duration of the after-effects depended on a complex function of the stimulus parameters. Prolonged suppression was induced by QPS-50 ms and QPS-100 ms, whose frequency corresponds to 20 Hz and 10 Hz; regular rTMS at those frequencies usually gives rise to trans-ient, short-lasting facilitation (less than a few minutes) (Pascual-Leone *et al.* 1994; Maeda *et al.* 2000). The difference in effects is presumably attributable to the number of pulses per train because the stimulus intensity and the total number of pulses used for our study were comparable to those in previous studies (Maeda *et al.* 2000; Arai *et al.* 2007). Consequently, the ISI and the number of pulses per train are critical determinants for the sign and duration of the after-effects in the present

experiments. Although it would be of value to investigate other parameters of the QPS protocol such as the ITI or the number of pulses per train, this is not possible at the present time. There are no devices which can combine more than four monophasic stimulators and the ITI cannot be shortened to less than a few seconds because of the re-charging time for a monophasic stimulator.

Stimulus–response function of QPS-induced plasticity

The principal finding of this study was that changing the ISIs of QPS induced various levels of plastic changes. As the ISIs of QPS were shortened, the duration of MEP suppression (i.e. produced by QPS-100 ms) first increased (i.e. QPS-50 ms, to more than 75 min) and then decreased (i.e. QPS-30 ms). Further reductions in ISI altered the sign and duration of the plastic changes; transient facilitation (i.e. QPS-10 ms) became stronger and longer-lasting

as the ISIs were further shortened (i.e. QPS-5 ms and QPS-1.5 ms). The stimulus–response function using the values of plastic changes obtained at 30 min after QPS exhibited a smooth transition from net suppression to net facilitation. Although the stimulation frequency (i.e. reciprocal of ISI) within one train generally determines the direction of rTMS-induced plasticity (Hallett, 2007), results of our study demonstrate that the ISI within a train not only defines the sign of the plastic changes, but also determines its duration in a non-linear form.

At first sight, the non-linear stimulus–response function appears very similar to those obtained in animal studies. Dudek & Bear (1992) demonstrated a frequency–response function with a smooth transition from net LTD to net LTP as the stimulation frequency was increased systematically, conforming to the proposal of the BCM theory, which states that the strength of synapses depends on postsynaptic activity in a particular non-linear form
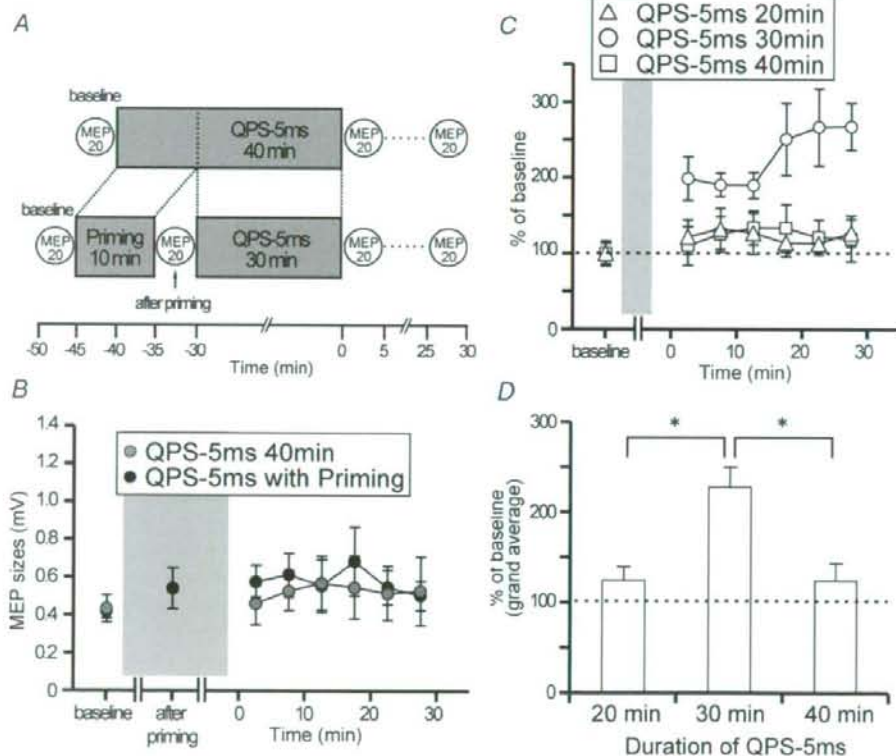
Figure 6.
A, timeline of experiment with QPS-5 ms for 40 min (see Methods). B, the after-effects of QPS-5 ms for 40 min (grey circles) did not differ from those of QPS-5 ms for 30 min with 10 min QPS-5 ms priming (black circles). C, time courses of the after-effects of QPS-5 ms with different conditioning durations. D, the grand average of normalized amplitudes of MEPs for 30 min post conditioning as a function of the conditioning duration of QPS-5 ms. *$P < 0.05$.